COMBINED EFFECT OF STATIC MAGNETIC FIELD AND METFORMIN ON WEIGHT LOSS IN OVERWEIGHT RATS

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"COMBINED EFFECT OF STATIC MAGNETIC FIELD AND METFORMIN ON WEIGHT LOSS IN OVERWEIGHT RATS"** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, was carried out by **Mr. KARTHICK.T.** in the Department of Pharmacology, Nandha College of Pharmacy, Erode-52 for the partial fulfillment for the degree of **MASTER OF PHARMACY** in Pharmacology under the supervision of **Dr.S.SENGOTTUVELU, M.Pharm., Ph.D.,** Professor, Department of Pharmacology, Nandha College of Pharmacy, Erode.

The work is original and has not been previously formed the basis for the award of any other Degree, Diploma, Associateship, Fellowship or any other similar title and the dissertation represent entirely an independent work on the part of the candidate.

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This work is original and has not been submitted in part or full for the award of any other degree or diploma of this or any other university.

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The work presented in this thesis entitled, **"COMBINED EFFECT OF STATIC MAGNETIC FIELD AND METFORMIN ON WEIGHT LOSS IN OVERWEIGHT RATS"** was carried out by me in the Department of Pharmacology, under the direct supervision of **Prof. Dr. S. Sengottuvelu, M. Pharm., Ph.D.,** Head, Department of Pharmacology, Nandha College of Pharmacy, Erode-52.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of this or any other university.

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CONTENTS

ABBREVIATIONS

Abbreviation	Expansion
ALP	Alkaline Phosphatase
AMPK	Adenosine Monophosphate activated Kinase
ANOVA	Analysis of Variance
AO	Abdominal Obesity
BMI	Body Mass Index
CO	Combined Obesity
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
FDA	Food and Drug Administration
FFA	Free Fatty Acid
GLP-1	Glucagon Like Peptide-1
GMF	Geomagnetic Field
GO	Generalized Obesity
HDL	High Density Lipoprotein

HFD	High Fat Diet
IAEC	Institutional Animal Ethics Committee
IAO	Isolated Abdominal Obesity
IGO	Isolated Generalized Obesity
LDL	Low Density Lipoprotein
NAFLD	Non Alcoholic Fatty Liver Disease
NHANES III	The Third National Health and Nutrition Examination Survey
NPY	Neuropeptide Y
OECD	Organization for Economic Co-operation and Development
PEMF	Pulsed Electromagnetic Field
POMC	Proprio Melanocortin
RF-EMF	Radio Frequency-Electromagnetic Field
SD	Standard Diet
SGOT	Serum Glutamate Oxaloacetate Transaminase

SGPT	Serum Glutamate Pyruvate Transaminase
SMF	Static Magnetic Field
TC	Total Cholesterol
TG	Triglycerides
TZDS	Thiazolidinediones
UKPDS	United Kingdom Prospective Diabetes Study
VLDL	Very Low Density Lipoprotein
WC	Waist Circumference
WHO	World Health Organization

INTRODUCTION

OBESITY

Overweight and Obesity is measured by Body Mass Index (BMI) through calculated as weight divided by height squared (Sivakumar *et al*., 2016).

Definitions

Overweight was defined as a BMI ≥23 kg/m^2 but <25 kg/m^2 for both genders (based on the World Health Organization Asia Pacific Guidelines) with or without Abdominal Obesity (AO). Generalized Obesity (GO) was defined as a BMI ≥ 25 kg/m^2 for both genders (based on the World Health Organization Asia Pacific Guidelines) with or without AO. AO was defined as a Waist Circumference (WC) ≥ 90 cm for men and ≥ 80 cm for women with or without GO. Isolated Generalized Obesity (IGO) was defined as a BMI ≥ 25 kg/m^2 with waist circumference of < 90 cm in men and < 80 cm in women. Isolated Abdominal Obesity (IAO) was defined as a WC of ≥ 90 cm in men or ≥ 80 cm in women with a BMI< 25 kg/m^2. Combined Obesity (CO): Individuals with both GO and AO. Non obese subjects: Individuals without either GO or AO (Pradeepa *et al*., 2015).

Classification

Based on degree of obesity they are classified into three types as follows.

Class I Obesity - BMI with 30 to 34.9 kg/m^2

Class II Obesity - BMI with 35 to 39.9 kg/m^2

Class III Obesity - BMI with more than 40 kg/m^2 (Sunyer *et al*., 2002).

Over the past 25 years prevalence of overweight and obesity has been increase worldwide concern of alarming proportion. According to World Health Organization (WHO) 2015, it shows overweight and obese people population were occupied by one third of world portion. Among the global hazards top 10 countries which contain more number of obese people, India is just behind United States and China. In India the occurrence of obesity were high in Tamilnadu (Sivakumar *et al*., 2016, Pradeepa *et al*., 2015).

At present, half or more individuals is now identified as overweight or obese were belongs to the countries involved in Organization for Economic Co-operation and Developement (OECD, Rukhsana *et al*., 2012). More than 90% of overweight or obese having type 2 diabetes in US (Lloret-Linares *et al*., 2008). Every year 3 lakhs adults were died due to these complications. Obesity and diabetes are the leading disorders which cause major fatal complications including hypertension, stroke, heart disease, high cholesterol, osteoporosis, Alzheimer's disease and certain types of cancers (Song *et al*., 2014). The projected diabetes could be raise from 171 million in 2000 to 366 million in 2030 including developed and developing countries (Modak *et al*., 2011).

Obesity is a common health disorder of carbohydrate and lipid metabolism due to multiple factor contributing to increased risk of type 2 diabetes, blood pressure, cardio vascular disorder, non alcoholic fatty liver metabolic disorder, stroke and certain cancer is the major cause for morbidity and mortality which could be associated with obesity. Since the etiology of obesity is multifactorial, the sedentary life styles, lack of physical exercise, particularly consumption of energy rich diets are play an important role for its development (Sudhakara *et al*., 2016). Individual's body weight increase with above 20% ideal weight shows adverse clinical consequences of obesity (Camila *et al*., 2006, Von Diemen *et al*., 2006). Thus currently obesity is a major health problem. Therefore it is important to maintain healthy life of obese person by the prevention of obesity.

Strategies involved in management of weight reduction in obese people, which includes diet control, exercise, pharmacological treatment, behavioural therapy and bariatric surgery or its combination with one another. Exercise, behavioural therapy and diet control were the first line treatment for overweight and obesity. Even safest method of management of obesity these are difficult to maintain for long time. Pharmacological treatment on reducing body weight may involves either by decreasing appetite or increase satiety, and also through reducing absorption of nutrient or enhance the utilization of energy. Though medications are available but these are having poor outcomes on long term weight management and are accompanied with multiple adverse effects. Bariatric surgery, generally used for obese individuals with BMI nearly or greater than 40 kg/m^2. This is effective for sustaining

the weight loss but often it may leads to complications such as increased mortality (Sivakumar *et al*., 2016).

The year 2013 could be the landmark for the field of obesity, because June 2013 American Medical Association recognizes obesity as a disease. With respect to future point of view, obesity is set to be the world's main cause for morbidity and mortality on 21st century (Parveen and Uma, 2015). Thus, there is an essential need of alternate therapy which provides moderate weight loss in overweight or obese with an effective and safety of obesity for reducing the fatal complications.

