EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF *Syzygium cumini* ON OXYTOCIN INDUCED PRETERM LABOUR

A Dissertation Submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

CHENNAI-600 032

In partial fulfillment of the requirement for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

OCTOBER-2017

DEPARTMENT OF PHARMACOLOGY

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPPATTI ROAD,

COIMBATORE-641048

EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF *Syzygium cumini* ON OXYTOCIN INDUCED PRETERM LABOUR

A Dissertation submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

CHENNAI-600 032

In partial fulfillment of the requirement for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

OCTOBER-2017

DEPARTMENT OF PHARMACOLOGY

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPPATTI ROAD,

COIMBATORE-641 048

EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF *Syzygium cumini* ON OXYTOCIN INDUCED PRETERM LABOUR

A Dissertation submitted to

THE TAMIL NADU Dr. M .G .R. MEDICAL UNIVERSITY

CHENNAI-600 032

In partial fulfillment of the requirement for the award of the Degree of

MASTER OF PHARMACY
IN
PHARMACOLOGY
OCTOBER-2017

Submitted by

Reg. No. 261525802

DEPARTMENT OF PHARMACOLOGY

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPPATTI ROAD,

COIMBATORE-641 048

Prof. Dr. A. Rajasekaran, M. Pharm., Ph.D.,
Principal,
KMCH College of Pharmacy,
Kovai Estate, Kalapatti Road,
Coimbatore - 641 048.
Tamil Nadu

CERTIFICATE

This is to certify that the dissertation work entitled **"EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF *Syzygium cumini* ON OXYTOCIN INDUCED PRETERM LABOUR"** was carried out by **Reg. No. 261525802**. The work mentioned in the dissertation was carried out at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, for the partial fulfillment for the degree of Master of Pharmacy during the academic year 2016-2017 and is forwarded to the Tamil Nadu Dr. M. G. R. Medical University, Chennai.

Date: **Prof. Dr. A. Rajasekaran, M. Pharm., Ph.D.,**

Place: Coimbatore **Principal**

GUIDE
Dept. of Pharmacology,
KMCH College of Pharmacy,
Kovai Estate, Kalapatti Road,
Coimbatore - 641 048.
Tamil Nadu

CERTIFICATE

This is to certify that the dissertation work entitled "**EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF *Syzygium cumini* ON OXYTOCIN INDUCED PRETERM LABOUR**" is a bonafide work carried out by **Reg. No. 261525802**. The work mentioned in the dissertation was carried out at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, under my supervision and guidance during the academic year 2016-2017.

This research work either in part or full does not constitute any of any thesis / dissertation.

Date: **Signature of the guide**

Place: Coimbatore

DECLARATION

I do here by declare that to the best of my knowledge and belief ,the dissertation work entitled **"EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF *Syzygium cumini* ON OXYTOCIN INDUCED PRETERM LABOUR"** submitted to the Tamil Nadu Dr. M.G.R. Medical university , Chennai, in the partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology,** was carried out at Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, during the academic year 2016-2017.

Date:

Place: Coimbatore **Reg. No: 261525802**

EVALUATION CERTIFICATE

This is to certify that the work embodied in the thesis entitled **"EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF *Syzygium cumini ON* OXYTOCIN INDUCED PRETERM LABOUR"** submitted by **Reg No: 261525802** to the Tamil Nadu Dr. M.G.R. Medical university, Chennai, in the partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work carried out by the candidate during the academic year 2016-2017 at KMCH College of Pharmacy, Coimbatore, Tamil Nadu and the same was evaluated by us.

Examination Center: KMCH College of Pharmacy, Coimbatore

Date:

Place: Coimbatore

Internal Examiner **External Examiner**

Convener of Examination

CERTIFICATES

DEDICATION

ACKNOWLEDGEMENT

INTRODUCTION

REVIEW OF LITERATURE

PLANT PROFILE

AIM AND OBJECTIVES

PLAN OF WORK

MATERIALS AND METHODS

RESULTS

DISCUSSION

CONCLUSION

BIBLIOGRAPHY

DEDICATED TO ALMIGHTY,
MY BELOVED PARENTS,
SIBLINGS AND FRIENDS

ACKNOWLEDGEMENT

*My dissertation entitled **"EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF Syzygium cumini ON OXYTOCIN INDUCED PRETERM LABOUR"** would not have been a feasible one without the grace of God almighty who gave me moral till the completion of my project.*

*Many thanks to **Almighty God**, for it He who began this work in me and carried it to completion. It is he who has blessed me with the people whose names I feel privileged to mention here.*

*First and foremost it gives me great pleasure to record my deep sense of gratitude and indebtedness to my esteemed guide, **Mr. M. Ramasamy, M.Pharm, Assistant Professor, Department of Pharmacology**, for his constant insight, personal advice, countless serenity and pain taking efforts in all stages of my study.*

*I would like to thank **Dr. K. T. Manisenthil Kumar, M.Pharm., Ph.D., HOD, Department of Pharmacology**, for suggesting and guiding me throughout. I will forever be grateful for his invaluable ideas and support. His patience has always encouraged me in learning many things in this work and in my life too.*

*With great pleasure I wish to place my indebtedness to **Dr. A. Rajasekaran, M.Pharm., Ph.D., Principal, KMCH College of Pharmacy** for his support and for giving me an opportunity to do my project work.*

*My respectful regards to our beloved chairman **Dr. Nalla G. Palaniswami** and respected trustee madam **Dr. Thavamani D. Palaniswami**, Kovai Medical Centre Research and Education Trust, Coimbatore for all the facilities that were provided to me at the institution.*

*I owe my debt of gratitude to our esteemed staffs **Mr. Ariharasivakumar, M.Pharm., (Ph. D.,)** Professor and **Mr. Saravanan J. M. Pharm., Ms. Sanju K. M.Pharm.,** for their immense support, timely help and valuable suggestions.*

This project would not be a resplendent one without the timely help and continuous support by my ever-loving buddies ***Anna Mathew, Anusree E, T. Boopathi, Neethu Devasia, and Parthipan S.***

I take this opportunity to extend my indebtedness to Kokila, Sangeetha, Alga, Chippy, Neenu, Arathi, Treasa, Abin, Andrews, and Asif.

I am greatful to Lab technicians Mr. Tamilarasan (Dept. of Pharmacology), Mrs. Anandhi, Mrs. Akhila, Mrs. Menaka, Mrs. Muneeswari, , librarian, Ms. Dhanalakshmi and chemical storekeeper Mr. Viji for their timely support and help during the course of the entire work.

Above all I dedicate myself before the unfailing presence, constant love, immense support and encouragement given to me by my beloved Mother, Sister, Sister in law, my Brothers and Ivan who deserves the credit of success in whatever work I did.

Thank you all for the support and motivation

Reg No: 261525802

LIST OF ABBREVIATIONS

SL. NO	ABBREVIATIONS	FULL FORM
1.	ABTS	2, 2, - azinobis (3-ethylbenzoline-6-sulfonic acid)
2	AESC	Aqueous seed extract of Syzygium cumini
3.	AMP	Adenosine monophosphate
4	ANOVA	Analysis of Variance
5	CAT	Catalase
6	CCl4	Carbon Tetrachloride
7	cGMP	Cyclic Guanosine Monophosphate
8	COX	Cyclooxygenase
9	dL	Decilitre
10	DPPH	1,1 diphenyl picryl hydrazine
11	GSH	Glutathione reduced
12	IUGR	Intrauterine Growth Restriction
13	KCl	Pottassium Chloride
14	LPS	Lipopolysaccharide
15.	MIF	Macrophage Migration Inhibitory Factor
16.	NO	Nitric oxide
17.	OTR	Oxytocin Receptor
18.	PAM	Peptidylglycine alpha-amidating Monooxygenase
19.	PPARγ	Peroxisome Proliferator –activated Receptor Gamma
20.	PPROM	Preterm premature rupture of the membrane
21.	PROM	Premature Rupture Of The Membranes
22.	PTD	Preterm Delivery
23.	PTL	Preterm Labour
24.	ROS	Reactive Oxygen Species
25.	S.C	*Syzygium cumini*

26.	SEM	Standard Error Mean
27.	SOD	Superoxide dismutase
28.	T11	11^{th} Thoracic Vertebra
29.	T12	12^{th} Thoracic Vertebra
30.	TBA	Thiobarbituric acid
31	TCA	Trichloroacetic acid

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Syzygium cumini is a well known bioactive plant which has been widely used for the treatment of various diseases in traditional and folk medicine. The present investigation was aimed to determine the tocolytic activity of the aqueous seed extract of *Syzygium cumini* (AESC) in animal models. The in vitro antioxidant activity of AESC was evaluated by DPPH and ABTS radical scavenging assay which showed the hydrogen donating and free radical scavenging activity of extract that aids in the prevention of preterm labour. In vivo tocolytic activity of AESC was evaluated. Group I was given normal saline, Group II oxytocin (1 IU) i.m, Group III atosiban (6mg/kg) i.p, Group IV AESC (200mg/kg) p.o and Group V AESC (400mg/kg), p.o. AESC shows significant reduction in the rate of preterm labour which was comparable with the standard treated group as well as the normal group. The level of in vivo antioxidant parameters such as catalase, SOD, and GSH were restored in the treated group compared to the control group. The study reveals that the extracts have been able to increase the endogenous antioxidant enzyme activities while reducing the lipid peroxidation. The AESC was subjected to pharmacological testing in vitro on a piece of isolated rat uterus previously pretreated with estradiol valerate, concentrations used were 25 mg/ml and 50 mg/ml. The concentrations (25 mg/ml) and (50 mg/ml) produce 50.9 % and 72.7 % inhibition respectively. In conclusion, the results indicate the presence of active principles in the aqueous seed extract of *Syzygium cumini* which may be responsible for the tocolytic activity.

Key words: *Syzygium cumini,* Tocolytic activity

1. INTRODUCTION

Plants have been of great importance to mankind due to their medicinal as well as nutritional properties. In 1985, World Health Organization (WHO) estimated that around 80% of the population depends on medicinal plants for their primary healthcare needs in developing countries. Medicinal plants encompass some secondary metabolites like alkaloids, glycosides, saponins, essential oils, bitter principles, tannins and mucilages in different parts of the plant which can cure various illnesses in humans and other animals. Epidemiological studies indicate that increased intake of leafy vegetables is associated with decreased risk of cancers, cardiovascular disease, cataract and other age-related diseases. [1]

Plants have been the source of a wide variety of current drugs which are available in the market today. Natural products, commonly termed as 'secondary metabolites' are an essential, reputable source of successful drug leads which originate from Earth's bio-diverse flora and fauna. [2] All cultures since long time use herbs as a proficient source of medicines. According to WHO, most of the human population still use plants as crucial remedial agents in the primary health care system. Even then there are a lot many plants and herbs that still remain concealed and are not been studied yet. It has been estimated that some 80% of the world's residents rely predominantly on the traditional medicines for the health care requirements and the traditional system mainly utilizes the plant extracts or their active ingredient. [3]

The need of herbal medicines are presently rising every day. About 500 plants with medicinal value are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is an infinite depository of medicinal plants that are used in traditional medical therapies. The use of herbal medicine fetching recognition due to the side effects and toxicity of allopathic medicines. This led to haste rise in the number of herbal drug manufactures. Herbal medicines as the chief therapy in traditional system of medicine have been used in medical practices since the distant past. The practices persist today as its biomedical benefits as well as place in cultural attitude in several parts of world and have made a great involvement towards maintaining human wellbeing. India has large number of plant species around the world. There are estimated

to be roughly 25,000 successful plants-based formulations, used in folk medicine and known to rural communities in India. There are about 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative areas. [4]

Although several allopathic drugs are available, number of reports also stated their detrimental side effects on body's functioning. Therefore, plant-based therapy is supposed to be more reliable. Plant parts and their extracts are potential agents for the management and treatment of oxidative stress-induced diseases. Because these medicines are not only economic and affordable but are also safer and are extensively using as effective medication. [5]

Herbal plants play an input role in the human physical condition. About 80% of the world populations rely on traditional medicine which is based on medicinal plants. Herbal drugs have gained significance in recent years because of their efficacy, low cost and effectiveness. These drugs are used as either single plant extracts or fractions or mixtures of extracts from different plants. These plant extracts are standardized for their safety and efficacy. [6]

PRETERM LABOUR

Preterm delivery is defined as the birth occurring before 37weeks of gestation or before 259 days from the last menstrual period. Prematurity is multifactorial and its incidence has increased during the last decade in most occidental countries, due to increased risk factors responsible for elective prematurity. [7]

The incidence of preterm birth (PTB), or delivery at <37 weeks, continues to increase and it accounts for about 75% of all neonatal mortality and morbidity. All physicians agree the fact that preterm delivery (PTD) following preterm labor (PTL) involves frequent uterine contractions which dilates the cervix. Contractions which do not encounter that rate of recurrence or contractions lacking any cervical change several hours after uterine activity starts are called as uterine irritability, or false labor. The contractions which are having no effect on the cervix are never called preterm labor. In order to postponement or prevent PTD, detection of true uterine contractions (cervical dilatation <4 cm) should be done sufficiently early, to allow reasonable success of

tocolytic drugs which can result in significant pregnancy maintenance. Sometimes, painful, irregular uterine contractions can also be associated with false labor or uterine irritability and even when they are strong, much less than 50% are perceived by patients. PTL can be acute (abruptly going from normal contraction frequency of 1-2/hr on average to >8 ctx/hr) or gradual (over days). [8]

The mechanisms for preterm labour are still uncertain. It can be associated either with a premature activation of the physiological contracting process or with a pathological factor accountable for uterine contractions, leading to preterm delivery. Uterine over expansion due to multiple pregnancies or polyhydramnios, placental ischaemia, cervical disease, immunologic and allergic phenomenon, decidual or retroplacental haemorrhage, fetal endocrine activation and inflammatory processes are among the known pathways for preterm labour. Elective prematurity due to maternal or foetal conditions is becoming a significant cause for preterm labour. Tocolytic drugs that are available till date are directed towards only to the effects and not to the causes of preterm labour.

Tocolysis aims to inhibit uterine contractions and also to allow a safe transfer of the pregnant patient to a tertiary care centre. It gives the opportunity to take corticosteroids for preventing neonatal risks associated with prematurity.

Risk factors for preterm birth

Genetic factors can influence preterm birth. Women whose mothers and sisters have had a preterm delivery are more likely to have a chance of giving birth prematurely which means that a family history of preterm birth is a key risk factor. There exist a small but significant association between the paternal genome to preterm labor; this is maybe because of the effect of paternally contributed fetal genes, as confirmed by isolation analyses and twin studies. Rates of preterm birth in the precise ethnic groups, with genetic variation arising from geographic history, also indicate a role for genetics in preterm birth. For example, a higher occurrence of preterm birth has been established when either the mother or father is an African American. Several studies point out that environmental risk factorsare not sufficient to explain racial differences in preterm birth.

These population based findings indicate a role for genetic variation in the timing of human birth and the risk for prematurity. [9]

Mechanisms of Tocolysis

Myometrial contractility is a complex procedure based on myocytes function. It involves the presence of many ions channels, hormonal receptors, intercell gap junctions, and regulatory proteins such as oxytocin, endothelin, tachykinin, and angiotensin. For the uterine smooth muscle contraction, the increase of intracellular calcium concentration is necessary.

As shown on Figure 1, uterine relaxation can be obtained by interfering with an intracellular messenger which is responsible for contractile proteins effects. The tocolytic drugs include β adrenergic receptor agonists, nitric oxide (NO) donors, magnesium sulphate and calcium channel blockers. Another pathway involves the inhibition of contracting factors synthesis or its effects. By interfering with endogenous myometrial stimulators, atosiban, an oxytocin receptor antagonist and prostaglandin-synthetase inhibitors have this result. [7]

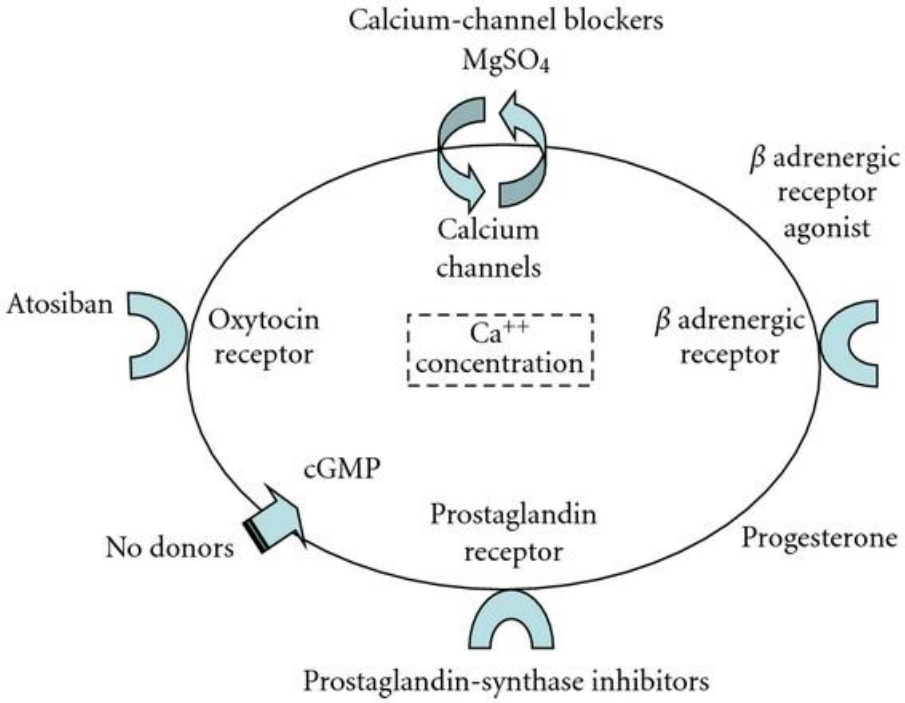


Figure 1: Mechanisms of action for tocolytics.