DISEASE ASSOCIATED WITH OBESITY (Sunyer *et al*., 2002)

- Insulin resistance / hyperinsulinemia
- Type 2 diabetes
- Hypertension
- Dyslipidemia
- Coronary heart disease
- Gallbladder disease
- Cancer (prostate, endometrial, uterine, cervical, ovarian, colon, kidney, gallbladder, and postmenopausal breast)
- Premature death
- Psychological distress
- Osteoarthritis
- Stroke
- Asthma
- Sleep apnoea
- Complications of pregnancy
- Menstrual irregularities
- Hirsutism
- Increases surgical Risk

Insulin Resistance / Hyperinsulinemia

A decrease in sensitivity to insulin can occur as consequence of obesity. Impact of obesity is independent of genetic factor. An obese individual has higher fasting insulin levels as compared with non-obese member which shows that lower

insulin sensitivity on 75 gm oral glucose tolerance test. Once insulin resistance and hyperinsulinemia is occurred, it initiates metabolic changes to cause diabetes, hypertension, heart disease and dyslipidemia. BMI is inversely proportional to insulin sensitivity.

Type 2 Diabetes

The risk of type 2 diabetes increases with increasing BMI. Weight gain is a significant determining risk of diabetes, selectively individuals with higher baseline BMI. In AO, waist to hip ratio is a strong predictor of diabetes than BMI alone. Subjects with low waist to hip ratio have decreased chance of developing diabetes than highest BMI. The risk of developing diabetes was 30 times higher than with increased BMI. Hence, type 2 diabetes is proportionally correlated with BMI.

Hypertension

The relative risk of developing hypertension also increases steeply with increasing BMI. The relative risk on BMI of 32 kg/m^2 or greater has been 4.8. In addition to BMI, weight gain also significantly increased risk of hypertension due to inadequate blood supply to all parts of the body and reduction in body weight could reduce such risk. A person with BMI of 30 kg/m^2 or higher were twice as likely to have hypertension as compared with normal individuals. The Third National Health and Nutrition Examination Survey (NHANES III) data also prove that obesity enhances risk of hypertension. There are multiple mechanisms were involved in the development of hypertension in obese people: increased renal absorption of sodium and water, activation of sympathetic nervous system, alteration in Na^+/H^+ - ATPase activity.

Dyslipidemia

Increased hepatic glucose output is not only the consequences of increasing Free fatty acid (FFA) levels in obesity. Higher FFAs also influence the lipid metabolism by enhancing the production of Very Low Density Lipoprotein (VLDL) by the liver, decreasing High Density Lipoprotein (HDL) levels and also promoting the production of most small, dense Low Density Lipoprotein (LDL) particles. These alterations occur in lipoprotein profile are associated with increased risk of congestive heart disease. The impact of obesity on lipid metabolism was higher as compared with

non obese subjects. In addition to that increased synthesis of triglycerides was also occurred in obese individuals.

Congestive Heart Disease

A relationship between obesity and Congestive Heart Disease (CHD) mortality was explained in the Nurses' Health Study. The relative risk of developing CHD death increased significantly with increasing BMI. Obese individuals with BMI of 29.0 to 31.9 kg/m^2 and 32.0 kg/m^2 or higher were at 4.6 and 5.8 times greater risk, correspondingly than those with BMI under 22.0 kg/m^2. However, the waist-to-hip ratio was strongly projecting the CHD mortality. Obese people with increased waist-to-hip ratio had a increasing relative risk of CHD death of 8.7 compared with those in the lowest waist to hip ratio.

Gall Bladder Disease

An independent relationship between obesity and gall bladder disease was shown by atherosclerosis risk in communities study. The risk of gallbladder disease was increased with increasing BMI those with greater than 25 kg/m^2 as well as influenced by increased waist to hip ratio. Gall bladder diseases were generally occurring higher in obese women than male.

Cancer

The relative risk of developing cancer also increases steeply with increasing BMI. Mortality rates of cancer was highest among than those who were overweight. The major impact of obesity on cancer was estimated by utilizing the prospective study. From this study mortality ratios for colorectal and prostate cancer in men and endometrial, uterine, cervical, ovarian, gallbladder, and breast cancer in women were observed. Cancer death rate of BMI who has more than 32 kg/m^2 were twice that of those BMI less than 19 kg/m^2. The increased death rate was predominantly because of higher mortality caused by colorectal, breast and endometrial cancers.

The other minor disorders caused by overweight and obesity are Premature death, Psychological distress, Osteoarthritis, Stroke, Asthma, Sleep apnoea, Complications of pregnancy, Menstrual irregularities, Hirsutism and Increases surgical risk.

DRUGS FOR OBESITY

Number of drugs has been approved for the treatment of obesity on past 2 decade. Currently, there are only few weight-loss drugs with a favourable side effect profile available. However, most of them have been withdrawn from the market because of their severe adverse effects, leaving only Orlistat, a lipase inhibitor. Drugs like Rimonabant have been promising in the recent past. However, severe side effects lead to their withdrawal from the market. Presently, GLP-1 (Glucagon Like Peptide-1) analogues such as Liraglutide are being promoted as a new strategy to loose weight without major side effects however at high costs (Seifarth *et al*., 2013).

Orlistat

Orlistat was approved by the Food and Drug Administration (FDA) in 1998 and is the currently available drug for long term therapy of obesity. Orlistat is an effective and reversible gastric and pancreatic lipase inhibitor preventing dietary fat absorption by approximately 30% , has been used for around two decades and proved to be useful in improving weight loss and weight maintenance. The beneficial effect on body weight is significant with improve number of cardio metabolic parameters, including WC, blood glucose levels, blood pressure and lipid profiles. Treatment with Orlistat was associated with improving cardiovascular risk after adjustment for weight loss. In addition to that Orlistat shows significant decrease in total cholesterol and also reducing the incidence of diabetics.

Although Orlistat has approved by FDA, studies showed it causes undesirable adverse effects, and severe health risks. The frequently experienced side effects are gastrointestinal and include diarrhea, flatulence, bloating, abdominal pain, dyspepsia and recently severe liver injury also has been reported. Current study also stated that the modest efficacy, undesirable adverse effects, and severe health risks combine to highlight the deficiencies of Orlistat and underscore the pressing need for alternate therapy for anti-obesity drug options (Sivakumar *et al*., 2016, Kang *et al*., 2012)

GEOMAGNETIC FIELD

As one of the physical factors of environment, like air pressure and temperature, Geomagnetic Field (GMF) 30-70 μT (micro Tesla) plays an important role on living and evolution for organism on earth (Lohmann, 2010 and ICNIRP

Guidelines, 2009). All living being are sensitive to magnetic field. This can be possible by two mechanism due to presence of magnetic nanoparticle like Fe_3O_4 (magnetite) and also influenced by chemical reaction occur in living organism.

Living things such as human beings, animals, plants and micro organisms which are born and grow under the presence of several physical fields including geomagnetic fields. In some way, living beings are affected by the physical characteristics of the GMF, because of their long time of relationship with GMF due to their sensitivity. The first experimental evidence of living beings can be influenced by the GMF was the discovery of magnetotactic bacteria in 1963 by Salvatore Bellini and later by Blakemore in 1975. GMF is not fully dipolar. But some areas on the Earth's surfaces were expected from a dipolar one (Belova and Acosta-Avalos, 2015).