Types of Tocolytic Treatment

β Adrenergic Receptor Agonists: Selective β 2 agonists such as ritodrine and salbutamol have been used in clinical practice for preterm labour since in the early 1980s.The intracellular cyclic AMP concentration was made weaken by these drugs and facilitate myometrial relaxation. These agents were more efficient than placebo for delaying preterm birth for two days which have been reported by randomized controlled studies and meta-analysis. Unfortunately, no benefit for long-term (tocolytic effect restricted to 7 days) and perinatal mortality and morbidity rate was found with these drugs. Furthermore, there are Significant maternal side effects such as tachycardia, dyspnoea, hypokalemia, hyperglycemia, and chest pain were reported even with selective β 2 adrenergic receptor agonists. In conclusion, regardless of their efficiency, β 2 agonist ssafety profile is a real concern responsible for therapy discontinuation and choosing substitute tocolytic drugs. [10]

NO Donors: NO is a potent vasodilator which is synthesized during an amino acid oxidation process catalyzed by NO synthase. It is present in myometrial NO Donors cells and by interaction with guanylyl cyclase, it increases cGMP content. There is a direct relation between NO production and uterine relaxation. Transdermal nitroglycerin administrations have a better effect than placebo on delaying delivery for two days. Its outcome was similar to ritodrine. NO is not used in clinical routine as there is no large randomized studies available. [11]

Magnesium Sulphate: The relaxant effect of Magnesium sulphate *in vivo* and *invitro* on human uterine contractility has been widely reported. Since magnesium is a calcium antagonist, it decreases calcium intracellular concentration and inhibits contraction process. There were concerns about fetal protection as the drug is crossing the placenta. In some trials using Magnesium sulphate treatment at high dosage, there were reports of an increased risk of perinatal death and neonatal adverse effects together with neurological and metabolic disorders. It can also affect maternal neuromuscular system. There is a high toxicity risk ensuing in respiratory depression and disappearance of reflexes, over a serum concentration of 9mg/Dl. There is no evidence any more to advise this drug as a first-line tocolytic agent. However, it was reported to have a neonatal neuroprotective effect when administered prophylactically at low dose, in a

randomized multicentre trial but this effect should be confirmed in the next future on large randomized controlled studies. [12]

Prostaglandin synthase inhibitors: For converting arachidonic acid to prostaglandins, prostaglandin-synthase or cyclooxygenase (COX) isoforms COX-1 and -2 are necessary enzymes. By enhancing myometrial gap junction and rising intracellular calcium concentration prostaglandins are well-known uterine contraction inducers. In studies and in a recent meta-analysis, Indomethacin a nonspecific COX inhibitor has been reported to be a capable tocolytic drug compared to placebo, significantly delaying preterm delivery. It can be administrated via rectally or orally. Due to fetal ductus arteriosus closure risk and decreased urine production accountable for oligohydramniosits use should be controlled in duration and limited to pregnancies below 32 weeks. Maternal side effects including gastric ulcer or asthma recurrence have been reported with these treatments. COX-2 inhibitors such as nimesulide or rofecoxib have been studied in animals but not yet in humans and are not recommended for preventing preterm labour in clinical practice. In conclusion, indomethacin is a competent tocolytic drug without any severe adverse drug reactions and is even indicated for short term effects during the second trimester of pregnancy. [13]

Oxytocin Receptor Antagonists: These agents are in competition with the myometrial and decidual oxytocin receptors. The only drug now used in clinical practice is atosiban. It blocks the intra cytoplasmic calcium release associated with contractions and down regulates prostaglandin synthesis. A comparable tocolytic effect but fewer adverse effects with atosiban were demonstrated in a first multicentric randomized trial comparing atosiban and ritodrine.

Because of its low side effects profile atosiban is widely used in clinical practice. A german meta-analysis based on 6 randomized trials, among them 3 double blind studies, confirmed a comparable tocolytic action for atosiban and β adrenergic receptor agonists. A significantly low incidence of adverse effects is reported. Furthermore, when compared to continuous fenoterol administration controls. Cost saving in terms of hospital length and extra tests for excluding morbidity causes is found for the atosiban treated patients. In conclusion, atosiban seems to be an ample therapeutic choice for effective tocolysis with a low fetal and maternal adverse effects profile. [14] [15]

Calcium-Channel Blockers: Calcium ions transfer through the myometrial cell membrane are interfered by these agents. They cause decrease in the intracellular free calcium concentration and stimulate myometrial relaxation. At a daily dose of 30–60 mg nifedipine is the most commonly used drug for preterm labour inhibition.

Unfortunately, there is no placebo-controlled study available to confirm it. Decreased number of deliveries within 7 days following treatment and also, a reduced occurrence of neonatal respiratory distress syndrome was reported in a Cochrane Database review meta-analysis published in 2003. A recent systematic review based on 26 trials and 2179 patients confirms a higher effectiveness and a lower side effects incidence in the nifedipine group compared to β adrenergic receptor agonists-treated patients group. With an easy oral route of administration, low neonatal complications rate and fewer adverse effects nifedipine can be confirmed as an efficient tocolytic agent.. However, as they may be a risk of pulmonary oedema and cardiac failure, it should be used with caution in patients with compromised cardiovascular condition. [16]

Progesterone and 17-α-Hydroxyprogesterone Caproate: Progesterone a steroid hormone is secreted by the corpus luteum and also by the placenta after 8 weeks of gestation. It has a physiological consequence on uterine quiescence which is mediated by a direct effect on intracellular calcium concentration and prostaglandin synthesis. Several randomized trials in patients at risk treated either with weekly intramuscular17-α-hydroxy progesterone caproate or daily vaginal micronized progesterone from 24 to 34 weeks reported a notably reduced incidence of preterm birth. But these treatments showed less or no benefit in terms of perinatal mortality and morbidity. Fewer side effects such as sleepiness and headaches were seen with the vaginal route of progesterone administration. Even though these treatments appear effective in patients with previous history of preterm birth or with a short cervix, it is essential to gather additional data in large randomized controlled trials for confirming its possible benefit in the prevention of preterm delivery. [7]

Antibiotics: Infection is one of the causal factors of preterm labour with an incidence of 20–40%, especially before 30 weeks. Antibiotics use for preventing preterm labour has been mostly studied. The prophylactic administration of antibiotics is not recommended as there is little proof of benefits in cases such as the presence of preterm

labour with whole membranes. But a meta-analysis based on 22studies including more than 6000 patients, shows a significant reduction of preterm delivery and chorioamnionitisrate in the treated group, if there is a preterm rupture of the membranes (PROM). Neonatal complications were also seemed to be lesser in this population. In bacterial vaginosis associated with pregnancy, antibiotics were found to eliminate infection but they showed no effect on the incidence of preterm delivery. In conclusion, antibiotics can be used only when preterm labour is only due to PROM. [17]

ANATOMY AND PHYSIOLOGY OF UTERUS

The uterus is the most important female reproductive sex organ of humans and also in most other mammals. The lower end of the uterus, the cervix, opens into the vagina, while the other end, the fundus, is linked to the fallopian tubes in case of humans. It is inside the uterus that the fetus develops during gestation period. In the embryo, the uterus develops from the paramesonephric ducts which then combines into a single organ known as a simplex uterus.

Structure

The uterus is located within the pelvic region and more or less overlying the bladder, and in front of the sigmoid colon region. The shape of human uterus is somewhat pear-shaped and about 7.6 cm (3 in.) long, 4.5 cm broad (side to side) and 3.0 cm thick. The weight of a typical adult uterus is almost about 60 grams. Anatomically the uterus can be divided into four regions: The fundus, corpus (body), cervix and the internal os. The cervix protrudes into the vagina. The uterus is detained in place within the pelvis by ligaments which include the pubocervical, transverse cervical ligaments or cardinal ligaments, and the uterosacral ligaments. It is enclosed by a sheet-like fold of peritoneum, which is the the broad ligament.

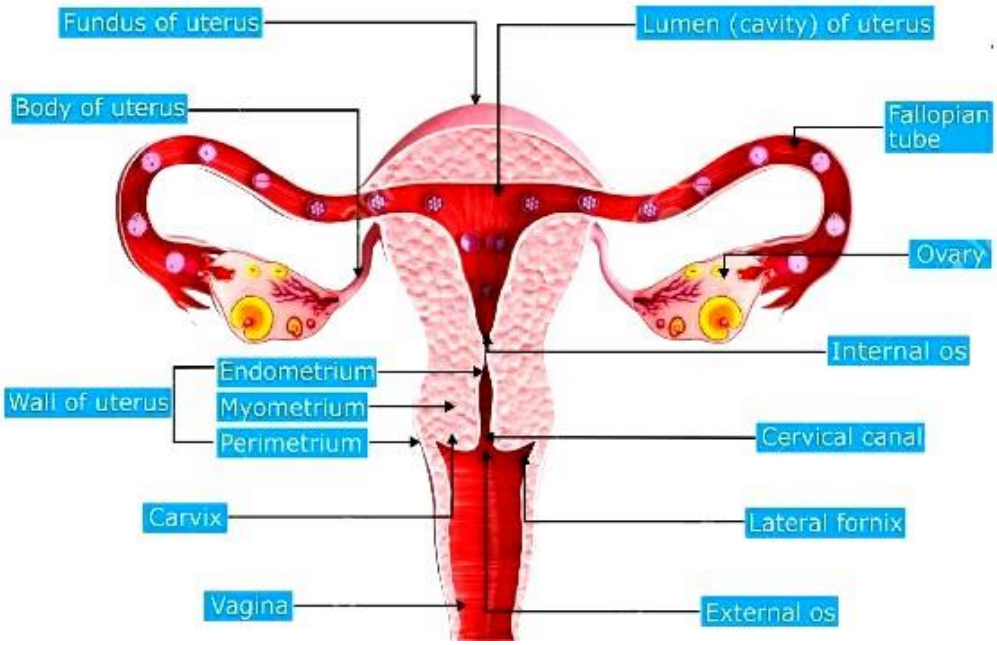


Figure 2: Diagram showing regions of the uterus

Histological Structure

The fundus and body of the uterus are composed of three tissue layers;

- **Peritoneum:** A double layered membrane, continuous with the abdominal peritoneum, also called as the perimetrium.
- **Myometrium:** The thick smooth muscle layer and the cells of this layer undergo hypertrophy and hyperplasia during pregnancy in preparation to force out the fetus during birth.
- **Endometrium:** An inner mucous membrane lining the uterus. It can be further subdivided into 2 parts – the stratum basalis and the stratum functionalis:

Deep stratum basalis: It is not shed at menstruation and it changes little throughout the menstrual cycle.

Superficial stratum functionalis: It becomes secretory in response to progesterone and proliferates in response to oestrogens. It is shed during menstruation and regenerates from cells present in the stratum basalis layer.

Ligaments

The main support for the uterus is provided by the tone of the pelvic floor. Some ligaments provide further support, which secures the uterus in place.

They are:

- **Broad Ligament:** This is a double layer of peritoneum which attaches the sides of the uterus to the pelvis. It acts as a mesentery for the uterus and helps to maintain it in position.
- **Round Ligament:** A remnant of the gubernaculum extending from the uterine horns to the labia major through the inguinal canal. The anteverted position of the uterus is maintained by this ligament.
- **Ovarian Ligament:** It connects the ovaries to the uterus.
- **Cardinal Ligament:** It is located at the bottom of the broad ligament; the cardinal ligament extends from the cervix to the lateral pelvic walls. It contains the uterine artery and vein.
- **Uterosacral Ligament:** It extends from the cervix to the sacrum and provides support to the uterus.

Position

The uterus is positioned in the center of the pelvic cavity in frontal plane (due to ligamentumlatum uteri). The fundus does not exceed the lineaterminalis, and also the vaginal part of the cervix does not extend below interspinal line. The uterus is movable and moves posteriorly under the pressure of a full bladder, or anteriorly under the pressure of a full rectum and if both are full, it moves upwards. Increased intra-abdominal pressure pushes it downwards. The mobility is conferred to it by musculo-fibrous apparatus which consists of suspensory and sustentacular part. Under normal

conditions the suspensory part keeps the uterus in anteflexion and anteversionand keeps it "floating" in the pelvis.

The changes of the position of the uterus due to pathological conditions are:

- retroflexion - if the uterus is fixed
- hyperanteflexion – slanted too forward
- anteposition, retroposition, lateroposition – these are the situations when the entire uterus is moved which is caused by parametritis or tumors
- elevation, descensus, prolapsed of the uterus
- rotation (the entire uterus rotates around its longitudinal axis), torsion (only the body of the uterus rotates around)
- inversion

Women may have the symptoms of pain during sexual contact, pelvic pain during menstruation, minor incontinence, urinary tract infections, fertility difficulties and difficulty using tampons in cases where the uterus is "tipped", also called as retroverted uterus.

Blood supply

Arterial blood both from the ovarian artery and uterine artery is supplied to the uterus. From anastomosis of these two arteries, another anastomotic branch may also provide blood to the uterus

Nerve supply

T11 and T12 are afferent nerves supplying uterus. Parasympathetic supply is from second, third and fourth sacral nerves. Sympathetic supply is from hypogastric plexus and ovarian plexus.

Development

Bilateral Mullerian ducts form during the early fetal life. In males, MIF secreted from the testes results in their weakening. In females, these ducts give rise to the fallopian tubes and the uterus. In humans, the formation of single uterus occurs when the lower segments of these two ducts joins together; however, in cases of uterine deformities this development may sometimes get troubled. Due to various degrees of

fusion of the two mullerian ducts, there is the formation of different uterine in various mammals.

Functions

By directing blood flow to the pelvis and ovaries, and also to the external genitals, including the vagina, labia, and clitoris. Uterus play an important role in sexual response.

A fertilized ovum which passes through the utero-tubal junction from the fallopian tube is accepted by the uterus, which is considered as its main reproductive function. The ovum divides to become a blastocyst, which implants into the endometrium, and get nourishment from blood vessels which develop solely for this purpose. The fertilized ovum becomes an embryo, which gets attaches to the wall of the uterus, creates a placenta, and develops into a fetus until childbirth.

Clinical significance

A hysterectomy is the surgical exclusion of the uterus which may be carried out for a number of reasons including the eradication of tumors both benign and malignant. A complete hysterectomy involves the complete removal of the parts of uterus which includes the body, fundus, and cervix of the uterus. A partial hysterectomy may just involve the removal of the uterine body while leaving the cervix intact. It is the most commonly performed gynecological surgical procedure.

Some pathological states include:

- Prolapse of the uterus
- Carcinoma of the cervix – known malignant neoplasm
- Carcinoma of the uterus – known as malignant neoplasm
- Fibroids – known as benign neoplasms
- Adenomyosis – which is the ectopic growth of endometrial tissue within the myometrium
- Endometritis– defined as the infection at the uterine cavity.
- Uterine malformations– congenital malformations which includes uterine Didelphys,bicornuate uterus and septate uterus.

- Asherman's syndrome– known as intrauterine adhesions which occurs when the basal layer of the endometrium is damaged by infection (e.g. endometrial tuberculosis) resulting in endometrial scarring followed by adhesion formation which partially or completely obliterates the uterine cavity.
- Hematometra–accumulation of blood within the uterus.
- Myometritis – inflammation of the muscular uterine wall. [18] [19]

OXYTOCIN

Oxytocin is a hormone which is made in the brain, in the hypothalamus. It is transported to and secreted by the pituitary gland, which is situated at the base region of the brain. Oxytocin is classed as a nonapeptide (a peptide containing nine amino acids) in chemistry and in biological classification it is considered as a neuropeptide. It acts both as a hormone and also as a brain neurotransmitter.

The release of oxytocin by the pituitary gland acts to control two female reproductive functions:

- Childbirth
- Breast-feeding**.**

The release of the hormone oxytocin during labor is responsible for increasing the uterine motility and making the muscles of the uterus (womb) contract. The release of oxytocin is stimulated by the widening of the cervix and vagina during labor and this effect is in turn increased by the subsequent contractions. It is released in large amounts during the time of labor, and after stimulation of the nipples. It is a facilitator for childbirth and breast-feeding.

Biosynthesis

The oxytocin peptide is synthesized as an inactive precursor protein from the *OXT* gene. This precursor protein also includes the oxytocin carrier protein which is the neurophysinI. The inactive precursor protein is gradually hydrolyzed into smaller fragments via a series of enzymes. The last hydrolysis that releases the active oxytocin nonapeptide is catalyzed by peptidylglycine alpha-amidating monooxygenase (PAM). The activity of the PAM enzyme system is dependent upon vitamin C which is an

essential vitamin cofactor. By chance, sodium ascorbate by itself was found to motivate the production of oxytocin from ovarian tissue over a range of concentrations in a dose-dependent manner.

Neural sources

In the hypothalamus, oxytocin is made in magnocellular neurosecretory cells of the supraoptic and paraventricular nuclei, and is stored in Herring bodies at the axon terminals in the posterior pituitary. It is then released into the blood from the posterior lobe (neurohypophysis) of the pituitary gland. These axons have collaterals that innervate neurons in the nucleus accumbens, a brain structure where oxytocin receptors are being expressed. The common release of the hormone through these collaterals is responsible for the endocrine effects of hormonal oxytocin and the cognitive or behavioral effects of oxytocin neuropeptides. Oxytocin is also produced by some neurons which is present in the paraventricular nucleus that project to other parts of the brain and to the spinal cord.

In the pituitary gland, oxytocin is packaged in large thick core vesicles, where it is bound to neurophysinI. It is a large peptide fragment of the larger precursor protein molecule from which oxytocin is derived by the enzymatic cleavage.

Electrical activity of the oxytocin cells in the hypothalamus regulates the secretion of oxytocin from the neurosecretory nerve endings. The action potentials generated by these cells propagate down axons to the nerve endings in the pituitary. Oxytocin gets released by the process of exocytosis when the nerve terminals get depolarized.

Non-neural sources

Endogenous oxytocin concentrations in the brain have been found to be as much as 1000 times higher than the peripheral levels. Besides the brain, oxytocin containing cells are present in several other tissues, in females in the corpus luteum and the placenta, and in males it is in the testes. It is also be present in the retina, the adrenal medulla, the thymus and the pancreas.

Male

In some species the Leydig cell have the biosynthetic mechanism to produce testicular oxytocin, to be specific, in rats (which can synthesize vitamin C

endogenously), and in guinea pigs, which, like humans, require an exogenous source of vitamin C in their diets.

Female

Oxytocin is synthesized by corpora lutea of several species, including ruminants and primates. Along with estrogen, it is involved in triggering the endometrial synthesis of prostaglandin $F_{2\alpha}$ which causes regression of the corpus luteum.[20]

RAT REPRODUCTIVE SYSTEM

The female reproductive system consists of the two ovaries and the female genital tract. The genital tract includes the oviducts, uterus, cervix and vagina. The female genital tract in mammals arises from the Mullerian ducts, commencing with the ostium of the oviduct. In the rat, this ostium forms a complete capsule called the ovarian bursa, which envelop the ovary. The oviducts are small, extremely coiled tubes. The uterus consists of two separated uterine horns, which enables the rat to have multiple offspring. The vagina of the rat opens directly to the exterior region.