The idea of electromagnetic fields in medical research was firstly given by Kolin and later Korchevskii and Marochnik (Kolin, 1936).

BIOEFFECTS OF MAGNETIC FIELD

Bioelectromagnetics is the study of interaction between non-ionizing electromagnetic field and biological system. In recent year several studies have suggested, possible bioeffect of magnetic fields on rodent. Application of magnetic field to treat disorder in human is one of the most promising complementary / alternative and conventional medicines. Since it provide non invasive, easy and safety. In fact, ancient China, Japan, and Europe use natural magnetic material to treat disease such as headache, oedema, and rheumatism, but also cure paralysis. The mechanism of treating disorders may be mediated by stimulation of increased blood due to alteration of calcium ion in muscle.

Past few decades hundreds of experiments have been carried out on animal to asses biological effect of exposure on different types of magnetic field such as permanent/ Static magnetic field (SMF), low frequency sine waves, Pulsed frequency electromagnetic field (PEMF), Radio frequency electromagnetic field (RF-EMF), transcranial electric/magnetic stimulation. In this, SMF of various types were included in this studies due to most of the work performed on these types of magnetic field while, others are excluded from this studies due to deficiency of data. Results

obtained from these studies shows most has beneficial effect and some has negative effect.

Under these conditions most experimental results carried over year for bioeffect of different magnetic field on rodents. Because rodents are similar in genetic resemble with human as well as some advantage in reproducibility and also well developed disorder model with low cost. To the best knowledge experiment on effect of various magnetic fields were rapidly increased after the Second World War (Markov, 2007).

Numerous publications discussed the possibilities of exogenous magnetic to initiate beneficial effect on various biological processes, which are importance for healing of different injuries and pathologies. Their results are discussed in table 3.1 and 3.2. According to their frequency, electromagnetic fields are classified into four types by European commission of non ionization radiation, which are mentioned in table 1. They are SMF, low frequency magnetic fields, intermediate frequency magnetic fields, RF-EMF (Lewczuk *et al*., 2014).

Table 1: Types of Magnetic Field and their frequency

S.No	TYPES OF MAGNETIC FIELD	FREQUENCY RANGE
1.	Static magnetic field	0HZ
2.	Low frequency magnetic field	0-300HZ
3.	Intermediate frequency magnetic field	300HZ-100KHZ
4.	Radio frequency electromagnetic field	100KHZ-300GHZ

Corresponding to therapeutic utility SMF is mainly discussed due to numbers of availability of results and focused on them to show integrated concept on biological system. Low frequency sine wave and transcranial magnetic stimulation are excluded due to data deficiency. Here we give brief introduction for static magnetic field on their bioeffect and more detailed sub types explained individually on their following topic.

Static Magnetic Field:

SMF can be created by various permanent magnets as well as by passing direct current through a coil (Markov, 2007). It can be characterized by frequency of (zero) 0 Hz and not varying with time during production of magnetic field (Elferchichi *et al*., 2011). Bioeffect of SMF on animal model since 1948 (Yu and Shang, 2014). Due to development of animal model for various disorders, examination on bioeffect of magnetic field rapidly increased.

SMF have different therapeutic effect in animals including anti inflammatory, pain relive, antibacterial effect, but SMF also have therapeutic effect in different system of rodent has been examined , which are seen in table 3 (Yadollahpour and Rashidi, 2014). The application of SMF has increased for treating some specific medical problems during last three decades. SMF can be easily penetrated into biological tissue through several physical mechanisms. So that SMF are widely used throughout the world (Lahbib *et al*., 2010). Several clinical studies are described in table 3.1 and 3.2. Due to diverse response is of SMF on biosystem of living organism, it can be classified as various intensity ranges are mentioned in table 2.

The application of permanent magnets for treating specific medical problems such as arthritis, chronic pain syndromes, wound healing, insomnia, headache and others has steadily increased during the last decade (Colbert *et al*., 2009).

Table 2: Types of SMF and its intensities (Elferchichi *et al*., 2010)

S.NO	SMF	INTENSITY RANGE (mT-milli Tesla, T-Tesla)
1.	Weak intensity	Less than 1mT
2.	Moderate intensity	1mT – 1T
3.	Strong intensity	1T – 5T
4.	Ultra strong intensity	More than 5T

Table 3.1: Bioeffects of Static Magnetic Field

TYPES OF SMF	BIOLOGICAL EFFECT	EXPOSURE PARAMETERS	REFERENCE
Weak Intensity SMF	Decrease volume of tumour and increase longevity	Mice - 42µT, 1h a day for 12 days	Novikov *et al*., 2009
	Influence of action on pineal gland	Rats – 80µT, 1h	Lerchl *et al*., 1991
Moderate Intensity SMF	Cause temporary diabetic like state	Rats – 1mT/10mT, 1h a day for 10 days	Gorczynska and Wegrzynowicz , 1991
	Changes occur in haematological and biochemical parameter	Rats – 128mT, 1h a day for 5/30 days	Amara *et al*., 2006[b]
	Alteration in glucose and lipid metabolism	Rats – 128mT, 1h a day for 5/15 days	Lahbib *et al*., 2010
	Changes in glucose and lipid parameters of Serum and skeletal muscle	Rats – 128mT, 1h a day for 15 days	Elferchichi *et al*., 2010
	Anti oxidant activity	Rats – 128mT, 1h a day for 30 days	Ghodbane *et al*., 2011[b]
	Biochemical parameters change due to up regulation of norepinephrine concentration	Rats - 128mT, 1h a day for 5 days	Abdelmelek *et al*., 2006
	Increasing immune function	Mice - 200-400mT, until death	Yang *et al*., 2009
	Changes occur in behavioural response	Rats - 128mT, 1h a day for 5 days	Ammari *et al*., 2008
	Alteration of inorganic ion content in spinal cord, medulla	Same as above	Miryam *et al*., 2010
	No influence on spermatogenesis	Rats - 128mT, 1h a day for 30 days	Amara *et al*., 2006[a]
	Antidiabetic effect	Diabetic mice - 2.8 to 476.7mT, 30 min a day for 12 weeks	Laszlo and Porszasz *et al*., 2011
	Produce Analgesic effect due to alteration in blood circulation	Rats - 200 mT for 4 weeks	Kanai and Taniguchi *et al*., 2012
	Anti oxidative function on blood	Rats - 128 mT, 1h a day for 5 days	Ghodbane S *et al*., 2011[a]
	Alters blood pressure associated with Nicardipine	Rats - 180 mT, 1h a day for 8/6 weeks	Okano and Ohkubo, 2005, 2006

Weak Intensity SMF

SMF produced is less than 1mT known as weak intensity SMF. This intensity magnetic field also expressed as μT (micro Tesla) range. Normal metabolisms in living organism are occurred, which are frequently exposed to this intensity range because of geomagnetic field 50μT (ICNIRP, 2009). Apart from that only limited numbers of studies are carried out by using weak intensity SMF.