Estrous cycle

The rat estrous cycle is short, which is lasting four to five days. It occurs throughout the year, without any seasonal effect. The first regular estrous cycle occurs about one week after the opening of the vaginal orifice, usually 33 to 42 days after birth. The cycle length increases slightly with age and last about 6 days near the ending of the reproductive life span**.**

The estrous cycle in the rat consists of four stages known as proestrus, estrus, metestrus and diestrus. Proestrus lasts approximately 12 h; estrus, 9 to15 h; metestrus, 21 h; and diestrus (the longest phase), over 57 h.

Hormones play significant roles in the estrous cycle of the rats. Gonadotrophins, which are secreted by the anterior pituitary gland, regulate the estrous cycle through luteinizing hormone (LH) and follicle stimulating hormone (FSH). Hormonal fluctuations result in ovarian and follicular changes, as well as changes in vaginal cytology. FSH stimulates follicle growth, while LH stimulates the follicles to ovulate and form the corpus luteum. Progesterone is secreted by the corpus luteum during metestrus

and declines during diestrus. During the follicular development, the level of estradiol-17β increases. The cycle ends when estrogen peaks during proestrus, stimulating gonadotropin release to trigger ovulation.

Identification of estrous cycle stages

Estrous cycle phases can be noticed by observing behavioral changes or vaginal cytology examination. The latter method is widely used and considered as a practical way to determine the phases of the estrous cycle. Precise phase identification depends on smears taken at fixed times in the day, as the cell populations vary throughout a 24-h period. Behavior and vaginal smear morphology during the different phases of estrous cycle as well as the duration of each phase are shown in figure 3. Estrus is defined as the period when the female accepts the male and allows copulation. Many behavioral changes occur during this phase, including increased running activity, lordosis and ear quivering. During estrus, dry vaginal wall and a swollen vulva can also be observed. The female accepts the male at the end of proestrus, while during metestrus and diestrus, the female does not accept the male. Vaginal cytology in estrus reveals cornified cells and nucleated cells. In metestrus, leukocytes, nucleated cells and cornified cells are seen. In diestrus, which is the longest phase, vaginal cytology principally reveals leukocytes. Nucleated cells are predominant in vaginal smears during proestrus**.**

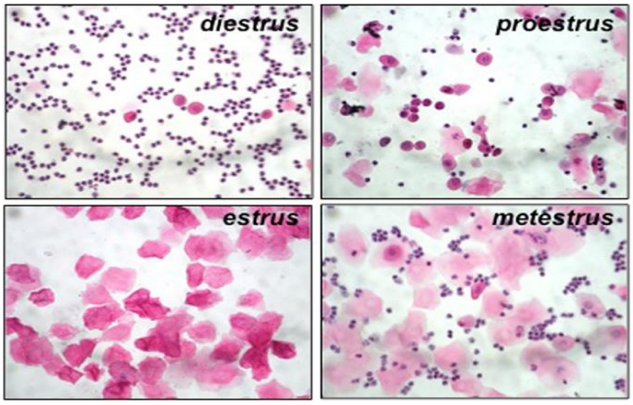


Figure 3: Diagram showing stages of estrus cycle in rats

Stages of the Estrous Cycle

The stages of the estrous cycle are identified by the absence, presence, or proportion of the described four basic cell types as well as by the cell density and also arrangement of the cells on the slide. The following will define and characterize the range in appearance of the four classic stages:

Proestrus

Proestrus is a short stage, lasting an average of 14 hr in rats and less than 24 hr in mice. The predominant feature of this stage is the presence of small, round, nucleated epithelial cells of relatively uniform appearance and size; these cells may stain deeply basophilic. Often they will be seen in cohesive clusters, sheets, or strands.

However, cohesive clusters, sheets, and strands are not always observed, especially in low cellularity samples, and should not be thought of as a prerequisite in the determination of proestrus; cells may only be seen individually throughout the smear. Typically, no neutrophils will be seen. Rare to occasional neutrophils can be found in early proestrus as the rodent would have recently transitioned from diestrus into proestrus stage. Relatively less numbers of large epithelial cells and keratinized anucleated cells may also be observed. As the cycle approaches estrus, keratinized cells will become more abundant. The presence of low numbers of neutrophils, or large and anucleated epithelial cells, does not preclude the diagnosis of proestrus when the predominating feature of the smear are the small, round epithelial cell population**.**

Estrus

Estrus duration ranges between 24 and 48 hr in rats and between 12 and 48 hr in mice. It is characterized by the presence of predominately anucleated keratinized epithelial cells. Numerous bacteria may be observed adhered to the cells or free in the background. Occasional nucleated epithelial cells can also be observed throughout the stage of estrus, and neutrophils are absent or occasionally observed in late estrus. The first and second half of estrus, while consisting mostly of anucleated keratinized cells, have distinct differences in their appearances and also differ between rats and mice. The second phase in rats occurs late in estrus and will not always.

Metestrus

Metestrus is comparatively a short stage of the estrus cycle which lasts for 6–8 hr in rats. This stage is characterized by a combination of keratinized epithelial cells (anucleated) and neutrophils. In mice, nucleated cells may appear occasionally throughout metestrus. In rats, the small and large nucleated cells of late estrus are present in low to moderate numbers throughout the stage. In early metestrus, neutrophils are interspersed with the epithelial cells and are sometimes tightly packed together or clumped around the cells; the epithelial cells usually predominate but may be in equal proportion to the neutrophils. As metestrus progresses, neutrophils become very high in number and the smear will be highly cellular and dense. Neutrophil and epithelial cell numbers decrease by late metestrus, with a decrease in smear cellularity, before the transition to diestrus.

Diestrus

Diestrus is the longest stage of the estrus cycle with an average duration of 48–72 hr in both mice and rats. This stage is characterized by a substantial decrease in the number of anucleated keratinized epithelial cells as the animal transitions out of metestrus. In general cellularity is moderate to low with a combination of neutrophils, small and large nucleated epithelial cells, and low numbers of anucleated keratinized cells. Neutrophil numbers can vary but are usually higher in number relative to the epithelial cells with smears sometimes being exclusively neutrophilic. Occasionally, in early diestrus, neutrophils may still appear in clumps. It is not unusual for diestrus smears to have a very low cellularity, especially on day 2 or 3 of diestrus, with only a sparse spreading of neutrophils and epithelial cells. In late diestrus, the epithelial cells may become more round or be organized in small clumps, indicating proestrus the next day. During diestrus, macroscopic vaginal excretions are low. When excreations are present, however, they can be viscous and stringy and this is especially true during times of persistent diestrus. This excretion is sometimes aspirated during vaginal cytology sample collection. It will stain and appear as a thick pink to blue-violet material with entrapped neutrophils and epithelial cells. The cells may be distorted or elongated in the strings of mucous. [21] [22]

OXIDATIVE STRESS

Oxidative stress is an imbalance between free radical production and antioxidant defenses and is associated with damage to a wide range of molecular species including lipids, proteins and nucleic acids. Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins and excessive exercise. These injured tissues produce increased radical generating enzymes (e.g., xanthine oxidase, lipogenase, cyclooxygenase) activation of phagocytes, release of iron, copper ion or a disruption of the electron transport chains of oxidative phosphorylation producing excess ROS. [23]

Risk factors of PTB and pPROM can be classified into two major categories, static and dynamic. Independently or in combination, the static risk factors can either predispose or cause the dynamic risk factors that are commonly diagnosed as clinical risks. Epigenetic changes that are independent of DNA base variations generated by complex interactions between various risk factors during pregnancy can also contribute to dynamic clinical risks by altering expression of certain genes. These changes can cause transition between static and dynamic risks. Static and dynamic risk factors produce pathways and pathophysiologies depicted in the inner circle with a unique biomarker profile contributing to labor-inducing changes, resulting in PTB or pPROM. The final effector pathways culminating in labor and delivery include inflammation and oxidative stress (OS). In normal pregnancies, these are generated by various fetal and maternal factors that signal the end of pregnancy. In PTB, the maternal–fetal signals and their causal origins are still unclear as they arise from complex etiologies and redundant pathways. [24]

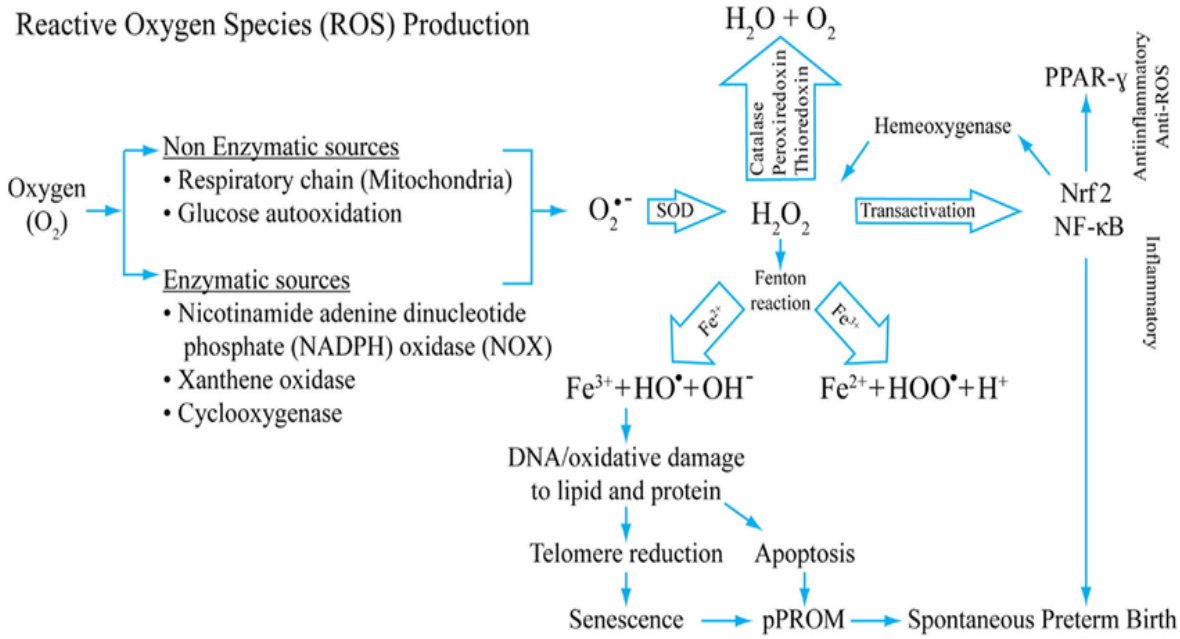


Figure4: A model of reactive oxygen production, its inactivation by antioxidant systems and potential consequences of redox imbalances

MATERNAL/FETAL ANTIOXIDANTISSUES

During pregnancy itself there is a burden of excess and unstable radicals on maternal tissues as well as those of the developing fetus and placenta. The subsequent in ability to vasodilate (or direct vasoconstriction) caused by these reactive species result in diminished blood flow and can lead to PTL, PPROM, preeclampsia, and primary IUGR (or secondary due to the preeclampsia).Numerous strategies are adopted to address this excess of oxidative radicals, particularly supplementation of one or two micronutrients in the hopes of reducing these serious maternal/fetal disorders. Such therapy is directed at decreasing the numbers of reactive oxygen/nitrogen species by supplementing vitamins A, C or E in hope of reducing vasoconstriction and/or organ damage. According to the hypothesis if vasoconstriction is reduced there would be less early deliveries both from PTL and PPROM as well as from indicated deliveries due to preeclampsia and severe fetal growth restriction.

ENZYMATICANTIOXIDANTDEFENSES

Antioxidant enzyme activities have been shown to increase in response to oxidative stresses. Antioxidant enzymes take part in a complex interaction of reducing and oxidizing molecules that defines the cellular milieu necessary for maintaining cellular, placental, fetal, postnatal growth. Such enzymes have small activity in preterm infants and cannot balance excessive ROS production. The most significant antioxidant enzymes are copper-zinc superoxide dismutases (SOD), which are found in cytoplasm as well as peroxisomes, and manganese SOD from mitochondria. SOD catalyzes the dismutation of superoxide anion to H_2O_2. Glutathione peroxidase (GPx) in mitochondria and catalase (CAT) in peroxisomes catalyze the reaction of H_2O_2 to molecular oxygen and water. These enzymes, together with vitamin E, play an important role in the peroxidation of polyunsaturated free fatty acids in cell membrane. [25]

2. REVIEW OF LITERATURE

Syama Hari Priya *et al.*, (2017) performed the antioxidant activity, phenolic-flavonoid content and high-performance liquid chromatography profiling of three different variants of *Syzygium cumini* seeds: A comparative study. *S. cumini* is widely used in traditional systems of medicines in India, such as Ayurveda, Unani, and Siddha. Different parts of *S. cumini* are reported to have several medicinal properties like antidiabetic, antimicrobial, anti-inflammatory and free radical scavenging potential. The seeds have been reported to possess compounds such as jambosine, gallic acid (GA), ellagic acid, corilagin, 3,6-hexahydroxy diphenoylglucose, 1-galloylglucose, 3-galloylglucose, quercetin, β-sitoterol and 4,6 hexahydroxydiphenoylglucose . The phenolic profiling of active fractions of all the variants indicated the presence of prominent phenolic compounds which were known for their antioxidant potential. [26]

Kuldip Singh *et al.*, (2016) studied the antioxidant and antimicrobial potential of *Syzygium cumini* leaves and found that high contents of phenol present in different extracts of *Syzygium cumini* leaves. The presence of high contents of phenol suggested the antioxidant properties to scavenging the production of ROS and lipid peroxidation. Antimicrobial activity of the various leaves extract was checked by Agar Well Diffusion and Micro Dilution Broth method. Antimicrobial activity was tested by four bacteria (Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus aureus) and two fungus (Aspergillus niger and Trichoderma viridae). The outcome showed that the activity of various leaves extracts of *Syzygium cumini* shows significant antibacterial and antifungal activities. These antimicrobial activities of extracts may be due to presence of different phytochemicals. [27]

Madhulika Pradhan (2016) studied phytochemistry, pharmacology and novel delivery applications of *Syzygium cumini* (L.). *S.C* is a widely used medicinal plant for the treatment of various ailments. The plant contains anthocyanins, glucoside, ellagic acid, isoquercetin, kaempferol, and myricetin as its chief active constituents. These active ions

isoquercetin, kaempferol impart multiple pharmacological activities to the plant which includes antidiabetic, anticancer, antioxidant, antibacterial, antifungal and antidiarrhoeal activity. [28]

Deepali Laxman Jaybhaye *et al.*, (2016) studied tocolytic plant *Tectona grandis* Linn. extended study on other systemic effect. *Tectona grandis* Linn. (T.G) is one of the well-known Indian herbs. In Ayurveda, T.G stem extract has tocolytic effect. Conventional tocolytic drugs are known to have cardiovascular, skeletal muscle toxicity. This study is undertaken to observe possible adverse effects of T.G stem extract especially on cardiovascular and musculoskeletal system. After analysis by *t*-test, it was observed that T.G causes vasodilatation same as that of the sodium nitrate, without causing any cardiac toxicity seen with nifedipine and no neuromuscular blockade as seen with magnesium sulfate. [29]

Shweta Sharma *et al.*, (2016) performed comparative study of alcoholic and aqueous extracts of *syzygium cuimini* on carbon tetrachloride-induced hepatotoxicity in wistar rats. Hepatotoxicity means chemical-driven liver damage since liver cells are main site of detoxification and drug metabolism, certain toxin/ drug might injure the organ. Hepatic injury was induced by single oral administration of CCl_4 mixed with olive oil as vehicle in 1:1 ratio (3 ml/kg of rat body weight). In this experiment, it was found that the aqueous extract has more hepatoprotective activity than alcoholic extract of *Syzygium cumini*. In addition, their protective efficacy was also found to be comparable to that of standard drug used. [12]

Sekar *et al.*, (2015) evaluated hepatotoxic effect of ethanolic extract of *Syzygium cumini. Linn* leaves on experimental animals. Ethanolic extract of *Syzygium cumini* have been reported to possess significant anti-inflammatory, diuretic, anti-hyperglycemic, hepatoprotective, chemopreventive and anti-diabetic. The present study reports hepatotoxicity of ethanolic extract of *Syzygium cumini* leaves on experimental rats at dose levels of 1250, 2500 and 5000mg/kg body weight for 28 days. The extract has reduced glucose levels considerably but the levels of body weights, RBC, WBC, Hemoglobin, AST and ALT were found to be elevated. [6]

Michelle C. Cora *et al.*, (2015) evaluated vaginal cytology of the laboratory rat and mouse:review and criteria for the staging of the estrous cycle using stained vaginal smears. The estrous cycle is generally divided into the four stages of proestrus, estrus, metestrus, and diestrus. On cytological evaluation, these stages are defined by the absence, presence, or proportion of 4 basic cell types as well as by the cell density and arrangement of the cells on the slide. Vaginal cytology can be evaluated immediately after collection as an unstained, wet mount preparation (direct cytology) or as a fixed and stained slide preparation. [17]

Swadhin Ranjan Behera *et al.*, (2014) studied the hepatoprotective activity of aqueous extract of *Syzygium cumini* seed on streptozotocin induced diabetes in rats. It was observed that aqueous seed extract of *S.C* (500mg/kg) showed significant hepatoprotective effect in diabetic rats compared to other groups. The present protective effect may be due to saponins, tannins and flavanoids present in seed extract. Standard oral hypoglycemic drug and high dose of seed extract showed almost normal levels of liver enzymes. But 250mg/kg do not show significant effect compared to standard and high dose plant extract administered groups. It indicates that low doses plant extract do not show hepatoprotection. This may be due to antioxidant property of seed extract. Antioxidants counteract the oxidants generated in the liver. Any changes between levels of oxidants and antioxidants cause development of liver damage. [30]

Ramkumar Menon (2014) studied oxidative stress damage as a detrimental factor in preterm birth pathology. Normal term and spontaneous preterm births (PTB) are documented to be associated with oxidative stress (OS) and imbalances in the redox system. A healthy pregnancy is characterized by a stable balance between ROS and antioxidants. Redox imbalance is an under- lying pathologic feature of many pregnancy complications. All of the PTB and pROM risk factors detailed above are capable of causing redox imbalance, leading to the production of superoxide, hydrogen peroxide, hydroxyl ions, and nitric oxide that can damage collagen matrix and consume antioxidant defenses. These events can trigger uterine contractions (labor), leading to PTB. [24]

Swadhin Ranjan Behera *et al.*, (2014) studied nephroprotective effect of aqueous extract of *Syzygium cumini* seed on streptozotocin induced diabetes in rats. 30 rats were divided into 5 groups. G-I (Control) normal saline was administered. 24 rats were administered streptozotocin to induce diabetes. After 72hr blood glucose was measured. G-II-diabetic control, G-III- Streptozotocin (45mg/kg/i.p/0day) + Glibenclamide (5mg/kg/orally/120days), G-IV-Streptozotocin (45mg/kg/i.p/0day) + Aqueous extract of *Syzygium cumini* seeds (250mg/kg/orally/120 days) and G-V- Streptozotocin (45mg/kg/i.p/0day)+ Aqueous extract of *Syzygium cumini* seeds (500mg/kg/orally/120days). It was observed that aqueous extract of S.C seed extract showed significant nephroprotective effect compared to other groups. This seed powder extract can use to treat patients having kidney problems along with diabetes. [31]

Katherine Y Bezold *et al.*, (2013) observed the genomics of preterm birth: from animal models to human studies. The use of animal models to study the events leading up to and throughout birth has provided significant insight into the mechanisms regulating parturition, at term and preterm. However, the applicability of current animal models of parturition to the physiological mechanisms of human pregnancy and birth has been limited, as the means by which these different species regulate and initiate parturition differ from each other and from humans. [32]

Naguib Salleh *et al.*, (2013) observed the *In-Vitro* effect of *Ficus deltoidea* on the contraction of isolated rat's uteri is mediated via multiple receptors binding and is dependent on extracellular calcium. *Ficus deltoidea*, is a perennial herb that is used to assist labor, firm the uterus post-delivery and to prevent postpartum bleeding. FDA-induced contraction of the isolated rat's uteri is mediated via multiple uterotonin receptors (muscarinic, oxytocin and prostaglandin F2α) and was dependent on the extracellular Ca2+. Contraction, however, was not dependent on the Ca2+ release from the internal stores. This *in-vitro* study provides the first scientific evidence on the claimed effect of *Ficus Deltoidea* on uterine contraction. [33]

Huda Yahia Hamid *et al*., (2013) studied reproductive characteristics of the female laboratory rat. The estrous cycle consists of four stages known as proestrus, estrus, metestrus and diestrus. Phases of the estrous cycle can be detected by observing behavioral changes or examining vaginal cytology. Detection of sperm in vaginal smear is an excellent predictor of pregnancy in rats. Implantation is initiated on day 5 and completed by day 7 of pregnancy. Gestation takes 21 to 23 days from copulation to parturition. Short estrous cycle and gestation period make the rat an ideal animal model for research on reproduction. On the other hand, detection of sperm in vaginal smear is an excellent predictor of pregnancy in rats. The day that sperm is detected in the vaginal smear is designated as day 1 of gestation. [34]

Lekha K Nair *et al*., (2013) studied the *invitro* antioxidant activity of the seed and leaf extracts of *Syzygium cumini*. The extract showed significant antioxidant activity in all antioxidant assays when compared to ascorbic acid. The results of this research work are promising thus indicating the utilization of the seed and leaf of *Syzygium cumini* as a significant source of natural antioxidants. [35]

Asiqur Rahaman *et al*., (2013) evaluated the memory related learning ability of old rats in eight arm radial maze using *Syzygium cumini* (L.) Seed extract .The study demonstrated that the oral administration of S. *cumini* seed extract improves radial arm performance in old rats concomitantly with the amelioration of the levels of LPO in the cerebral cortex and hippocampus. Radial maze behavior allows the simultaneous measurement of reference memory and working memory without any harmful effects on the rats. [36]

Bhaskar Sharma et al., (2013) studied Liver protective effects of aqueous extract of *Syzygium cumini* in Swiss albino mice on alloxan induced diabetes mellitus. Eighteen Swiss albino mice (weighing 28–32 g) were randomly divided into control, alloxan treated and *S. cumini* treated mice group. Diabetes was induced in mice by injecting intraperitoneally alloxan monohydrate at dose of 150 mg/kg body weight. Aqueous extracts of *S. cumini* seed at dose of 250 mg/kg body weight were given orally in diabetic

mice daily for three weeks after established LD_{50} value. In diabetic mice, the SGOT, SGPT, Bilirubin and serum glucose levels were significantly increased in comparison with the control groups. Statistical analysis ($p < 0.05$) of the data indicated that aqueous extract of *S. cumini* were significantly decrease serum contents of liver enzymes (SGOT, SGPT and Bilirubin) as well as serum glucose in treated groups. [37]

Md. Rashedul Alam *et al.*, (2012) studied the antidiabetic phytochemicals in *Syzygium cumini*(L.) skeels. The present study was carried out to identify the putative antidiabetic constituents from the S. cumini leaves. From the NMR data four different compounds, Lupeol, 12-oleanen-3-ol-3ß-acetate, Stigmasterol, ß- sitosterol were identified from n-hexane fraction of plant extract. These compounds have potential antidiabetic activities which support the traditional use of the leaves as being remedy for treating diabetes. [38]

Shweta sharma *et al.*, (2012) evaluated the pharmacological activity of *Syzygium cumini* extract using different solvent and their effective doses. In this review it is concluded that the plant having potential of treating various highly dangerous disease which are responsible for mortality in different age group. *S.C* reduces the radiation induced DNA damage in the cultered human peripheral blood lymphocytes in preliminary level study in the dose of 100µg/ml. Plant extracts have been examined for their antidiabetic properties in an effort to identify alternative treatment strategies pose less of a risk for diabetes. S.C ameliorates insulin resistance and β-cell dysfunction via modulation of PPARγ, dyslipidemia, oxidative stress and TNF-α in type 2 diabetic rats. *S.C* skeels are effective on post prandial blood glucose levels in non diabetic rats and rats with streptozocin –induced diabetic mellitus. [39]

Robert A. Knuppel *et al.*, (2012) studied oxidative stress and antioxidants: preterm birth and preterm infants. It was found that the oxidative stress during pregnancy yields free radicals and other oxidative molecules exceeding the available antioxidant buffering capacity in the mother and growing fetus. This results in cellular damage, which is associated not only with PTL and delivery, but also preeclampsia, PPROM and IUGR as

well as several serious post delivery issues for the premature infant. Antioxidant enzymes participate in a complex interaction of reducing and oxidizing molecules that defines the cellular milieu necessary for maintaining cellular, placental, fetal, and postnatal growth. Such enzymes have low activity in preterm infants and cannot balance excessive ROS production. The most important antioxidant enzymes are copper-zinc superoxide dismutases (SOD), which are found in cytoplasm as well as peroxisomes, and manganese SOD from mitochondria. [25]

Yavuz Simsek *et al.*, (2012) studied elevated cardiac oxidative stress in newborn rats from mothers treated with atosiban. The animals were treated from days 15 to 20 of gestation. One group acted as a control group, and received intraperitoneal (i.p.) injections of saline in a daily dose volume of 6 mg/kg/day. The second group received 6 mg/kg/day i.p. atosiban. There was no significant difference in birth weight or in the number of pups between two groups. Newborns from atosiban-treated mothers showed significantly increased oxidative stress in the plasma and heart tissue than that of controls. [40]

Prashant B. Shamkuwar *et al.*, (2012) evaluated the antidiarrhoeal activity of seeds of *Syzygium cumini* L. Aqueous *Syzygium cumini* extract (125, 250, 500 mg/kg, po) was tested for its antidiarrhoeal, antimotility and antisecretory activity in mice. The method of castor oil induced diarrhoea was used to evaluate antidiarrhoeal activity; while charcoal meal test and castor oil induced intestinal secretions were used for testing antimotility and antisecretory activity in mice. Aqueous *Syzygium cumini* extract (ASC) produced a significant and dose dependent antidiarrhoeal,antimotility, and antisecretory effect. It can be concluded that ASC possesses antidiarrhoeal effect may be due to its antimotility and antisecretory effect. [41]

C. Hubinont *et al.*, (2011) evaluated prevention of preterm labour: 2011 update on tocolysis. Preterm delivery is defined by a birth occurring before 37 weeks of gestation or before 259 days from the last menstrual period. Tocolytic therapy includes β adrenergic receptor agonists, NO donors, magnesium sulphate, prostaglandin-synthase inhibitors, oxytocin receptor antagonists, calcium channel blockers, progesterone, 17-α-

hydroxyprogesterone caproate, and antibiotics. Atosiban, an oxytocin receptor antagonist and prostaglandin-synthetase inhibitors have uterine relaxation effect by interfering with endogenous myometrial stimulators. The only drug used in clinical practice is atosiban. It blocks in a reversive manner the intra cytoplasmic calcium release associated with contractions and downregulates prostaglandin synthesis. [7]

Nikolaos Vrachnis *et al*., (2011) evaluated the oxytocin-oxytocin receptor system and its antagonists as tocolytic agents. Oxytocin, a hormone involved in numerous physiological processes, plays a central role in the mechanisms of parturition and lactation. It acts through its receptor, which belongs to the G-protein-coupled receptor super family, while Gq/phospholipaseC (PLC)/inositol 1, 4, 5-triphosphate (InsP3) is the main pathway via which it exerts its action in the myometrium. Oxytocin receptor antagonist that blocks OT binding to OTR and is the only oxytocin antagonist used today for the treatment of preterm labor. [42]

Christine K. Ratajczak *et al*., (2010) evaluated preventing preterm birth: the past limitations and new potential of animal models. The use of humans in controlled studies on parturition is severely limited by ethical considerations. However, studies of other animals, notably sheep and mice, have made key contributions to the understanding of parturition. The use of each of these model systems has its own advantages and disadvantages. Although animal models are not without their limitations in modeling human parturition biology, they have made important contributions to our current understanding of this complex process. Differences in how parturition is executed in common animal models and humans have been identified, but these differences should not discourage investigators from conducting further research to identify additional important similarities. [43]

Omonkhelin j. owolabia *et al*., (2009) evaluated the tocolytic activity of ethanol extract of the stem bark of ficus *capensis* thunb. (moraceae). The ethanolic extract obtained by maceration technique was subjected to pharmacological testing *in vitro* on a piece of isolated rat uterus previously pretreated with stilbestrol, suspended in De Jalon at 37^{O}C.

The higher concentration of the extract (80 mg/mL) significantly ($p < 0.05$) exerted smooth muscle relaxant activity on the uterus. The results indicate the presence of active principles in the bark extract of *Ficus capensis* which may be responsible for some of the applications in traditional medicines as an anti-abortifacient and as a remedy against threatened abortion. [44]

A. Kumar *et al*., (2008) studied anti-inflammatory activity of *Syzygium cumini* seed. This study was intended to evaluate the anti-inflammatory activity of ethyl acetate and methanol extracts of *S. cumini* seed in carrageenan induced paw oedema in wistar rats at the dose level of 200 and 400 mg/kg administrated orally. Both the extracts exhibited significant anti-inflammatory activity, which supports the traditional medicinal utilization of the plant. [45]

P. Suresh Kumar *et al*., (2006) studied the in vitro tocolytic activity of *Sarcostemma brevistigma Wight*. The effect of a chloroform soluble fraction (F-A) of the acetone extract of twigs of *Sarcostemma brevistigma Wight* on contractions induced by oxytocin and KCl, in the isolated rat uterine smooth muscles, has been evaluated. At concentrations of 32.8 μg/ml, the F-A significantly inhibited ($P<0.001$) the contractions induced by 60 mM KCl in Ca^{2+}, containing physiological salt solution to the extent of 88.7 ± 2.2%. The F-A, at concentrations of 26.3 μg/ ml, completely inhibited the rhythmic contractions induced by oxytocin in Ca^{2+}, containing physiological salt solution. However, it failed to inhibit the contractions induced by oxytocin in Ca^{2+} free PSS. These results suggest that fraction F-A exhibits uterine relaxant activity, by interfering with the extracellular Ca^{2+}. [46]

Ochiogu *et al*., (2006) performed a new and simple method of confirmatory detection of mating in albino rats (Rattus norvegicus). The method involved the gross observation of grey to yellowsh protein coagulates (remnants of the copulatory plug) on vaginal smears of mated females made on clean glass slides. The copulatory plug, also known as the vaginal plug, is a white or grey to yellowish waxy coagulated mass of proteins which is

usually deposited by males in the female reproductive tract at mating in some mammalian species including rats. This new method is simple, easy to apply and does not interfere with fertilization and pregnancy, and also does not involve either the use of specially designed rat cages or microscopy of vaginal smears, which were the constraints of the former methods of confirming mating in rats. [47]

Ronald F. Lamont (2003) studied the development and introduction of anti-oxytocic tocolytics. The perfect tocolytic agent, which is completely safe for both the mother and fetus and, which will inhibit uterine contractions and stop preterm labour in every case does not exist and the search continues. The analogue, atosiban, was found to be more potent and so was chosen for clinical evaluation in dysmenorrhoea and preterm labour. Atosiban was found to be at least as effective as the beta agonists as a tocolytic agent, but significantly less likely to result in maternal cardiovascular side effects or the need to discontinue therapy as a result of unacceptable side effects. [48]

Masatoshi Sakai *et al.*, (2001) evaluated the tocolytic effect of a selective cyclooxygenase -2 inhibitor in a mouse model of lipopolysaccharide induced preterm delivery. The inflammatory process is known to cause preterm birth. In this study the COX-2 inhibitor celecoxib was studied for its tocolytic effect and its side effect on dams and pups using a lipopolysaccharide (LPS)-induced preterm delivery mouse model. With administration of 10 or 100mg/kg celecoxib the fetal ductus arteriosus was constricted significantly in preterm and near term rats, although constriction rates preterm rats were significantly lower than those in near-term rats. Reproductive and renal functions in offspring whose mothers where treated with LPS and celecoxib were normal. These data demonstrate that celecoxib can be used as new therapy for preterm labour. [49]

3. PLANT PROFILE

Figure 5: *S. cumuni* (seed)

3.1 Description of the plant: [50, 51, 52]

Botanical name : *Syzygium cumini*

Family : Myrtaceae

Genus : *Syzygium*

Species : S. *cumini*

Synonyms : Calyptranthes caryophyllifolia Wild.

3.2 Vernacular name:

Spanish : Ciruleo de Java

Portuguese : jamelao

French : Jambolanier

Hindi : Jamun

Tamil : Naval

Malayalam : Njaval

3.3 Species and distribution

This species is distributed in tropical and subtropical Asia to Queensland. It is thought to be indigenous to India, Myanmar, Sri Lanka and the Andaman islands. It was introduced long time ago to Malaysia, Indonesia and the Philippines and has become naturalized.

3.4 Plant parts used:

All parts of the plants are used medicinally especially leaves, seed, stem bark

3.5 Morphological Characters

S. cumini may reach 30 m tall in India and Oceania or up to 12-15 m in Florida, USA, with a wide crown up to 11 m in diameter and a trunk diameter of 0.6-0.9 m though it usually has a multi-stemmed form branching close to the ground. Bark is rough, fractured, flaking and discoloured on the lower part of the trunk, becoming smooth and light-grey higher up. Evergreen leaves have a turpentine smell, and are opposite; pinkish when young, becoming leathery, glossy, dark-green above, lighter beneath, with a conspicuous, yellowish midrib when grown-up. Flowers are fragrant and appear in clusters, white at first, becoming rose-pink, detaching rapidly to leave only the numerous stamens. Fruit appear in clusters of just a few or 10-40, are round or oblong. The skin is thin, smooth, glossy, and adherent. The pulp is purple or white, very juicy, and normally encloses a single, oblong, green or brown seed, up to 4 cm long, though some fruits have 2-5 seeds tightly dense within a leathery coat, and some are seedless.

3.6 Chemical constituents in leaf:

The plant leaves contain an essential oil with pleasing odour. The oil contains terpenes, 1-limonene and dipentene (20%), sesquiterpenes of cadalane type (40%), and sesquiterpenes of azulene type (10% or less). Yield and physical uniqueness of the oil varies according to the season of collection. This essential oil is reported to be responsible for the antibacterial activity of the leaves.

3.7 Chemical constituents in seed:

The plant seeds are rich in protein and calcium. The seeds enclose tannins (19%), ellagic acid, gallic acid (1-2%). A glycoside- Jamboline, starch, Myricyl alcohol in the unsaponified fraction of seeds and a small quantity (0.05%) of pale yellow essential oil (specific gravity20: 0.926, [α] D- 5.42o) are also present.This constituents may be responsible for the hypolipidaemic, hypoglycemic and antianemic effect.

3.8 Chemical constituents in stem bark:

Stem bark contains pentacyclic triterpenoid betulinic acid (m.p. 306-310^{o}C). Betulinic acid is a naturally occurring triterpenoid, which has demonstrated selective cytotoxicity against a number of specific tumors and active against a variety of infectious agent like HIV, malaria, immunomodulatory and inflammation.

3.9 Traditional Uses

The bark of the plant has various properties like astringent, refrigerant, carminative, diuretic, digestive, antihelminthic, febrifuge, constipating, stomachic and antibacterial activity. The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection. The leaves are antibacterial and used to make stronger the teeth and gums. The leaves have also been widely used to treat diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy and to inhibit blood discharge in the feces. The barks, leaves and seeds extracts of S. Cumini have also been reported to possess anti- inflammatory, antibacterial and antidiarraheal property. Powdered seeds are used as a remedy in diabetes and in menorrhagia. It has been also showed before that the leaf, bark, stem and pulp of *S. cumini* plants possess potent antidiabetic activity

4. AIM AND OBJECTIVES

4. 1. AIM:

Syzygium cumini L. family Myrtaceae is a well-known common fruit in India. The seed is used in various alternative healing systems like Ayurveda, Unani and Chinese medicine for digestive ailments. The literature study done so far revealed that there is a lack of scientific data concerning the tocolytic evaluation of the *S.cumini* seed extract on animal models.

The aim of the current study is to assess the tocolytic effect of the aqueous seed extract of *Syzygium cumini* on oxytocin induced preterm labour.

4. 2. OBJECTIVES:

The objectives of the present study include:

- Evaluation of *in- vitro* antioxidant activity of the *S.cumini* seed extract.
- Tocolytic activity of *S.cumini* seed extract.
- Evaluation of *in -vivo* antioxidant activity of the *S.cumini* seed extract
- Studies on isolated rat uterus

5. PLAN OF WORK

The present study examines the efficacy of aqueous seed extract of *Syzygium cumini* as a tocolytic agent. The effect of the drug was evaluated on female wistar rats.