Moderate Intensity SMF

Moderate intensity SMF is characterized by permanent magnet which produces the magnetic field limit of 1mT-1T. Normal whole human body safety exposure limit were set up to 400mT of moderate intensity SMF (ICNIRP, 2009). Number of the studies were conducted between these exposure limit cause biological effects on most of the system in rodents. According to researchers (Gorczynska and Wegrzynowicz 1991, Elferchichi *et al*., 2011, Laszlo *et al*., 2011, Amara *et al*., 2006, Elferchichi *et al*., 2010, Lahbib *et al*., 2010), experiment conducted by using magnetic field less than 200mT is suitable for metabolic alteration in living organism. In the past decade, there has been substantial and growing evidence that moderate intensity Static magnetic fields (SMF) ranging 1mT-1T (Rosen, 2010) can influence physiological processes.

Strong Intensity SMF

Strong intensity SMF is characterized by permanent magnet which produce magnetic field from 1T to 5T. During past few decades only limited number studies are carried on this type SMF to express their bioeffect on rodent.

Ultra Strong Intensity SMF

This type of SMF can be categorized based on magnetic field intensity more than 5T produced by permanent magnet. During past 30 years least number of studies is conducted on this type of SMF.

Table 3.2: Bioeffects of Static Magnetic Field

TYPES OF SMF	BIOLOGICAL EFFECT	EXPOSURE PARAMETERS	REFERENCE
Strong Intensity SMF	Decrease food and water consumption, decrease in body weight	Mice - 5T for 24h, 48h	Tsuji *et al*., 1996
	Alteration in pain response	Mice - 3T for 30 min	Laszlo and Gyires, 2009
	Changes occur in primary bone marrow cells	Mice - 1.4T for 1h	Bhatia, 1999
	No changes observed in treated and control group	Mice - 4.7T for 2 days	Okazaki *et al*., 2001
	Alteration in bone marrow	Mice - 3T, 4.7T for 3 days	Suzuki *et al*., 2001
	Activation of intracellular signalling transduction pathways	Rats- 1T for 1h	Prina-Mello *et al*., 2006
Ultra Strong Intensity SMF	Change in locomotors activity	Rats - 14.1T for 30/5 min	Houpt *et al*., 2007, 2011
	Behavioural changes	Rats - 14T for 30 min	Cason *et al*., 2006
	Changes blood flow	Rats - 8T for 5 min	Ichioka *et al*., 2003
	Changes behaviour response	Rats - 7T for 30 min	Houpt *et al*., 2003
	No changes observed	Rats - 9.4T for 10 weeks	High *et al*., 2000
	Effects on sciatic nerve	Rats - 8T for 1ms	Sekinz *et al*., 2006

ORAL HYPOGLYCAEMIC AGENT

Oral hypoglycaemic agent is used for reducing raised blood glucose in type 2 diabetic patient. Several types of oral hypoglycaemic agents are associated with increased body weight, including insulin secretagogues (sulphonylureas and meglitinides) and thiazolidinediones (TZDs). Two classes of oral hypoglycaemic agents have not been associated with weight gain: the biguanides (of which Metformin is now effective) and α-glucosidase inhibitors (Golay, 2008).

Metformin is one among the oldest oral hypoglycaemic medications not only reduces type 2 diabetes but also produces modest and durable weight loss effect

among the other pharmacological agents in diabetic patients and it has been also shows weight loss in obese non diabetic populations. Several randomized studies reported that Metformin has neutral effect on weight gain (Priscilla, 2007). It is frequently chosen for well tolerated and for not causing hypoglycaemia. Furthermore, Metformin is the only oral antidiabetic drug that was proven to reduce cardiovascular risk (Mitri and Hamdy, 2009). Studies on Metformin were reported that, it shows beneficial effects in correcting disturbances in lipid metabolism in individuals. Metformin is reported to have beneficial effect in weight reduction of obese diabetic patients and high fat diet induced overweight in wistar rats. It is also commonly prescribed for patients with polycystic ovary syndrome and its use resulted in weight reduction. Further use of Metformin is reported to show protective action against obesity associated Non-Alcoholic Fatty Liver Disease (NAFLD), hepatic steatosis, kidney and cardiovascular problems (Golay, 2008 & Sudhakara *et al*., 2016).

Metformin causes weight loss by decreasing food intake. Metformin primarily acts on the central nervous system to reduce appetite by attenuating hypothalamic Adenosine Monophosphate-activated Protein Kinase (AMPK) activity, which decreases Neuropeptide Y (NPY, Orexigenic) and increases Proprio Melano Cortin (POMC, Anorectic) expression. In addition to that Metformin has food lowering effects by increasing leptin and insulin sensitivity, improving GLP-1 levels, and affecting gut flora. It also reduces ectopic lipid depots (i.e. liver and skeletal muscle) through increased fat oxidation and decreased lipid synthesis, which may be regulated to some extent by circadian clock genes (Malin and Kashyap , 2014).

Recently studies also shown, it is involved in energy expenditure and body fat mass regulation and effectively controls weight gain, obesity and associated heart disease. In the United Kingdom Prospective Diabetes Study (UKPDS), Metformin did not cause change in body weight after 3 years of regular use (Mitri and Hamdy, 2009).

Based on literature and to compensate the SMF induced side effects, we aimed to evaluate the efficacy and safety of Metformin and SMF alone and the effect of combination therapy on High Fat Diet (HFD) induced overweight and compare them with standard anti-obesity i.e., Orlisat of both on lipid profiles and liver function parameters, blood glucose level, body weight, food & water intake, body temperature and locomotor activity of control and experimental rats.

REVIEW OF LITERATURE

A review of revealed literature is an fundamental aspect of scientific research. It involves the systematic detection, scrutiny and outline of written material that contains information on a research problem. It broadens the understanding and gain an insight of a wide conceptual content into which the problem fits.

Weak Intensity Static Magnetic Field:

- Novikov *et al*., 2009 have used an experimental model of carcinoma bearing mice to examine the effect of weak intensity SMF. Particularly weak intensity SMF exposed to normal animal shows no harmful effects on major organs and tissue. In addition, no pathological deviations were observed. Weak intensity SMF exposed on experimental model of carcinoma bearing mice cause degradation of tumor size initiated by autolytic enzyme and then cells are lysed. Simultaneously it activates antitumor immunity; particularly it stimulates the production of tumor necrosis factor this leads to elevate the level of local concentration of reactive oxygen species. There by producing anticancer activity. Finally the result showed that exposed mice had a decrease the incidence of tumor growth and an increase in longevity.

- Research by Lerchl *et al*., 1991 have suggested that application of 80μT SMF cause effect in mammalian pineal gland. Because pineal gland is sensitive to magnetic field of spatial orientation. Based on their finding the result suggested that artificial SMF cause change in pineal Indole metabolism. When a tissue exposed to altered magnetic field cause production of eddy current. An induced eddy current may affect nervous system.

Moderate Intensity Static Magnetic Field:

- Gorczynska and Wegrzynowicz, 1991 have researched the biological effect of SMF on rats exposed to 1mT and 10mT. The data showed that, it cause temporary diabetic like state due to hyper function of

adrenal, thyroid and pituitary glands and also pancrease. Hence, the release of insulin got decreased while glucagon content was increased. It seems that metabolism of glucose is similar to diabetics. It has been suggested that reduction of insulin release may be decrease in calcium efflux of islet cell caused by magnetic field. Further magnetic field creates hydrophobic property of cell membrane and this will affect the glucose transport across cell membrane.