1. Review of Literatures.

2. Selection, collection and authentication of plant material.

3. Extraction of dried seeds with water.

4. Preliminary phytochemical analysis.

5. Quantification of total phenol and flavonoid content.

6. In-vitro antioxidant study.

- DPPH radical scavenging assay
- ABTS radical cation scavenging assay

7. Tocolytic activity

8. In-vivo antioxidant study.

- Enzymatic antioxidants(SOD,CAT)
- Non-enzymatic antioxidant(GSH)
- Estimation of Lipid Peroxidation (LPO)

9. Study on isolated rat uterus tissue

10. Statistical analysis of the results.

11. Discussion

12. Conclusion

6. MATERIALS AND METHODS

6.1 MATERIALS USED FOR THE STUDY

Table 1: List of instruments

Sl. No	Instruments	Manufacturer
1.	Analytical weighing balance	Shimadzu
2.	Cooling centrifuge	Remi
3.	UV spectrometer	Pharmaspec UV-1700, Shimadzu
4.	Kymograph	INCO instruments and chemicals PVT.LTD
5.	Rotary evaporator	IKA RV 10
6.	Microscope	Dollar US -4

Table 2: Requirements of the study

DRUGS	CHEMICALS	OTHERS
1.Oxytocin (Pfizer Ltd) 2.Atosiban(Emcure pharmaceuticals Ltd) 3.Estradiol valerate (Akums Drugs and Pharmaceuticals Ltd)	1.ABTS 2. DPPH 3.Phosphate Buffer 4.Quercetin 5. Potassium Persulfate 6.Potassium Ferricyanide 7.Ferric Chloride 8.Gallic Acid 9.Sodium Carbonate 10.Folin Ciocalteau Reagent	1.Distilled water 2.Sterile water for injection 3.Normal saline 4.Syringe 5.Needle 6.Gloves 7.Droppers 8.Microscopical slides

6.2 PHARMACOGNOSTIC STUDIES

6.2.1 Collection of Plant material and authentication

The plant material was collected and authenticated by **Dr. K. Madhava Chetty. Ph. D**., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh**.** The seeds were processed, powdered coarsely and coarse seeds were used for extraction

6.2.2 Extraction of the seeds [41]

The dried seeds were coarsely powdered. The powdered seeds (200 gm) were taken in a round bottom flask and were extracted with water for 48 hr at room temperature. After 48 hrs, the solution was filtered and the extract was concentrated in a rotary evaporator.

6.2.3 Preliminary Phytochemical Analysis of the Extract

The information about the constituents present in the plant clarifies the medicinal uses of the plant. Identification and evaluation of herbal extracts is a fundamental procedure and parts of quality control protocol. The aqueous extract of *Syzygium cumini* seed was subjected to phytochemical evaluation and identified the various plant constituents present in the test samples by qualitatively and quantitatively. The following studies were carried out in phytochemical analysis.

- Qualitative chemical test
- Estimation of total phenol
- Estimation of total flavanoid

6.2.3.1 Qualitative chemical tests [53] [54] [55] [56]

The qualitative chemical tests were carried out for the extract and identified the various secondary metabolites present in the aqueous extract of *Syzygium cumini* seed.

Preparation of test sample

500 mg of the extract was dissolved in 5ml of distilled water and then filtered. The filtrate was tested to detect the presence of various phytochemical constituents in the sample.

TEST FOR CARBOHYDRATES

- **Molisch's test**

A few drops of Molisch's reagent were added to 2-3ml of filtrate, and concentrated sulphuric acid was added along the sides of the test tube. Violet colour ring formed at the junction of two liquids indicates the presence of carbohydrates.

- **Fehling's test**

Equal volume of Fehling's- A [copper sulphate in distilled water] and Fehling's- B [potassium tartarate and sodium hydroxide in distilled water] reagents were mixed in a test tube and boiled for one minute. 1ml of sample was added to the above mixture and heated for few minutes. Brick red precipitate formed confirms the presence of sugars.

- **Benedict's test**

The test sample was mixed with equal volume of Benedict's reagent (alkaline solution containing cupric citrate complex) in a test tube and heated for few minutes. Formation of brick red precipitate confirms the presence of sugars.

TEST FOR ALKALOIDS

Small amount of extract was mixed with few ml of dilute hydrochloric acid and filtered. The following tests were performed with the filtrate:

- **Dragendorff's test**

Few drops of the Dragendorff's reagent (Sodium iodide, basic bismuth carbonate, glacial acetic acid and ethyl acetate) were added to 2-3ml of filtrate. Development of orange brown precipitate indicates the presence of alkaloids.

- **Mayer's test**

The filtrate was treated with few drops of Mayer's reagent (mercuric chloride and potassium iodide). Formation of yellowish buff coloured precipitate indicates the presence of alkaloids.

- **Wagner's test**

A few drops of filtrate were treated with Wagner's reagent (solution of iodine in

potassium iodide) and a reddish brown precipitate obtains to indicate the presence of alkaloids.

- **Hager's test**

A few drops of Hager's reagent were added to 2-3 mlof filtrate. Development of yellow precipitate indicates the presence of alkaloids.

TEST FOR TRITERPENOID

- **Libermann-Burchard test**

A small quantity of extract was treated with few drops of acetic anhydride, followed by a few drops of concentrated sulphuric acid. A brown ring was formed at the junction of two layers and the upper layer turns green colour, infers the presence of phytosterols and formation of deep red colour indicates the presence of triterpenoids.

- **Salkowski test**

A small quantity of the extract was treated with chloroform and a few drops of concentrated sulphuric acid and allowed to stand for few minutes. Yellow colour at the lower layer indicates the presence of triterpenoids.

TEST FOR GLYCOSIDES

- **Legal's test**

2ml of extract was dissolved in pyridine. Sodium nitroprusside solution was added to it and made alkaline. Pink red colour formed indicates the presence of glycosides.

- **Keller-Killiani test**

2ml of extract was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of two liquids, a reddish brown color formed, which gradually became blue colour due to the presence of glycosides. ß Baljet test 2ml of extract was added to sodium picrate solution. Yellow to orange colour was formed indicating the presence of glycosides.

TEST FOR STEROIDS AND STEROLS

- **Liebermann- Burchard reaction**

The test sample was dissolved in 2ml of chloroform in a dry test tube. Few drops of acetic anhydride were added followed by 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

- **Salkowski reaction**

Extract was dissolved in chloroform and concentrated sulphuric acid was added to it. Bluish red, cherry red and purple colour was noted in chloroform layer, whereas acid layer was marked with green fluorescence.

TEST FOR TANNINS

- **Lead acetate test**

1ml of alcoholic solution of extract was diluted with 5ml of distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow colour precipitate was formed which indicates the presence of phenols.

TEST FOR SAPONINS

- **Foam Test**

About 1ml of test sample was diluted separately with distilled water to 20ml and shaken in a graduated cylinder for 3 minutes. Foam of 1cm after 10minutes indicates the presence of saponins.

- **Froth test**

A drop of sodium bicarbonate was added to 5ml of the test sample. The mixture was shaken vigorously and kept for 3minutes. A honey comb like froth was formed which shows the presence of saponins.

TEST FOR PHENOLS

- **Ferric chloride test**

1ml of the alcoholic solution of the extract mixed with 2ml of distilled water followed

by few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols. ß Lead acetate test 1ml of alcoholic solution of extract was diluted with 5ml of distilled water and, few drops of 1% aqueous solution of lead acetate was added. A yellow colour precipitate was formed which indicates the presence of phenols.

TEST FOR FLAVONOIDS

- **Alkaline reagent test**

A few drops of sodium hydroxide solution was added to the extract. Intense yellow colour turned to colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

- **Shinodas test [Magnesium hydrochloride reduction test]**

A small piece of magnesium ribbon and few drops of concentrated HCl were added to the alcoholic solution of extract and heated. Appearance of crimson red or occasionally green to blue colour infers the presence of flavonoid.

TEST FOR PROTEINS AND AMINO ACIDS

- **Biuret test**

1ml of test sample was mixed with 1ml of 40 % sodium hydroxide and 2 drops of 1% copper sulphate. Formation of violet colour indicates the presence of proteins.

- **Ninhydrin test**

1ml of test sample heated with 2 drops of freshly prepared 0.2% Ninhydrin reagent. Development of blue colour indicates the presence of proteins, peptides or amino acids.

6.2.3.2 Estimation of total phenol content [57]

The determinations of total phenol content of the extracts were done by Folin-Ciocalteu (F-C) assay with some modifications. The Folin–Ciocalteu reagent produces blue colour complex when reacted with polyphenol compounds if present in the sample. The assay relies on the transfer of reducing electrons in the alkaline medium, from phenolic compounds to phospho-molybdic acid complexes, manifested in the formation of blue colour that are estimated by UV-visible spectrophotometer (Thermo Fischer model Evolution 201).

100 µL sample was taken in 2ml centrifuge tube followed by 0.5ml F-C reagents (1:10diluted with distilled water) was added and allowed to react for 5 min before adding 0.4ml 20% Na_2CO_3.The above solutions were mixed and allowed to stand 15 min at room temperature then measured absorbance of sample at 765nm.The blank was prepared in similar manner without sample and standard. Calibration curve was plotted using Gallic acid as standard (10, 20, 40, 60, 80,100µg/ml).The results were expressed as milligram of Gallic acid equivalents (GAE) per gram of extract.

6.2.3.3 Estimation of total flavanoid content [57]

Total flavanoid content was estimated for all the extracts by Aluminium chloride colorimetric assay with some modifications. 100µL aliquot of appropriately diluted sample or standard solution of quercetin (10, 20, 40, 60, 80 and 100µg/ml) was mixed with 50µL of $NaNO_2$ in 2ml micro centrifuge tube. After 6 min, 50µL of a 10% aluminium chloride solution was added and allowed to stand for 6 min, and then 50µl 1M potassium acetate solution was added to the mixture. The final volume was made up with distilled water to 2ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm against prepared blank. Blank was prepared in the same above manner omitting sample and standard. All values were expressed as milligrams of quercetin equivalent per 1g of sample.

6.3 *IN VITRO* ANTI-OXIDANT STUDY

Various methods are used to investigate the antioxidant property of samples. In the present study the antioxidant properties of the extract was evaluated by *in vitro* methods. The antioxidant properties could not be concluded based on the single antioxidant test method. It is in practice that generally several *in vitro* test procedure are carried out to conclude the antioxidant properties of the sample. Among various free radical scavenging methods DPPH and ABTS assays was carried out in the present study.

6.3.1 DPPH Radicalscavenging activity [1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl)] [58]

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517nm (purple

colour). When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical- scavenging antioxidant) and is reduced to DPPH and a consequence the absorbance's decreased from the DPPH radical to the DPPH-H form results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization more is the reducing ability. Also the lower absorbance of the reaction mixture indicates higher free radical scavenging activity. This test has been most accepted model for evaluating the free radical scavenging activity of any new drug.

(DPPH) + H-A → DPPH-H + A

(Purple) (Yellow)

Procedure:

0.3mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 1ml of various concentrations of sample (10, 20, 40, 60, 80 and100 µg/ml) and the reference compound (5, 10, 15, 20, 25 and 30µg/ml), were shaken vigorously and left to stand in the dark at room temperature for 30min and then absorbance was measured at 517nm. A control reaction was carried out without the test sample. Quercetin was used as standard and all the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Antiradical activity was expressed as inhibition percentage (I %) and calculation using the following equation:

Percentage inhibition (I %) = {($Abs_{control}$—Abs_{sample})÷$Abs_{control}$}x 100

The different sample concentrations were used in order to obtain calibration curves and to calculate the EC50 values (EC50: concentration required to obtain a 50% radical scavenging activity).The IC 50 value was defined as the concentration (in µg/ml)of extracts that inhibits the formation of DPPH radicals by 50%.

6.3.2 ABTS radical cation scavenging activity [59]

Principle

ABTS decolourization assay is an inhibition method. The peroxidase substrate 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), forms a relatively stable

radical ($ABTS^{+}$) upon one electron oxidation. This assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical and inhibits the absorption of the radical cation which has characteristic long-wavelength absorption spectrum showing maxima at 660,734, and 820nm. The relatively stable ABTS radical has a green colour and is quantified spectrometrically at 734nm.

Procedure

ABTS radical scavenging activity of the extract was measured by Rice-Evans method. ABTS was dissolved in water to a 7mM concentration. ABTS radical cation (ABTS+) was produced by reacting ABTS stock solution with 2.45mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12- 16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the study, ABTS solution was diluted with phosphate buffer saline pH 7.4 (PBS) to an absorbance of 0.70 (± 0.02) at 734nm and equilibrated at 300C. After addition of 1ml of diluted ABTS solution to various concentrations of sample (1, 2, 4, 6, 8, and 10 µg/ml) or reference compound (0.25, 0.5, 0.75, 1, 1.25 and 1.5 µg/ml) the reaction mixture was incubated for 6min and then absorbance was measured at 734 nm against a blank. A control reaction was carried out without the sample. All the tests were performed in triplicate in order to get the mean values. The percentage inhibition of ABTS+ by the sample was calculated according to the formula.

Percentage inhibition (I %) = (Abs control- Abs sample /Abs control) X 100

Different sample concentrations were used in order to obtain calibration curves and to calculate the IC_{50} values. (IC_{50} - concentration required to obtain a 50% radical scavenging activity).

6.4 PHARMACOLOGICAL STUDY

6.4.1 Animals and Diet

Female wistar rats, weighing 150-200g were obtained from KMCH College of Pharmacy, Coimbatore. The animals were housed under controlled conditions of temperature (20-25^{0}C) and photoperiod 12-h light/dark cycle. All the rats were fed a pelletized commercial chow diet and fresh water *ad libitum* throughout the experimental

period, and weight gain measured weekly. All animal procedures were performed after approval from the ethics committee and accordance with the recommendations for the proper care and use of laboratory animals.

6.4.2 TOCOLYTIC ACTIVITY

Experimental design

Thirty female wistar albino rats of 150-200 gm were used for the study. Rats were divided into five groups of 6 animals each.

Table 3: Experimental design for oxytocin induced preterm labour

GROUPS	TREATMENT
Group I	Normal saline
Group II	Oxytocin (1 IU), i.m
Group III	Atosiban (6mg/kg),i.p
Group IV	AESC (200mg/kg), p.o
Group V	AESC (400mg/kg), p.o

Procedure

The rats were divided into five groups of six animals each. The group I was treated with normal saline, group II was treated with oxytocin (1 IU i.m) only, group III was treated with atosiban (6mg/kg), i.p, group IV and group V were treated with 200 and 400 mg/kg of AESC respectively.

Requirements for obtaining vaginal smear: [60]

Normal saline: The dropper needs only a small volume of normal saline (0.2–0.25ml) for flushing. Distilled water can also be used without markedly distorting the cells enough to impair identification. Only a drop or two needs to be placed on a slide.

Slides: Microscopic slides can be used.

Microscope: A standard laboratory compound microscope is perfectly sufficient to evaluate a vaginal smear.

Stain: 0.1% methylene blue.

Procedure: [34], [60]

The thirty (30) female rats were randomly distributed into 10 cages such that each cage contained 3 female rats. Each of the rats was identified with an indelible marker. After the distribution and identification of the female rats, a vaginal smear of each of them was made on a labelled clean glass slide.

The smear was collected by carefully with droppers, although moistened cotton swabs have also been used. Dropper tips should be smooth and tapered. If a single dropper is used for more than one animal, it should be thoroughly rinsed between lavages to remove any residual cells from the dropper wall. When inserting the tip of the dropper into the vaginal orifice, it is important that the penetration be relatively shallow, approximately 1cm.

Immediately after withdrawal from the vaginal cavity, the content was smeared onto a labelled clean glass slide. The smear was observed grossly to check for the presence of protein coagulates (remnants of the copulatory plug).

After the initial smears were collected from the female rats, the male rats were introduced into 10 cages, such that the male: female ratio in these 10 cages was 1:3. After the introduction of males into the 10 mating cages, vaginal smears were made as described above for each of the females in all the 10 cages daily in the morning, and the smeared slides were observed grossly for protein coagulates. The observation of grossly visible protein coagulates on the vaginal smear of each female was recorded as evidence of mating. The mating was also confirmed by the presence of sperms in the vaginal smear. The sperm was observed by staining the smear with 0.1% methylene blue.

The presence of sperm in the vaginal smear or observation of a vaginal plug indicates the occurrence of mating. The day that sperm is detected in the vaginal smear is designated as day 1 of gestation. After 10 days of gestation, the fetuses can be palpated, but palpation is more accurate after day 12. By day 13 of gestation, the abdominal enlargement is visible, and mammary development and nipple enlargement can be

observed on day 14 of gestation. Once the protein coagulates and sperms were observed on the vaginal smear of each rat, the rat was thereafter weighed at four-day intervals to check the progress of the pregnancy.

6.4.3 *IN VIVO* ANTIOXIDANT ACTIVITY

Blood Collection

After the end of the treatment period the animals were anaesthetized with diethyl ether (inhalation) and its blood was collected by retro orbital puncture without adding EDTA.

Separation of serum [61]

The serum was prepared using a standard method. Briefly, the method used is as follows. The blood was allowed to clot for 30 minutes and then centrifuged at 2500rpm for 15 minutes and serum was harvested.

ESTIMATION OF TOTAL PROTEIN [62]

Total protein content of the sample was determined by following method:

Requirements

Alkaline copper reagent

- Solution A: 2% sodium carbonate in 0.1 N NaOH.
- Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate
- Solution C: 50 ml of solution A was mixed with 1 ml of solution B just before use.
- Folin's phenol reagent (commercial reagent, 1:2 dilutions) Bovine serum albumin (BSA).

Principle:

This method involves two steps; Step 1: Protein binds with copper in alkaline medium and reduces it into Cu++. Step 2: The Cu++ formed catalyses the oxidation reaction of

aromatic amino acids by reducing phosphomolybdo tungstate to heteropolymolybdanum, which leads to the formation of blue colour and absorbance was measured at 640 nm.

Procedure:

0.1 ml sample was made up to 1 ml with distilled water. 5 ml of alkaline solution was added, mixed well and allowed to stand for 10 min. Then, 0.5 ml Folin's reagent was added, mixed well and incubated at room temperature for another 10 min. The blue colour developed was measured at 640 nm against blank. Bovine serum albumin (1 mg/ml) served as the standard and from the standard graph obtained the amount of protein in the sample was calculated.

6.4.3.1 Enzymatic antioxidant activity

6.4.3.1.1 Estimation of superoxide dismutase (SOD) [63]

Requirements

- Adrenaline
- Carbonate Buffer (pH – 10.2)
- 0.1mM EDTA

Principle

Superoxide dismutase is an endogenous enzymatic antioxidant which catalyzes the dismutation of superoxide free radical. This method is based on the inhibition of the spontaneous oxidation of the adrenaline to adrenochrome by the enzyme superoxide dismutase.

Procedure

To 0.5 ml of the sample, 1.5 ml of carbonate buffer and 0.5 ml of 0.1mM EDTA was added and mixed. To this 0.4 ml of adrenaline was added at the time of measurement of the optical density at 480 nm. The antioxidant activity of SOD enzyme was expressed as units/min/mg protein.

6.4.3.1.2 Estimation of Catalase (CAT) [64]

Requirements

- Dichromate acetic acid reagent
- 0.01M Phosphate Buffer (pH – 7.0)
- 0.2M Hydrogen peroxide

Principle

The normal antioxidant activity of the enzyme catalase is to accelerate the decomposition of hydrogen peroxide to water and oxygen. This method is based on the principle of measuring the rate of decomposition of hydrogen peroxide by the enzyme Catalase, which was measured spectrophotometrically at 570nm, since hydrogen peroxide has the absorbance at this range.

Procedure

To 1 ml of sample, 4ml of hydrogen peroxide and 5 ml of phosphate buffer was added and mixed. From this 1ml of solution was taken and mixed with dichromate acetic acid reagent and allowed to incubate for 30 minutes at room temperature. The absorbance was measured at 570 nm. The activity of Catalase was expressed as µmole of H_2O_2 consumed/min/mg protein.

6.4.3.2 Non Enzymatic anti-oxidant activity

6.4.3.2.1 Estimation of reduced glutathione (GSH) activity [65]

Requirements

- 5% TCA
- 0.6mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate
- 0.2 M Phosphate buffer at pH 8.0

Principle

DTNB is a disulfide compound, which was reduced by sulfhydryl groups present in GSH. This reduction leads to formation of yellow colour, which was measured at 412nm.

Procedure

To 1ml of the sample, 1 ml of TCA solution was added and centrifuged. The supernatant was collected and the precipitate formed was removed. To 0.5 ml of supernatant 2 ml DTNB was added, the volume was made up to 3 ml with phosphate buffer. Then absorbance was read at 412nm. The amount of glutathione was expressed as µg/mg protein.

6.4.3.3 Determination of lipid peroxidation (LPO) [66]

Requirements

- Thiobarbituric acid 0.37%
- 0.25 N HCL
- 15% TCA

Principle

This assay is based on the reaction between thiobarbituric acid with malonyldialdehyde which is a formed as a result of polyunsaturated fatty acid oxidation. This reaction leads to the formation of pink colored TBA-MDA complex which is measured at 532 nm.

Procedure

To 0.1 ml of sample, 2ml of TBA-TCA-HCL reagent (ratio of 1:1:1) was added, mixed and kept in a water bath for 15 minutes. Afterwards the solution was cooled and supernatant was removed and absorbance was measured at 535 nm against reference blank. The level of lipid peroxides was given as moles of MDA formed/mg protein.

6.4.4 *IN VITRO* STUDY OF ISOLATED RAT UTERUS [67], [68], [44]

Female non-pregnant wistar rats were pretreated subcutaneously with estradiol valerate 0.1 mg/kg of 24 h prior to the actual experiment for uterine sensitization. The rats were killed by cervical dislocation and exsanguinations. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a strip of the horn about 1 - 2 cm was cut out. A thread was then attached to one end of the isolated strip of uterus and was tied to the aerator tube in the organ bath containing 25 ml De Jalon's physiological salt solution having the following chemical composition: NaCl-9 g/L,

$NaHCO_3$ - 0.5 g/L, D-glucose - 0.5 g/L, KCl-0.402 g/L, $CaCl2^{\times}2$ H_2O -0.08 g/L. Another thread was attached to the other end of the isolated uterus and fixed to a lever system. The tissue was aerated and temperature was maintained at 30-32^{O}C, with a pH of 7.4.

The tissue was allowed to equilibrate for 30 min before the start of the experiment. A lever system was used to record the uterine contractions and relaxations on a smoked glossy paper in kymograph drum, moved at 2.5mm/sec speed which was varnished after and calculated the data. The response of uterine tissue to oxytocin (0.01 IU/ml) before and after incubation with aqueous seed extract of *Syzygium cumini* (25 and 50 mg/ml) for one minute were recorded along with standard drug atosiban (5 mg/ml).

6.5 STATISTICAL ANALYSIS

Data were analyzed by one way ANOVA followed by Tukey's multiple comparison test using Graphpad 5.0 software. The values were expressed as Mean ± SEM.

7. RESULTS

7.1 EXTRACTIVE YIELD

Percentage Yield of AESC

Coarsely powdered seeds of *Syzygium cumini* were extracted with water and the percentage yield was found to be 14.5% w/w.

7.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

Table 4: Qualitative Chemical Tests

Sl.No	Phytochemical Constituents	AESC.
1	Carbohydrates	+
2	Alkaloids	+
3	Glycosides	+
4	Triterpenoids	+
5	Flavonoids	++
6	Phenols	++
7.	Tannins	+
7	Steroids and sterols	+
8	Saponins	+
9	Proteins and amino acids	++

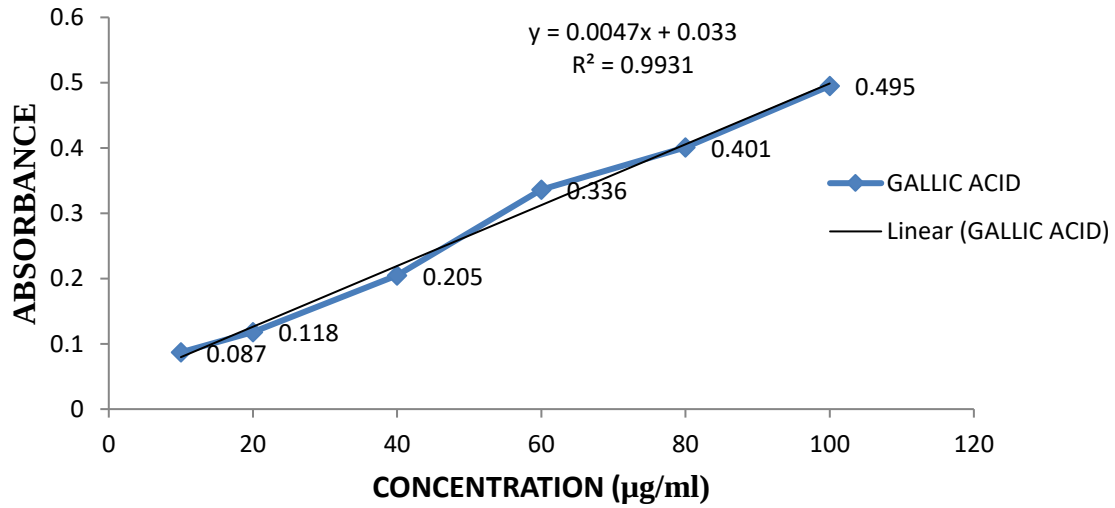
+= Present, ++ = More present

7.3 QUANTIFICATION OF TOTAL PHENOL AND FLAVONOIDS

Table 5: Estimation of total phenolic content of AESC

Sample	Concentration (µg/ml)	Absorbance at 725 nm
Standard (Gallic Acid)	10	0.087
	20	0.118
	40	0.205
	60	0.336
	80	0.401
	100	0.495
AESC	100	0.2494

Figure 6: Standard graph for Gallic acid for the estimation of total phenolic content

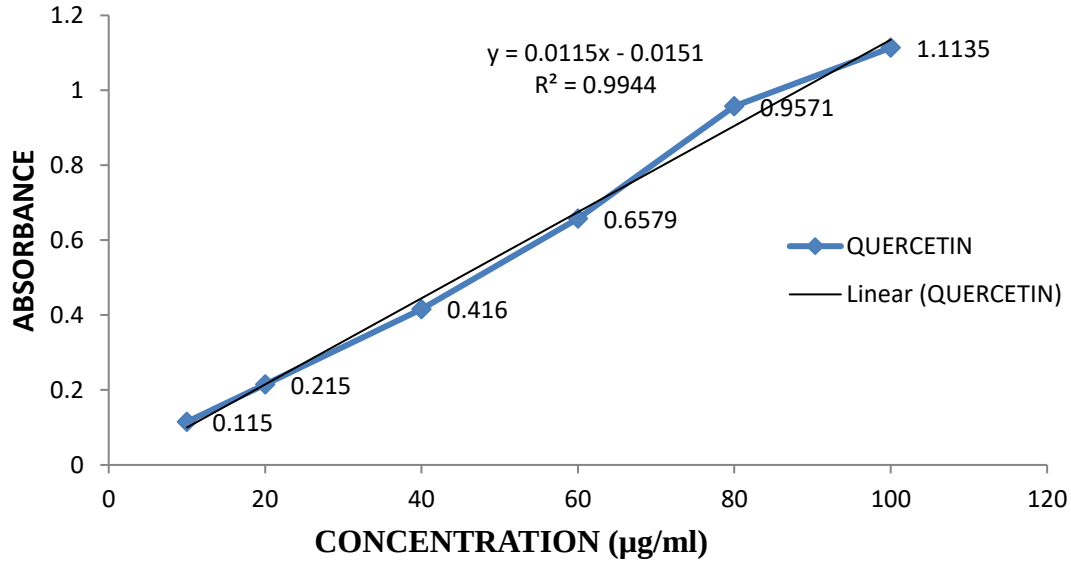


The total Phenol content present in AESC was found to be 54.1mg/g equivalent to gallic acid.

Table 6: Estimation of total flavonoid content of AESC

Sample	Concentration (µg/ml)	Absorbance at 415nm
Standard (Quercetin)	10	0.1150
	20	0.2150
	40	0.4160
	60	0.6579
	80	0.9571
	100	1.1135
AESC	100	0.5362

Figure 7: Standard graph of quercetin for the estimation of total flavonoid content



The total flavonoid content in AESC was found to be 50.1 mg/g of extract calculated as Quercetin equivalent.

7.4 *IN VITRO* ANTI OXIDANT STUDY

7.4.1 DPPH RADICAL SCAVENGING ACTIVITY

Table 7: Percentage inhibition and IC50 values of DPPH radical by quercetin and AESC

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Standard (Quercetin)	5	32.20	10.79
	10	42.14	
	15	53.36	
	20	68.33	
	25	86.65	
	30	94.56	
AESC	10	26.63	33.15
	20	38.70	
	40	48.21	
	60	57.33	
	80	74.22	
	100	85.12	

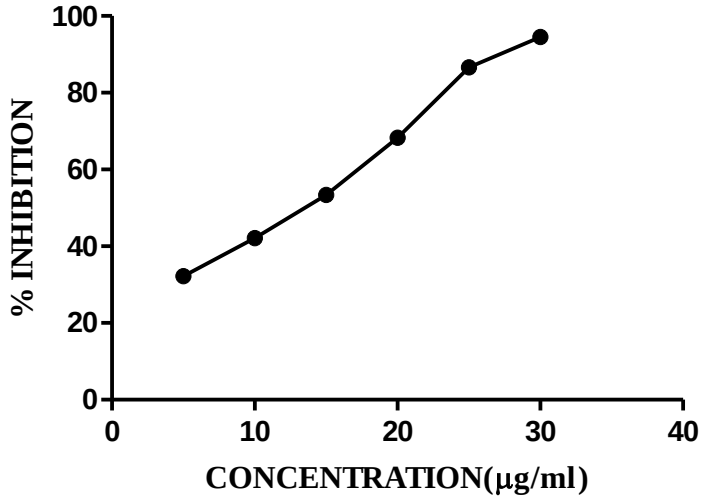


Figure 8: DPPH radical scavenging activity of quercetin

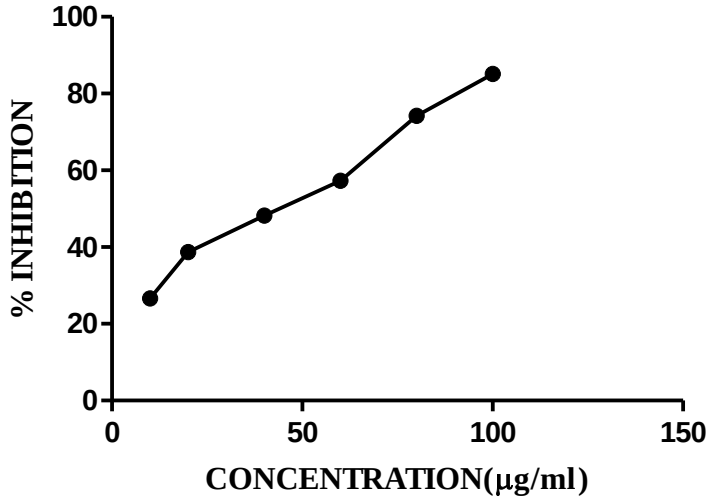


Figure 9: DPPH radical scavenging activity of AESC

7.4.2 ABTS RADICAL SCAVENGING ACTIVITY

Table 8: Percentage inhibition of ABTS radical by quercetin and AESC

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Standard (Quercetin)	0.25	42.56	0.3869
	0.5	50.34	
	0.75	65.12	
	1.0	79.84	
	1.25	90.23	
	1.5	96.41	
AESC	1	19.43	4.43
	2	27.82	
	4	39.9	
	6	53.12	
	8	70.24	
	10	83.56	

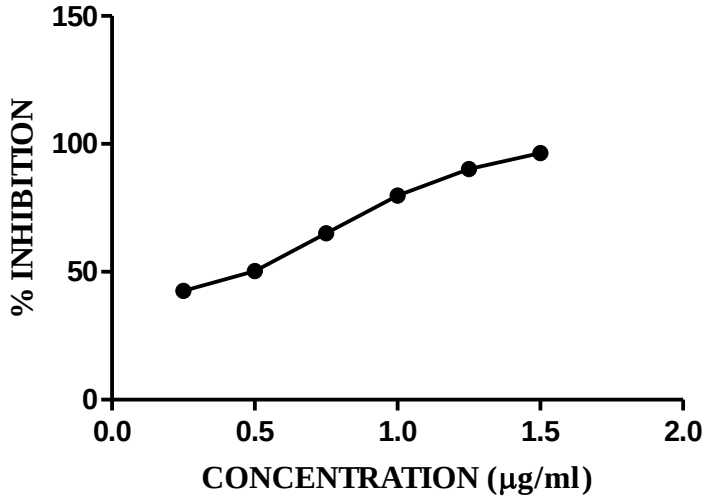


Figure 10: ABTS radical scavenging activity of quercetin

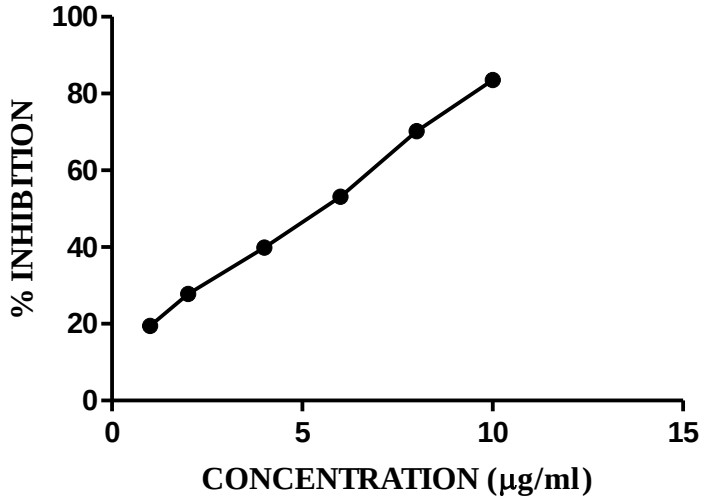


Figure 11: ABTS radical scavenging activity of AESC

7.5 TOCOLYTIC ACTIVITY

Table 9: Effects OF AESC and atosiban on oxytocin induced preterm labour on rat model

Group	Total number of rats	Numbers of rat delivered					Rates of term delivery	Delivery day (Mean±SEM)
		Preterm			Term			
		Day 17	Day 18	Day 21	Day 22	Day 23		
Normal	6	0	0	0	0	6	100	23±0
Control (oxytocin 1 IU, i.m)	6	4	2	0	0	0	0	17.3±0.210***
Control+ Atosiban (6mg/kg, i.p)	6	0	0	0	3	3	100	22.5±0.223***
Control+ AESC (200mg/kg,p.o)	6	0	0	2	2	2	66.6	22±0.365***
Control+ AESC (400mg/kg,p.o)	6	0	0	1	1	4	83.3	22.5±0.341***

Statistical comparison: values represent mean ± SEM, n=6. Statistical analysis was done by one way analysis of variation (ANOVA) followed by Tukey's multiple comparison test. ***P<0.001, denotes comparison of oxytocin control group with normal and all other groups were compared with oxytocin control group.