- Elferchichi *et al*., 2010 concluded that there were significant difference in treated and control group after exposure to 128mT SMF using rat. This shows prediabetic like state, when metabolic alteration were induced by moderate intensity SMF could develop. Moreover, it is important to note that hyperglycemia due to conformational changes occur in insulin by magnetic field exposed animals. It will leads to reducing the binding capacity of insulin particularly at hepatocytes and results in hyperglycemia. Additionally, raised in blood glucose also caused by epinephrine.

- Study conducted by Laszlo *et al*., 2011 observed the decrease in blood glucose level on repeated exposure of moderate intensity SMF in diabetic mice. This indicating the promising application of SMF for future treatment subsequently this study shows improved diabetic wound healing rate. From the above studies both positive and negative effects in glucose metabolism influenced by SMF, because it was based on magnetic field strength.

- Amara *et al*., 2006[b] conducted an experiment on rat blood which is exposed to 128mT intensity. Sub acute exposure of SMF reduces body weight of animal and the same treatment will cause increase of white blood cells, red blood cells, platelets and haemoglobin concentration while hematocrit level unchanged. This could be probably due to hypoxia status. Whereas increased in blood glucose level observed in magnetic field exposed rat could be related to the structural and functional changes in pancrease. The results were shows that alteration

in haematological and biological parameter due to proliferation of blood cells and enzymes release in blood related to duration of exposure.

- Similar work has been carried out by Ghodbane *et al*., 2011[a] in addition with selenium were included in their study. Because magnetic field treatment reduces selenium in major organs like kidney, brain and muscle. Hence combine effect of selenium and SMF should increases the antioxidant activity minimize the oxidative stress which is induced by SMF in rat. The result proposed by means of examine the antioxidant materials like tochopherol, retinol, glutathione peroxidise.

- Lahbib *et al*., 2010 Elferchichi *et al*., 2010 observed the glucose and lipid parameters (glucose, cholesterol, phospholipids, triglycerides,) in serum and skeletal muscle, and also glycogen content in liver and muscles by using rat as an experimental model exposed to moderate intensity SMF of 128mT. This cause hyperglycaemia attributed due to increased release of hyperglycaemic hormone (glucagon) and/or inhibition of hypoglycaemic hormone (insulin). Whereas lipid metabolism also altered because of membrane integrity. The decrease of body weight might be due to reduction in body fluid and protein content including hormonal changes and relatively loss in liver weight were also observed. In tissues, SMF exposure showed significant alteration in enzyme activities. The data showed that SMF effects on glucose and lipid metabolism and in addition, the conducted investigation on rat to examine the effect of SMF on loss in body weight. Therefore moderate intensity SMF seems to have anti-obese effect.

- A study was carried out by Abdelmelek *et al*., 2006 on skeletal muscle of rats were induced by moderate intensity SMF for 5 days. The result showed that 128mT cause effects in regulation of norepinephrine concentration. This may affect increase in blood pressure but no changes were observed in growth rate. The basic mechanisms involved

in changing the norepinephrine concentration were caused by magnetic field of electro sensitive ion channels on plasma membrane.

- Yang *et al*., 2009 have used an experimental model of mice to examine the effects of moderate intensity SMF. Mechanism of antitumor activity may be mediated by enhancing the cell proliferation and decreasing the affected cell number significantly. The findings suggested that, life time was prolonged significantly in leukaemia infected mice by upregulating the function of immune system for certain period of time.

- Amara *et al*., 2006[a] studied the sub chronic effect of SMF exposure on testicular function by examine the sperm count, genital organ weight and sperm motility. Finally they concluded that there were no significant changes occur between control group and treated group on spermatogenesis in rat testes.

- Ammari *et al*., 2008 studied the relationship of rat behavioural response and moderate intensity SMF using 128mT fields 1h a day for 5 days, behavioural response were checked by an experimental model of learning abilities in morris water maze, emotional behavioural testing, elevated plus maze and open field. They found some effects of moderate intensity SMF on rats.

- Miryam *et al*., 2009 they perform similar exposure condition on the effect of moderate intensity SMF in medulla spinalis. The treatment has resulted in variation of ion concentration of medulla spinalis which shows Ca^{2+}, Fe^{+} concentration increased rapidly while no change in magnesium and copper. Moderate intensity SMF cause raised blood circulation and bone mineral density which is mediated by absorption of Ca^{2+} ion in oesteoblast of bone, this leads to increasing locomotors activity. Therefore moderate intensity SMF has analgesic action Kanai & Taniguchi studied the influence on experimental rat models. These authors suggest that exposure to SMF may induce cholinergic nerves

by reducing cholinesterase activity and that results in release of acetylcholine induces elevated blood flow.

- A study by Okano and Ohkubo 2005, 2006 investigated the influence of combined effect of moderate intensity SMF upto 180mT and Nicardipine (Ca^{2+} channel blocker). The results found that they cause significant change in hypertension which can be mediated by clogging Ca^{2+} flux and up regulation of nitrous oxide metabolites. In case of animal exposed to magnetic field alone were significantly reduced or delayed the hypertensive development. But combined effect of SMF and Nicaripine shows increased reduction of blood pressure on rats. SMF induces raised NO level in plasma with Nicardipine. The reasons beyond these effects were due to elevation of plasma NO synergistically with Nicardipine.

- Politanski *et al*., 2010 conducted the experiments on mouse to investigate the effects of noise, when mice exposed to 5mT, 2h a day for 2 weeks. The result shows that limited application in hearing.

Strong Intensity Static Magnetic Field:

- Tsuji *et al*., 1996 researched the mice behaviour when exposed to 5T for 2 days. They examine weights of major organs in body like kidney, brain, heart, liver, spleen and lungs. However measuring the weights of major organ after magnetic field exposure shows no significant difference on it. But body weight, blood glucose and blood urea nitrogen were altered significantly due to fluid shift and also affect drinking and eating behaviour due to changes in circadian rhythm by exposed SMF. The result showed that SMF affect mice behaviour by decrease eating and drinking and lower body weight due to fluid loss cause increase blood sugar level and blood urea nitrogen after 2 days. Findings of this study show 5T cause direct effect on central nervous system.

- Prina-Mello *et al*., 2006 have conducted a study for investigating the effectiveness of strong intensity SMF of 1T cause activation of intracellular signalling transduction pathways by means of Extracellular Regulated Kinase and C-Jun N Terminal Kinase on primary cortical neurons were determined. Laszlo and Gyires, 2009 studied that strong intensity SMF cause alteration in pain response.

- Bhatia, 1999 assessed the strong intensity SMF in mice on 1.4T cause some changes in membrane and receptors of primary bone marrow cells. In this study temperature dependent effect of magnetic field has been observed. Finding shows phagocytic action on bone marrow cell mediated by action of body temperature of animal not on sex dependent manner.

- Study by Suzuki *et al*., 2001 investigated that strong intensity SMF on mice of 3T, 4.7T for 3 days were influence the alteration in bone marrow cells. Because this study is evaluated for wide spread application of magnetic resonance imaging, nuclear magnetic resonance, electron spin resonance by human beings.