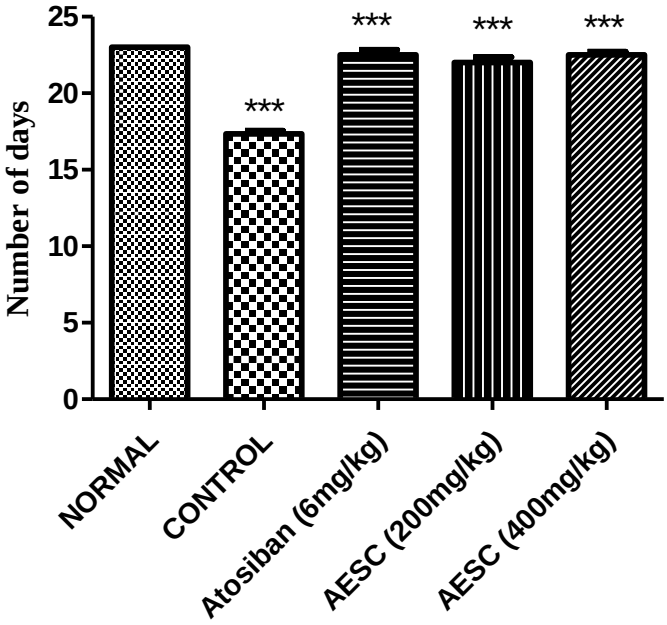


Figure 12: Effect of AESC on preterm labour

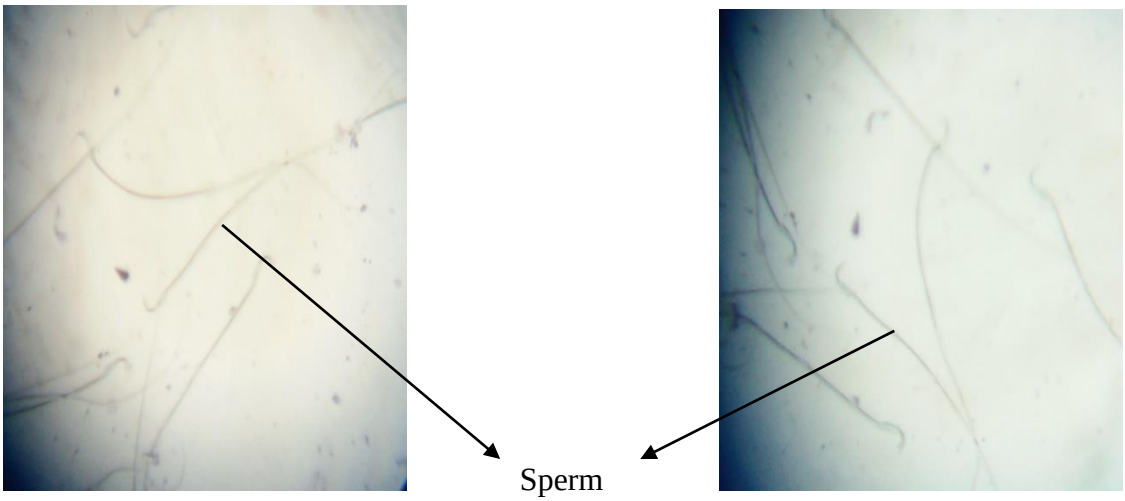


Figure 13: Microscopical examination (100x) of vaginal smear of mated animal showing sperms

7.6 INVIVO ANTIOXIDANT STUDY

Table 10: Effect of AESC on enzymatic and non enzymatic antioxidant levels

GROUP	Total protein (mg/dl)	SOD (Unit/ mg protein)	CAT (µmol of H_2O_2 consumed/ mg protein)	GSH (Glutathione µg/mg	LPO (nmol of MDA/mg protein)
Normal	3.9±0.08	7.4±0.24	53.3±0.37	12.8±0.09	2.8±0.09
Control oxytocin (1 IU)	9.8±0.44***	2.4±0.19***	28.4±0.36***	6.7±0.19***	8.8±0.11***
Control+ Atosiban(6mg/kg)	2.6±0.54***	5.84±0.13***	31.75±0.88**	11.9±0.10***	3.7±0.16***
Control+ AESC(200mg/kg)	4.8±0.21**	$3±0.02^{ns}$	31.07±0.38*	7.52±0.23*	6.8±0.09***
Control+ AESC(400mg/kg)	3.9±0.04***	3.36±0.24*	32.05±0.65**	11.8±0.122***	5.9±0.02***

Statistical comparison: values represent mean ± SEM, n=6. Statistical analysis was done by one way analysis of variation (ANOVA) followed by Tukey's multiple comparison test. *P<0.05, **P<0.01, ***P<0.001 and ns- non significant, denotes comparison of oxytocin control group with normal and all other groups were compared with oxytocin control group.

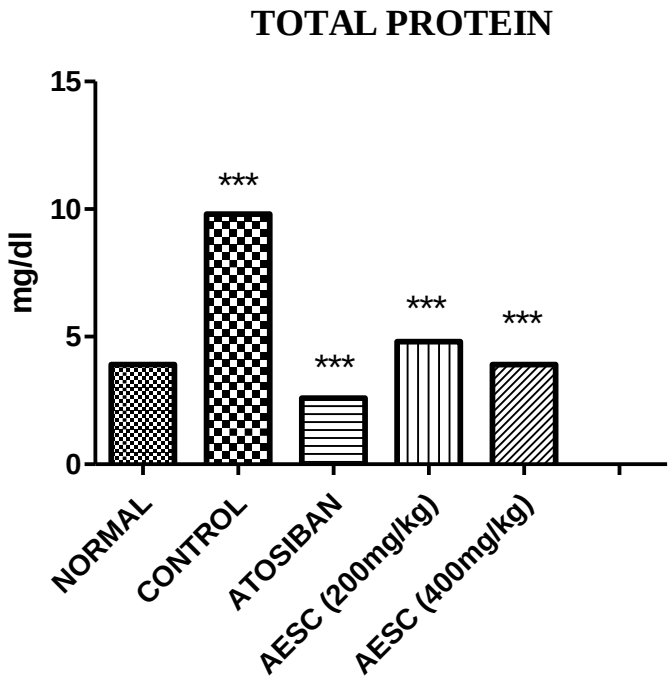


Figure 14: Effect of AESC on serum total protein

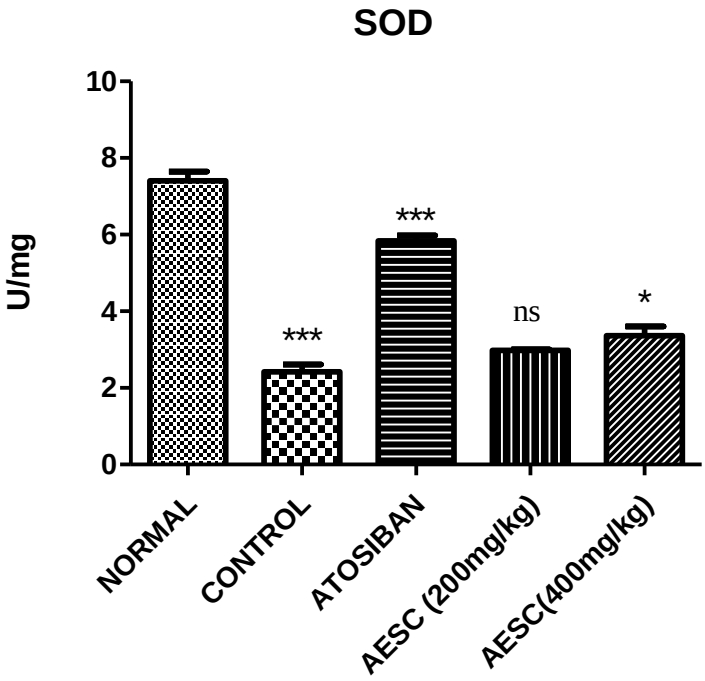


Figure 15: Effect of AESC on serum SOD

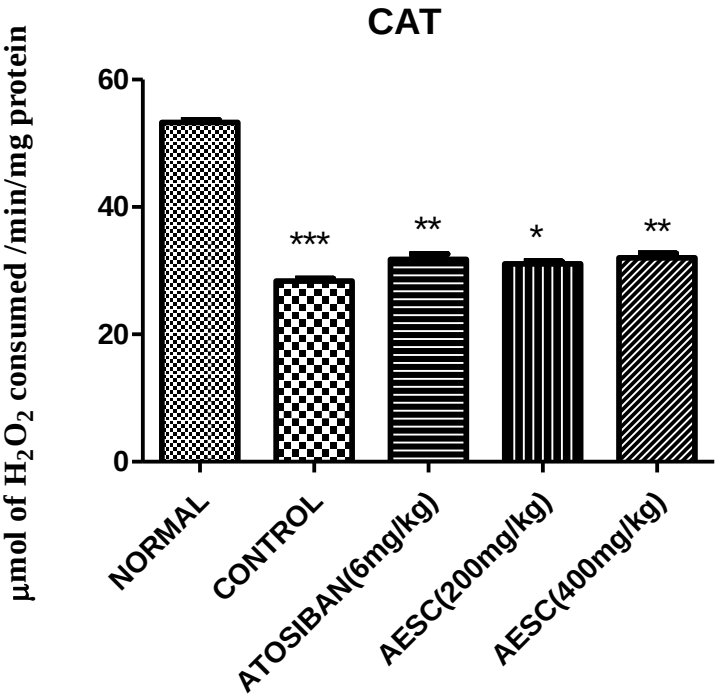


Figure 16: Effect of AESC on serum CAT

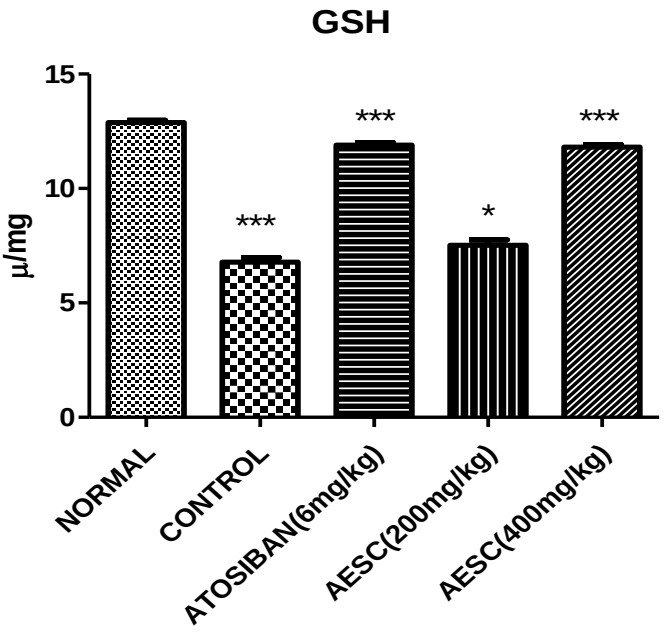


Figure 17: Effect of AESC on serum GSH

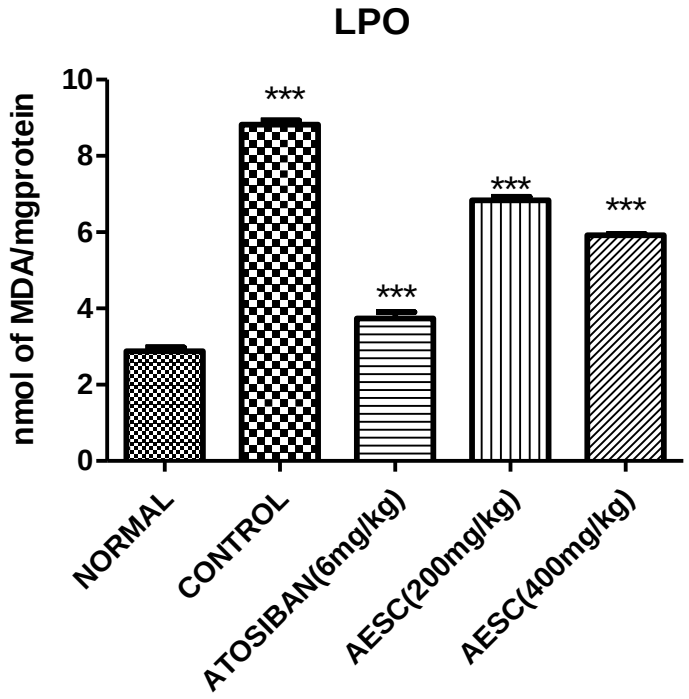


Figure 18: Effect of AESC on serum LPO

Statistical comparison: values represent mean ± SEM, n=6. Statistical analysis was done by one way analysis of variation (ANOVA) followed by Tukey's multiple comparison test. *P<0.05, **P<0.01, ***P<0.001 and ns- non significant, denotes comparison of oxytocin control group with normal and all other groups were compared with oxytocin control group.

7.7 INVITRO STUDY OF ISOLATED RAT UTERUS

Table 11: Effect of AESC on oxytocin induced contraction in rat uterus

Group	Drug	Concentration	Height of Contractions(cm)	% Inhibition
I	oxytocin	0.01 IU/ml	5.5	-
II	AESC extract + oxytocin	25 mg/ml + 0.01 IU/ml	2.7	50.9
III	AESC extract + oxytocin	50 mg/ml + 0.01 IU/ml	1.5	72.7
IV	Atosiban + oxytocin	5 mg/ml + 0.01 IU/ml	0.57	89.6

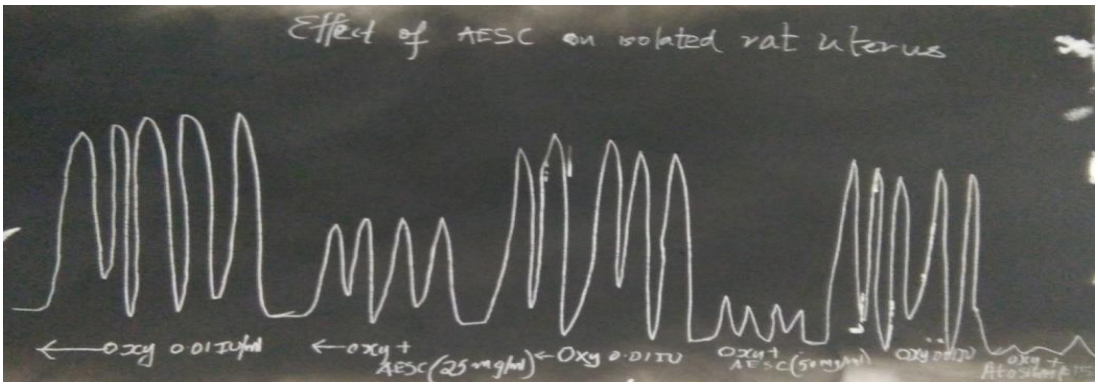


Figure 19: Effect of AESC and Atosiban on isolated rat uterus

8. DISCUSSION

Preterm birth is the most common cause of neonatal morbidity and mortality world- wide .Almost 75% of perinatal deaths occur in infants born before 37 weeks gestation [69].

The rationale for treating preterm labour is to reduce perinatal morbidity and mortality by increasing the gestational age at delivery. Assuming tocolysis is beneficial, the choice of drug remains controversial. Magnesium sulphate, calcium channel blockers, prostaglandin synthase inhibitors, nitric oxide donors, β-sympathomimetics and oxytocin antagonists have all been suggested to be effective. Given the problems of efficacy and adverse effects with each tocolytic, particularly in view of the potentially devastating consequences of preterm delivery, it is not surprising that new drugs have been developed and tested in clinical trials [70].

The study aims at the evaluation of the tocolytic activity of aqueous seed extract of *Syzygium cumini* on oxytocin induced preterm labour as well as its antioxidant property.

The preliminary phytochemical screening of AESC were performed and the results revealed the presence of carbohydrates, flavonoids, triterpenoids, phenols, glycosides, tannins, steroids, alkaloids, saponins, proteins and amino acids. The various phytoconstituents present in the extract is shown in the Table 4.

Phenolics and flavanoid normally scavenge the free radicals and play an essential role in prevention and therapy of PTL, and many more diseases by inducing antioxidant defense system, drug metabolizing enzymes, modulating diverse events in cellular level and inhibiting inflammation, hyperplasia, proliferation and oxidative DNA damage. Poly phenolic compounds (quercetin, gallic acid, rutin) are natural antioxidants which decreases oxidation of bio molecules essential for life [71]. The total Phenol content present in *Syzygium cumini* seed extract was found to be 54.1 mg/g equivalent to gallic acid. The total flavanoid content in AESC was found to be 50.1 mg/g of extract calculated as Quercetin equivalent.

Herbal drugs containing radical scavengers are gaining importance in treating oxidative stress related diseases. [72] DPPH radical scavenging activity of the extract at different concentration was compared with the standard quercetin. Smaller IC_{50} value indicates a higher antioxidant potential. The IC_{50} value of the standard and test extract was found to be 10.79 µg/ml and 33.15µg/ml respectively. The higher IC_{50} values of the sample indicated lesser scavenging activity of the sample when compared with the standard. The study showed the promising radical scavenging activity of the extract due to hydrogen-donating ability of the extract.

The ability of the extract to donate hydrogen atoms or electrons to scavenge the radical cation was reflected by the decolourization of ABTS radical cation. The radical scavenging activity of the extract was compared with that of the standard and the IC_{50} values obtained were 0.3869µg/ml and 4.43µg/ml for standard and extract respectively.

Premature labour still a health challenge world-wide, thus huge efforts were done and required to found a solution for it, this work was an attempt to find a natural solution.

However, the efficacy and safety of tocolytics are not adequate, new agents are therefore required including substances from natural sources. [1]

In the current study, a significant increase in the in the rate of preterm delivery of the control animals was observed when compared with the normal group. The AESC treated group has showed a significant reduction in the rate of preterm delivery which was comparable with the standard treated group as well as the normal group.

Living organisms have developed several effective mechanisms to get protection from the reactive oxygen species. The antioxidant defense mechanisms of the body include enzymes such as SOD, GSH, CAT and GPx.

In the present study it was observed that there was a crucial depletion of the activity of all the enzymes and a marked rise of lipid peroxidation. The study reveals that

the extracts have been able to increase the endogenous antioxidant enzyme activities while reducing the lipid peroxidation.

The cells containing Super Oxide Dismutase enzyme scavenges superoxide ion and prevents its accumulation so that cells are protected from oxidative stress. SOD catalyses this dismutation of superoxide in which one O_2* is oxidized to O_2 while the other is reduced to H_2O_2. The formed H_2O_2 is able to damage the cellular components and are instantly removed by enzymes like catalase and GPx. A significant decrease in SOD levels was observed in control (oxytocin) rats when compared to normal rats. The levels get increased in the rats treated with both low dose (200 mg/kg) and high dose (400mg/kg) of AESC when compared to SOD levels in control rats.

Oxidative stress has a major role in preterm labour. The catalase enzyme is an endogenous antioxidant enzyme that neutralizes reactive oxygen species by converting H_2O_2 into H_2O and O_2. Among the other antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, catalase is an elementary defense against oxidative stress. In oxytocin control rats, a significant decreased level of catalase was observed as compared to normal rats. The low dose (200 mg/kg) and high dose (400mg/kg) of AESC treated groups showed significant increase in catalase levels when compared to control rats.

Reduced Glutathione prevents free radical induced oxidation of SH groups of various proteins to disulfide derivatives. It also protects haemoglobin from getting oxidized by H_2O_2. A significant decrease in the GSH levels was observed in the control rats when compared to normal control rats. Rats treated with both low dose (200 mg/kg) and high dose (400mg/kg) of AESC showed significantly higher GSH levels when compared to control rats [24], [25].

Lipid peroxidation indicates the oxidative degradation of lipids in which the free radicals steals electrons from the lipids in the cell membrane and leads to cell damage. This process proceeds by a free radical chain reaction mechanism. Radical reaction consists of initiation, propagation and termination processes. A significant increased level of LPO was observed in the control rats when compared to normal control rats. The

low dose (200 mg/kg) and high dose (400mg/kg) of AESC treated rats showed significant decreased LPO levels when compared to control rats.