- Okazaki *et al*., 2001 reported that there were no significant difference observed in treated and control group when mice exposed to 4.7T for 48h. Simultaneously no adverse effects were investigated on fetal development.

Ultra Strong Intensity Static Magnetic Field:

- Houpt *et al*., 2007 and 2011 have found the relationship of SMF and behaviour of rat using 14.1T, 7T for 30 or 5 min. The behavioural change was observed by analysing locomotors activity, food and water intake. Decrease in locomotors activity and rearing cause reduction of drinking may due to lower mobility. Finally the concluded that high strength magnetic field cause reduction of food intake. The result showed that ultra strong intensity SMF exposed is directly proportional to altering the behavioural response on experimental rat models.

- Cason *et al*., 2006 studied the action of rat for 14T SMF exposed for 30 min shows behavioural changes. Ichioka *et al*., 2003 showed that decrease in skin temperature were measured. This may due to influence in water evaporation when rat exposed to 8T for 5 min. Sekino *et al*., 2006 have performed an experiment for describe the effect of SMF on rats of 8T for 1ms. The data shows that ultra strong intensity SMF cause effects on sciatic nerve potential.

- High *et al*., 2000 have conducted a study for explaining the effects of ultra strong intensity SMF on rat of 9.4T for 10 weeks observed no significant changes on spatial memory test, body weight, food and water consumption, gross pathological findings, heart rates, terminal hematologic, blood biochemical and urine parameters, feeding ratio and major organ weights. From the past studies we concluded that ultra strong intensity SMF of most of them cause influence in biological effect, while some shows no change in bioeffects.

AIM AND OBJECTIVE

Today large number of population suffers from overweight and obesity disorder. It has been increasing due to change in life style of community on developing and developed countries. An increase in body weight is complex processes that results from sedentary life style, lack of physical exercise, particularly consumption of energy rich diets are play an important role for its development.

The primary treatments used for protecting the body weight by diet control and exercise. But this is too hard to maintain and also weight regain could be possible. Though medication like Orlistat is available but these are having poor outcomes on long term weight management and are accompanied with various adverse effects. Further, progress in the study of the behavioural and biological manifestations of reducing body weight, there is no satisfactory drugs to use in clinical therapy.

Preventive measures are mostly taken from alternative sources. An alternative method of treatment is getting more importance in overweight and obesity because the modern medicine does not find curative treatments.

Currently there are various chemical and physical methods are available individually to the treatment of obesity but these methods can effective after long term treatment. In this research work we tried to use combination of static magnetic field with Metformin in experimental animal model as more effective and enhanced weight loss on subacute treatment. This can be obtained by synergism and it's also safe as the strength of glucose and lipid metabolism can be reduced without compromising on decreased body weight.

The present study will help to improve the biophysical knowledge among the researchers on weight loss with lesser side effects, which are affordable, non invasive and more effective with safety in the management of overweight and obesity.

SCOPE OF WORK

Scope of this Research Study

The scope of this research work has been explained under these following headings:-

Reason for targeting this particular disease - overweight and obesity

Overweight and Obesity is a health condition in which the body weight is persistently elevated (high BMI). Persistent increase in body weight is one of the risk factors for type 2 diabetes, hypertension, cardiovascular disorders, dyslipidemia and certain types of cancers. Even moderate elevation of body weight leads to shortened life expectancy.

The World health statistics 2013 report that one-third of world's population was overweight and obese. According to WHO 2015, shows that there are at least 2.3 billion peoples will be overweight and more than 700 million people worldwide who have elevated BMI (obese). In the developed countries like United States, shows about 13% of people have elevated BMI of world's obese population, while India and China together accounted for 15% of world's obese population. The WHO rates overweight and obese as one of the most important causes of premature death worldwide and the problem is growing.

Obesity is a risk factor for diabetes and the single most important risk factor for type 2 diabetes. It causes about 90% of obese individuals where increases the risk of type 2 diabetes. In addition to type 2 diabetes, complications of overweight and obesity includes dyslipidaemia, NAFLD, certain types of cancer, hypertension, coronary heart diseases and stroke.

The condition is often associated with significant morbidity and mortality. Because of this, it is one of the most significantly under-diagnosed and undertreated medical conditions.

Reason for choosing magnetic field and oral hypoglycemic agent for overweight and obesity

Overweight and obesity is one of the most pandemic disease states in the world today, and chronic, life-long drug therapy is often required for the maintenance of acceptable body weight levels. Diet and physical exercise are a first line treatment for overweight and obesity, as they reduce calorie intake and increase utilization of calories, thereby lowering elevated body weight, without exhibiting serious-adverse effects. But their maintenance in reducing body weight is difficult and weight regain is possible once discontinue the treatment.

Permanent/SMF, PEMF, low frequency electromagnetic field and transcranial magnetic field are used widely, not only for bone disorders, but also for the treatment of disease states such as parkinson's disease, Alzheimer's disease and hemiplegia. They are effective alternative medications with generally safe use.

Development of SMF of moderate intensity considered beneficial in terms of lowering the elevated body weight, with the aim of improving patient compliance and subsequent therapeutic outcomes.

Oral hypoglycemic agent of certain types shows significant lowering of elevated body weight on their treatment. In addition to that other types were not shows significant alteration in body weight were reported.

Reason for choosing a moderate intensity SMF and Metformin

Moderate intensity comes under second types of SMF which exhibits the highest degree of reducing body weight compared to other SMF, while strong intensity have an less action on decreasing body weight but low intensity and ultra high intensity being completely have no action on body weight.

Moderate intensity is a selective SMF for the treatment of mild and moderate elevated body weight and also for long term management. Moderate intensity SMF is one such magnetic field that is used for the treatment of several disorders. It may be used alone or in combination with western medicine. The magnetic field is generally exposed alone or combination therapy for enhancing its action. These causes decrease the cost of treatment and make patient convenience with highly effective and safety.

Therefore, moderate intensity SMF is an ideal magnetic field candidate for magnetic field treatment.

Metformin is a well tolerated oral hypoglycemic agent which is used for first line treatment of Type 2 diabetes and not cause severe hypoglycemic on overdose. Past decade's Metformin shows significant weight loss in non diabetic obese person were reported. Hence we are aimed to doing research on combined effect of SMF and Metformin for examine its synergetic action of decreasing body weight in HFD induced overweight rats.

PLAN OF WORK

This research works was carried out as under:-

- Exhaustive literature survey
- Computational modelling and simulation
- Selection and maintenance of animal
- Ethical consideration
- Experimental induction of overweight
- Design of Static Magnetic Field device
- Static Magnetic Field and Metformin treatment
- Pharmacological evaluation

  Anti-obesity activity

  - ✓ Determination of blood glucose level (mg/dl)
  - ✓ Parameter measured
    - Body weight(gm)
    - Food intake (gm)
    - Water intake (ml)
    - Body temperature (◦C)
  - ✓ Determination of Locomotor activity
  - ✓ Estimation of biochemical parameters:
    - SGOT, SGPT, ALP (IU/l)
    - TC, TG, LDL, VLDL, HDL (mg/dl)

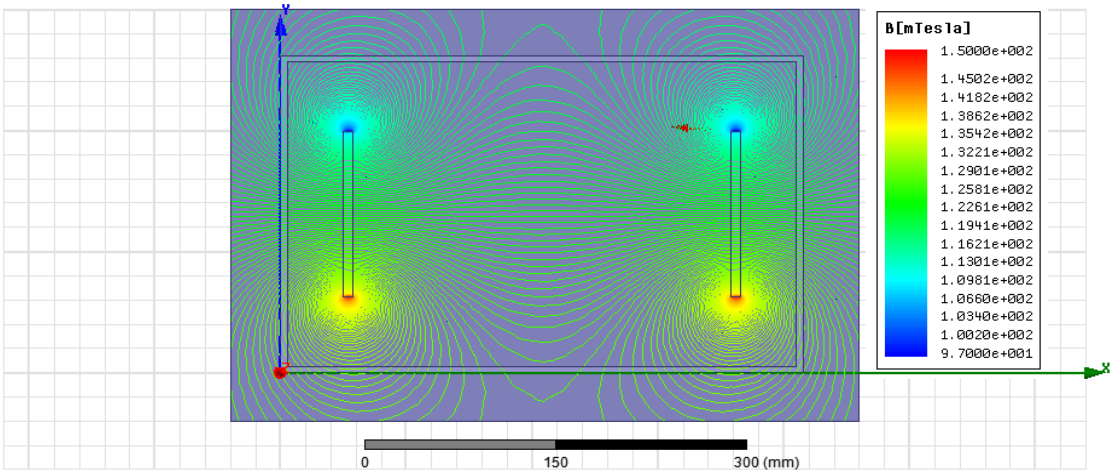
- Statistical analysis

MATERIALS AND METHODS

Computational modelling and simulation

Computational modelling and simulation of moderate intensity SMF exposure system prior to actual practical design and development process is very essential for designing flexibility and efficiency. 3-D Ansys Maxwell engineering software, 2014 version was used for modelling and simulation of SMF exposure device. This has been utilised to computationally model and simulate permanent magnetic field exposure system for most effective uniform distribution of magnetic flux density. Our model has utilised 2D application mode which present in this software.

Figure 1: Computational modelling and simulation of moderate intensity SMF



Permanent magnet plate made of alnico material could be modelled and designed for investigation of rectangular shape with dimension of 137mm length, 80mm width, and 8mm thickness. Two rectangular magnetic plates of previously mentioned parameters to be placed between gap of 300mm on rectangular rat cage of dimension 415mm length, 262mm width and 165mm height. By establishing the geometric model, setting boundary conditions and obtaining numerical solutions. We can find that the magnetic flux density on rat behavioural palne (XY plane) was uniform and the peak magnetic field intensity of average about 128mT was observed. The reason for selecting this particular moderate intensity SMF was that it had been reported to be effective in weight reducing on whole body which were performed by our study group over a short period of time.

Selection and maintenance of animals

Totally thirty male *Wistar albino rats* of 60 days age weighing between 150 - 180 g were used for this study. The animals were checked for disease, only healthy rodent is accepted for the experiments. The animals were obtained from the animal house of Nandha College of Pharmacy, Erode-52, Tamil Nadu, India. Animals were randomly grouped in polypropylene cages with paddy husk as bedding. A temperature of 25±2 ˚C and relative humidity of 30-70% was maintained. A 12 hours light and 12 hours dark cycle were strictly followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (**Reg No: 688/PO/Re/S/02/CPCSEA**) of Nandha College of Pharmacy, Erode-52 and were accordance with the guidelines of the IAEC.

Ethical consideration

The study was conducted after obtaining the approval from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC), proposal number **NCP/IAEC/2016-2017-01**.

Experimental induction of overweight

The acclimatized animals were checked for initial body weight and then animals subjected to increasing body weight were fed with HFD composition of 60% Kcal fat, 20% Kcal carbohydrate and 20% Kcal protein and rest of animals were fed with Standard Diet (SD). After 14 days rats which consuming HFD with body weight of 250 gm or above to be included in the study. HFD induces increasing body weight in laboratory animals.

Composition of High Fat Diet

Casein (20%) - 200 gm; Starch - 425 gm; Sucrose - 100 gm; Cellulose - 50 gm; Ground Nut oil - 175 gm; Mineral mix - 35 gm; Vitamin Mix - 10 gm; L-Cystine - 3 gm; Choline - 2 gm (Sivakumar *et al*., 2016).

Design of Static Magnetic Field device:

The SMF exposure device (Length: 137mm; Height: 80mm; Width: 8mm) was composed of a pair of rectangular magnetic plates made by alnico material, internally placed parallel to each other and at the air gap of 30 cm from two sides of standard rat cage (Length: 415 mm, Height: 165 mm, Width: 262 mm). The mean flux density at the centre of a cage was 128 mT (range = 125 - 132mT), respectively.

Static Magnetic Field treatment:

After confirmation of increased body weight the overweight rats were divided into different groups as mentioned below.

Grouping of animals:

Group I : Normal control (SD + 0.5% NaCMC 10ml/kg, p.o)

Group II : Negative control (HFD + 0.5% NaCMC 10ml/kg, p.o)

Group III : Positive control (HFD + Orlistat 200mg/kg/day, p.o)

Group IV : Test I (HFD + Metformin 20mg/kg/day, p.o)

Group V : Test II (HFD + SMF 128 mT/ hr/day + 0.5% NaCMC,10ml/kg, p.o)

Group VI : Test III (HFD + SMF 128 mT/hr/day + Metformin 20mg/kg/day, p.o)

The drug was dissolved in 0.5% NaCMC and administered orally via a standard orogastric cannula, then animals to be exposed to magnetic field could placed in static magnetic field exposure device.

PHARMACOLOGICAL EVALUATION

Anti-Obesity Activity

Determination of blood glucose level (Lahbib *et al*., 2010)

Blood samples were collected from the tip of the tail vein on initial day, 7th day and 15th day from tail vein by snipping off the tip of the tail and the blood glucose was measured by a glucometer (Accu-Chek active Roche, Switzerland).

Parameters Measured (Dixit *et. al*., 2012)

Body weight

The body weight (gm) was recorded on day 1 and then on alternate days for 15 days in High Fat Diet induced overweight rats.

Food and water intake

The daily food and water intake was measured for 15 days in HFD induced overweight rats in each groups on cage basis.

Body temperature

The body temperature was recorded on day 14 in HFD induced overweight rats using rectal telethermometer before and after treatment at 30, 60, 90 and 120 min time interval with contact time of 1 min.

Determination of Locomotor Activity (Dixit *et. al*., 2012)

Locomotor activity was recorded on day 14 in HFD induced overweight rats. Each rat was placed individually in the actophotometer with 10 min observation time after treatment and basal activity score was obtained. The movement of the animal cuts off a beam of light falling on the photocell and a count was recorded and displayed digitally.

Estimation of Biochemical Parameters (Sivakumar *et al*., 2016)

The biochemical parameters were determined after 24 hour of the last dose of treatment. On day 15 of experimentation, blood was withdrawn from retro-orbital plexus under pentobarbitone sodium (50mg/kg/i.p) anaesthesia and then blood samples were allowed to clot for room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 15 minutes and utilized for estimation of biochemical parameters including serum lipid profile, Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP). Lipid profile like Total cholesterol, Triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Very Low Density Lipoprotein (VLDL) levels were measured from serum sample by using the biochemical kits (Span diagnostic Ltd, Mumbai, India).