The uterus is spontaneously active, which means that, with or without any nervous/hormonal stimulation, a piece of isolated, pregnant or non- pregnant, uterus will produce regular spontaneous contractions. [2]

The results of *Invitro* study showed that the aqueous extract of *Syzygium cumini* at 25 mg/ml and 50 mg/ml produced significant inhibition of oxytocin, induced contractions of the uterine smooth muscle in non pregnant rats.

It is envisaged that the active ingredients (compounds) will have a potential for being added to the present list of tocolytic agents used clinically. To improve the safety of this traditional herbal remedy, additional research is needed to define the stability and bioactivity of this product. Therefore, further studies are needed for the isolation and characterization of the active constituents.

9. CONCLUSION

Syzygium cumini Linn (family Myrtaceae) is a well known bioactive plant in Ayurvedic system of medicine. The present investigation was aimed at determining the tocolytic activity of aqueous seed extract of *Syzygium cumini*.

AESC showed significant *invitro* antioxidant activity by terminating the actions of free radicals. The extract was studied for its tocolytic activity using oxytocin induced preterm labour in which atosiban (6mg/kg) was used as the standard.

The study revealed that the aqueous extract of *Syzygium cumini* seed possesses significant tocolytic activity which was evident with reduction in the incidence of preterm delivery.

AESC showed tocolytic activity that is mediated by its effects on rates of preterm delivery, anti-oxidant (SOD, CAT, GSH and, LPO), and *invitro* studies. The aqueous extract of S.cumini possesses tocolytic activity probably due to the presence of active constituents present in the extract. In vitro studies of AESC significantly inhibited the frequency and amplitude of spontaneous uterine contractions on the isolated non pregnant rat uterus preparations.

Hence, from the present study it can be concluded that the AESC has the potential to prevent preterm delivery as well as it can reduce the complications of the same. It seems promising that these data obtained from the study can be further validated in the future studies which eventually can be developed as a formulation that offers a high degree of protection from preterm delivery.

10. BIBLIOGRAPHY

1. Adhikari BS, Babu M M, Saklani PL, Rawat GS. Medicinal plants diversity and their conservation status in Wildlife Institute of India (WII) Campus, Dehradun. Ethnobotanical Leaflets 2010; 14: 46-83.
2. David B, Wolfender JL, Dias DA. The pharmaceutical industry and natural products: historical status and new trends. Phytochemistry Reviews. 2015 Apr 1; 14(2):299-315.
3. Tomlinson TR, Akerele O, editors. Medicinal plants: their role in health and biodiversity. University of Pennsylvania press; 2015 Jun 30.
4. Verma R K, Mishra G, Singh P, Jha K K, Khosa RL. Anti-diabetic activity of methanolic extract of Alpinia galanga Linn. aerial parts in streptozotocin induced diabetic rats. Ayu. 2015 Jan; 36(1):91.
5. Shweta Sharma, Mehta Bk. Comparative study of alcoholic and aqueous extracts of *Syzygium cumini* on carbon tetrachloride-induced hepatotoxicity in wistar rats. Asian J Pharm Clin Res. 2016 August 17;9(3):1-4
6. M. Sekar, C.Ayyanna, R. Niranjan Kumar, A. Siva kumar, G. Madhavi lakshmi, N.Siva, Mallika Supraja. Hepatotoxic effect of ethanolic extract of *Syzygium cumini. Linn* leaves on experimental animals. Int. J. of Res. in Pharmacology & Pharmacotherapeutics. 2015 Sep; 4(3):288-294.
7. C. Hubinont and F. Debieve. Prevention of Preterm Labour: 2011 Update on Tocolysis. Journal of Pregnancy. 2011 March.
8. John C. Morrison1, John P. Elliott and Stephen Jones. Uterine contraction monitoring, maintenance tocolysis, and preterm birth. Preterm Birth - Mother and Child. 2012. 201-212.
9. Christine K. Ratajczak, Justin C. Fay and Louis J. Muglia. Preventing preterm birth: the past limitations and new potential of animal models. Disease Models & Mechanisms.2010; 3:407-414.
10. Worldwide Atosiban versus Betaagonists Study Group. Effectiveness and safety of the oxytocin antagonist atosiban versus beta adrenergic agonists in the

treatment of preterm labour. British Journal of Obstetrics and Gynaecology. 2001; 108(2): 133–142.

11. Deepali Laxman Jaybhaye, Sushilkumar Varma, Prashant Chaudhary, Vijay Bonde, Amol Gite. Tocolytic plant *Tectona grandis* Linn. extended study on other systemic effect. Journal of Mahatma Gandhi Institute of Medical Sciences.2016; 21:122-123.
12. Shweta Sharma, Mehta Bk. Comparative study of alcoholic and aqueous extracts of *Syzygium cumini* on carbon tetrachloride-induced hepatotoxicity in wistar rats. Asian J Pharm Clin Res. 2016 August 17;9(3):1-4
13. Kumar, R. Ilavarasan, T. Jayachandran, M. Deecaraman, R. Mohan Kumar,P. Aravindan, N. Padmanabhan and M. R. V. Krishan. Anti-inflammatory activity of *Syzygium cumini* seed. Afr. J. Biotechnol. 2008 April 17; 7(8): 941-943.
14. P. Suresh Kumar, K. Soni and M. N. Saraf. The *In Vitro* tocolytic activity of Sarcostemma brevistigma Wight. Indian Journal of Pharmaceutical Sciences. 2006 April; 68 (2): 190-194.
15. Christine K, Ratajczak, Justin C. Fay and Louis J. Muglia. Preventing preterm birth: the past limitations and new potential of animal models. Disease Models & Mechanisms, 2010 Dec; 3(1):407-414.
16. Naguib Salleh and Vivi Noryati Ahmad. *In-Vitro* effect of *Ficus deltoidea* on the contraction of isolated rat's uteri is mediated via multiple receptors binding and is dependent on extracellular calcium. BMC Complementary and Alternative Medicine.2013; 13(359):1-8.
17. Syama Hari Priya, Nisha Prakasan, Jayamurthy Purushothaman. Antioxidant activity, phenolic-flavonoid content and high-performance liquid chromatography profiling of three different variants of *Syzygium cumini* seeds: A comparative study**.** J Intercult Ethnopharmacol. 2017 January 03; 6(1):107-108.
18. http://teachmeanatomy.info/pelvis/female-reproductive-tract/uterus
19. https://en.Wikipedia.org/wiki/uterus.
20. http://www.medicalnewstoday.com/articles/275795
21. Huda Yahia Hamid and Md Zuki, Abu Bakar Zakaria. Reproductive characteristics of the female laboratory rat. Afr. J. Biotechnol. 2013 May 8; 12(19): 2510-2511.

22. Michelle C. Cora, Linda Kooistra, and Greg Travlos. Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. Toxicologic Pathology.2015; 43: 776-793.
23. Cord JM .The evolution of free radicals and oxidative stress. American Journal of Medicine. 2000; 108: 652–659.
24. Ramkumar Menon. Oxidative stress damage as a detrimental factor in preterm birth pathology. Frontiers in Immunology.2014 Nov 12; 5(557):1-14.
25. Robert A. Knuppel, Mohamed, Hassan, James J. Mc Dermott, J. Martin Tucker and John C. Morrison. Oxidative Stress and Antioxidants: Preterm Birth and Preterm Infants. Preterm Birth - Mother and Child.2012 Jan 27.
26. Syama Hari Priya, Nisha Prakasan, Jayamurthy Purushothaman. Antioxidant activity, phenolic-flavonoid content and high-performance liquid chromatography profiling of three different variants of *Syzygium cumini* seeds: A comparative study**.** J Intercult Ethnopharmacol. 2017 January 03; 6(1):112-114.
27. Kuldip Singh, Ramandeep Kaur and Arvind Preet Kaur. Studies on antioxidant and antimicrobial potential of *syzygium cumini* leaves. RJPBCS. 2016 April; 7(2):677.
28. Madhulika Pradhan. Phytochemistry, Pharmacology and Novel Delivery-applications of *Syzygium cumini* (L.). International Journal of Pharmacy and Pharmaceutical Research. 2016 Aug 26; 7 (1): 659-675.
29. Deepali Laxman Jaybhaye, Sushilkumar Varma, Prashant Chaudhary, Vijay Bonde, Amol Gite. Tocolytic plant *Tectona grandis* Linn. Extended study on other systemic effect. Journal of Mahatma Gandhi Institute of Medical Sciences.2016; 21:122-129.
30. Swadhin Ranjan Behera, Sekkizhar M, Sarath Babu. K. Hepatoprotactive activity of aqueous extract of *Syzygium cumini* seed on streptozotocin induced diabetes in rats. International Journal of Ayurvedic and herbal Medicine.2014; 4(2):1470-1477.
31. Swadhin Ranjan Behera, Sekkizhar M and Sarath Babu K. Nephro-protective Effect of Aqueous Extract of *Syzygium cumini* Seed on Streptozotocin Induced Diabetes in Rats. International Journal Chemistry & LifeScience.2014 Feb 17; 3 (02):1285-1288.

32. Katherine Y Bezold, Minna K Karjalainen, Mikko Hallman, Kari Teramo, and Louis J Muglia. The genomics of preterm birth: from animal models to human studies. Genome Medicine.2013 April 29; 5(4):34.
33. Naguib Salleh and Vivi Noryati Ahmad. *In-Vitro* effect of *Ficus deltoidea* on the contraction of isolated rat's uteri is mediated via multiple receptors binding and is dependent on extracellular calcium. BMC Complementary and Alternative Medicine.2013; 13(359):3-5.
34. Huda Yahia Hamid and Md Zuki Abu Bakar Zakaria. Reproductive characteristics of the female laboratory rat. Afr. J. Biotechnol. 2013 May 8; 12(19): 2512-2514.
35. Lekha K Nair, Maleeka Begum, Geetha. S. Invitro-Antioxidant activity of the seed and leaf extracts of *Syzygium cumini*. IOSR-JESTFT. 2013 December; 7(1):54-62.
36. Asiqur Rahaman, Shahdat Hossain, Mijanur Rahman, Ibrahim Hossain, Taslima Nahar, Borhan Uddin and Ibrahim Khalil. *Syzygium cumini* (L.) Seed extract Improves Memory Related Learning Ability of Old Rats in Eight Arm Radial Maze. Journal of Pharmacognosy and Phytochemistry. 2013; 1(6):85-94.
37. Bhaskar Sharma Md., Sufiyan Siddiqui, Shiv Shanker Kumar, Gurudyal Ram, Manisha Chaudhary. Liver protective effects of aqueous extract of *Syzygium cumini* in Swiss albino mice on alloxan induced diabetes mellitus. Journal of Pharmacy Research. 2013 July.
38. Md. Rashedul Alam, Akib Bin Rahman, Md. Moniruzzaman, Mohammad Fahim Kadir, Md. Ahsanul Haque,Mohammad Razi-Ul-Hasan Alvi, Md.Ratan. Evaluation of antidiabetic phytochemicals in *Syzygium cumini* (L.)Skeels (Family:Myrtaceae). Journal of Applied Pharmaceutical Science. 2012 October; 2 (10):94-98.
39. Shweta sharma, B.K Mehta, Darshna Mehta, Hemant Nagar, Aditya Mishra. A review of pharmacological activity of *syzygium cumuni* extracts using different solvent and their effective doses. IRJP. 2012; 3(12):54-58.
40. Yavuz Simsek,Onder Celik,Abdullah Karaer,Ercan Yılmaz,Mehmet Gul,Elif Oze rol. Elevated cardiac oxidative stress in newborn rats from mothers treated with

atosiban. Archives of Gynecology and Obstetrics. 2012 March; 285(3):655–661.

41. Prashant B. Shamkuwar, Deepak P. Pawar, Shital S. Chauhan. Antidiarrhoeal activity of seeds of Syzygium cumini L. Journal of Pharmacy Research. 2012 September 17; 5(12):5537-5539.
42. Nikolaos Vrachnis, Fotodotis M. Malamas, Stavros Sifakis, Efthymios Deligeoroglou and Zoe Iliodromiti. The Oxytocin-Oxytocin Receptor System and Its Antagonists as, Tocolytic Agents**.** International Journal of Endocrinology. 2011; 1-8.
43. Christine K, Ratajczak, Justin C. Fay and Louis J. Muglia. Preventing preterm birth: the past limitations and new potential of animal models. Disease Models & Mechanisms. 2010 Dec; 3(1):407-414.
44. Omonkhelin J. Owolabia, Zulekhai A. Nworgua, Abiodun Falodunb, and Charles N. Nwakod. Evaluation of tocolytic activity of ethanol extract of the Stem bark of *Ficus capensis* thunb. Acta Poloniae Pharmaceutica - Drug Research. 2009 May; 66(3): 293-296.
45. Kumar, R. Ilavarasan, T. Jayachandran, M. Deecaraman, R. Mohan Kumar,P. Aravindan, N. Padmanabhan and M. R. V. Krishan. Anti-inflammatory activity of *Syzygium cumini* seed. Afr. J. Biotechnol. 2008 April 17; 7(8): 941.
46. P. Suresh Kumar, K. Soni and M. N. Saraf. The *In Vitro* tocolytic activity of *Sarcostemma brevistigma Wight*. Indian Journal of Pharmaceutical Sciences. 2006 April; 68 (2): 190-194.
47. Ochiogu, Izuchukwu Shedrack, Uchendu, Chukwuka Nwocha and Ihedioha, John Ikechukwu. A new and simple method of confirmatory detection of mating in albino rats (Rattus norvegicus). Animal Research International. 2006 Dec 24; 3(3): 527 – 530.
48. Ronald F. Lamont. The development and introduction of anti-oxytocic tocolytics. BJOG: an International Journal of Obstetrics and Gynaecology. 2003 April; 110(20):108–112.
49. Masatoshi Sakai, Kyoko Tanebe, Yasushi Sasaki, Kauo MommA, Satoshi Yoneda andShigeru Saito. Evaluation of the tocolytic effect of a selective

cyclooxygenase -2 inhibitor in a mouse model of lipopolysaccharide- induced preterm delivery. Molecular Human Reproduction. 2001; 7(6):595-602.

50. Abhishek Kumar Sah and Vinod K. Verma. *Syzygium cumini*: An overview. J. Chem. Pharm. Res. 2011; 3(3):108-113.
51. Shrikant Baslingappa Swami1, Nayan Singh J. Thakor, Meghatai M. Patil, Parag M. Haldankar. Jamun (*Syzygium cumini* (L.): A Review of Its Food and Medicinal Uses. Food and Nutrition Sciences. 2012; 3: 1100-1117.
52. http://www.cabi.org/isc/datasheet/52426
53. Kirtikar K.R, Basu. B. D. Indian medicinal plants. 2006; 2: 936-938.
54. Khandelwal K.R. Text book of practical pharmacognosy. 11th ed. Pune: Nirali prakashan. 2004; 149-155.
55. Trease G, Evans W. Textbook of Pharmacognosy, 13th edtn., BaillIere Tindal, London. 1989; 799 – 803.
56. Kokate C.K. Practical Pharmacognosy.4th edition.Nirali Prakashan; 1994:108-109.
57. Raghavendra H Lakshmanashetty, Vijayananda B Nagaraj, Madumathi G, Hiremuth and Vadalapudi Kumar. Invitro antioxidant activity of Vitex negundo L. Leaf extract. Chiang. Mai. J. Sci. 2010; 37(3): 489-497.
58. Md. Nur Alam, Nusrat Jahan Bristi, Md. Rafiquzzaman. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal. 2013 May; 21: 143–152.
59. V. Katalinic, D. Modun, I. Music, M. Boban. Gender differences in antioxidant capacity of rat tissues determined by 2, 2'-azinobis (3-ethyl benzothiazoline 6-sulfonate; ABTS) and ferric reducing anti-oxidant power (FRAP) Assays. Comparative Biochemistry and physiology. 2005; 140: 47-52.
60. Jerome M. Goldman, Ashley S. Murr, and Ralph L. The Rodent Estrous Cycle: Characterization of Vaginal Cytology and Its Utility in Toxicological Studies Birth Defects Research (Part B). 2007;80:84-97
61. H.B.Yesufu, P.U. Bassi, I.Z. Khaz, F.I Abdulrahaman, and G.T. Mohammed. Phytochemical screening and hepatoprotective properties of aqueous root bark extract of *Sarcocephalus latifolius* (smith) *Bruce* (African peach).Archives of Clinical Microbiology. 2010;1(2):1-5.

62. Lowry O H, Rosebrough N J, Farr L A, Randall A J. Protein measurement with the folin phenol reagent. Journal Biol Chem. 1951; 193:265–75.
63. Y. Sun, L. W. Oberly and Y. Li. A simple method for clinical assay of superoxide dismutase. Clinical Chemistry. 1988; 34(3):497-500.
64. Samuel Okwudili Onoja, Yusuf Ndukaku Omeh, Maxwell Ikechukwu Ezeja and Martins Ndubuisi Chukwu. Evaluation of the *in vitro* and *in vivo* antioxidant potentials of *Aframomum melegueta* methanolic seed extract. Journal of Tropical Medicine. 2014 may 15.
65. S. E. Atawodi. Evaluation of the hypoglycemic, hypolipedemic and antioxidant effects of methanolic extract of Ata-of a polyherbal tea (a-polyherbal) in alloxan –induced diabetic rats. Drug Invention Today. 2011; 3:270-276.
66. H. H. Draper and M. Hadley. Malondialdehyde determination as index of lipid peroxidation. Methods in enzymology. 1990; 186:421-431.
67. Indrani Chakraborty, Subhasish Ghosal, Nirmal Pradhan. *Jussiaea repens* (L) acts as an uterotonic agent - an *in vitro* study. Int. J. Pharm. Sci. Rev. Res.2014 July; 27(2): 368-372.
68. S. K. Kulkarni. Handbook of experimental pharmacology. 3rd revised and enlarged edition. 109-111.
69. Carolien Roos, Marc E. A. Spaanderman, Ewoud Schuit, Kitty W. M. Bloemenkamp, Antoinette C. Bolte. Effect of maintenance tocolysis with nifedipine in threatened preterm labor on perinatal outcomes. American Medical Association. 2013 January 2;309(1):41-47
70. Steven Thornton, Manu Vatish and Donna Slater. Oxytocin antagonists: clinical and scientific considerations. Experimental Physiology.2001; 86(2):297–302.
71. Narayanaswamy N, Balakrishnan KP. Evaluation of some medicinal plants for their antioxidant properties. Int J PharmTech Res. 2011 Jan; 3(1):381-385.
72. Larson RA. The antioxidants of higher plants. Phytochemistry. 1988 Dec 31; 27(4):969- 78.