Serum Glutamate Oxaloacetate Transaminase (Tietz and Saunders, 1997)

Principle

Aspartate amino transferase (AST) catalyses the transamination of L-Aspartate and α-Keto glutrate to form oxaloacetate and L-Glutamate. Oxaloacetate so formed is coupled with 2, 4-dinitrophenyl hydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown coloured complex in alkaline medium and this can be measured calorimetrically.

Procedure

SGOT in serum was estimated by MOD-IFCC method using an Asritha diagnostic kit. Pipetted out the serum sample in to clean dry test tube labeled as test. The test sample tube containing working reagent and serum sample. Mixed well and the initial absorbance after 1 min at 340nm was checked and repeated the absorbance reading after 1,2 & 3 min. calculate the mean absorbance changed per minute.

Calculation : SGOT activity in U/L $37^{o}C = \Delta A/min \times 1746 \times Tf$

Serum Glutamate Pyruvate Transaminase (Wolf *et al*., 1972)

Principle

Alanine amino transferases (ALT) catalyses the transamination of L-Alanine and α- Ketoglutarate to form pyruvate and L- glutamate. Pyruvate so formed is coupled with 2,4-dinitro phenyl hydrazine (2,4 DNPH) to form a corresponding hydrazone, a brown colour complex in alkaline medium and this can be measured colorimetrically.

Procedure

SGPT in serum was estimated by MOD-IFCC method using an Asritha diagnostic kit. Pipetted out the serum sample in to clean dry test tube labeled as test. The test sample tube containing working reagent and serum sample. Incubated at the assay temperature for 1 min at 340 nm then added sample (serum). Mix well and read the initial absorbance after 1 min and repeated the absorbance changed per min (ΔA/min.).

Calculation :SGPT activity in U/L 37^{o}C=ΔA/min×1746.

Alkaline Phosphatase (Wilkinsons and Winsten, 1969)

Principle

Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenyl so formed reacts in alkaline medium with 4-aminoantipyrine I presence of the oxidizing agent Potassium ferricyanide and forms an orange red coloured complex, which can be measured calorimetrically. The colour intensity is proportional to the enzyme activity.

Procedure

ALP in serum was estimated by PNPP method using an Asritha Diagnostic Kit. Pipetted out the serum sample into a clean dry test tube labeled as test. The test The test sample tube containing working reagent and serum sample. Mixed well and the initial absorbance after 1 min at 340nm was checked and repeated the absorbance reading after 1,2 & 3 min. Calculate the mean absorbance changed per minute.

Calculation : ALP activity in U/L 37^{o}C = ΔA/min×275×Tf

Lipid profile:

Total cholesterol (Roeschlau *et al*., 1974)

Total cholesterol in serum was determined by a colorimetric method. The assay principle is based on enzymatic hydrolysis and oxidation of cholesterol and the indicator compound, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The reagents consisted of 4-aminoantipyrine (0.03 mmol/l), phenol (6 mmol/l), peroxidase (≥0.5 U/ml), cholesterol esterase (> 0.15 U/ml), cholesterol oxidase(> 0.1 U/ml) and pipes buffer (80 mmol/L pH 6.8). The serum sample (10 µl) was mixed with 1 ml of reagent, incubated at 37^{o}C for 5 min, and absorbance measured at 500 nm against the reagent blank. The cholesterol standard was 5.17 mmol/l (200 mg/dl). The concentration of total cholesterol in the sample was calculated by

Total cholesterol = ΔA sample/ ΔA standard x concentration of standard.

Triglycerides (Tietz, 1990)

Serum Triglycerides (TG) were determined by a colorimetric method. The assay principle is based on the enzymatic hydrolysis of TG with lipases and the indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic activity of peroxidase. The enzyme reagent consisted of 4-aminophenazone (0.5 mmol/l), ATP (1.0 m.mol/l), lipases (≥150 U/ml), glycerol-kinase (≥0.4 U/ml), glycerol-3-phosphate oxidase (≥1.5 U/ml), peroxidase (≥0.5 u/ml). The serum sample (10 µl) was mixed with 1000 µl of enzyme reagent, incubated at 37^{o}C for 5 min and absorbance measured at 500 nm against the reagent blank. The TG standard was 200 mg/dl (2.29 mmol/l). The concentration of TG in the serum was calculated by

Triglycerides = ΔA sample / ΔA standard x concentration of standard.

HDL (Lopes-Virella *et al*., 1977)

Serum HDL cholesterol was determined by a colorimetric method. The assay principle is based on the following: the Low Density Lipoproteins (LDL and VLDL) and chylomicron fraction is precipitated quantitatively by the addition of phophotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined. The precipitation reagents consisted of phosphotungstic acid (0.55 mmol/l) and magnesium chloride (25 mmol/l). The serum sample (200 µl) was mixed with 500 µl of precipitation reagent and centrifuged at 4000 rpm for 10 min. The supernatant (100 µl) was mixed with reagent (CH 200 1 ml), incubated at 37^{o}C for 5 min and absorbance measured at 500 nm against the reagent blank. The cholesterol standard was 200 mg/dL (5.17 mmol/l). The concentration of cholesterol in the supernatant was calculated by.

HDL = ΔA sample / ΔA standard x concentration of standard.

LDL & VLDL (Friedewald *et al*., 1972)

Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) were calculated according to Friedwald formula.

LDL = TC – HDL – VLDL

VLDL = Triglycerides / 5.

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. Statistical analysis was carried out using one way Analysis of Variance (ANOVA) followed by Dunnett's '*t*' test. P value <0.05 was considered as significant.

RESULTS

Table 4: Effect of Static Magnetic Field and Metformin on fasting blood glucose (mg/dl) level in control and experimental rats

Groups	Initial Day	7th day	15th day
Standard Diet	89.00±1.14	90.00±1.09	88.00±0.84
HFD Overweight Rat	120.00±5.00	115.00±4.40	128.00±2.24
HFD + Orlistat (40 mg/kg)	108.00±4.64	105.00±1.70	108.00±1.70
HFD + Metformin (200 mg/kg)	120.00±4.40	111.00±1.30	118.00±5.00
HFD + SMF (128 mT/hr/day)	125.00±4.40	140.00±2.98*	160.00±2.98***
HFD + SMF (128 mT/hr/day) + Metformin (200 mg/kg)	130.00±2.45*	110.00±1.84	105.00±1.84

The Data Represented as mean ±SEM (n=5)

*P<0.05, **P<0.01 and ***P<0.001 Vs Induced Control

The blood glucose levels measured in normal and experimental rats in initial and at the 7th and 15th days of treatment are given in Table 4 and Figure 2. HFD induced overweight rats showed significant increase in the levels on blood glucose as compared to SD fed rats. Oral administration of Orlistat (40 mg/kg) and Metformin (200 mg/kg) shows no significant change in blood glucose level, while the SMF (128 mT/hr/day) exposed group shows a significant increase ($p<0.05$), ($p<0.001$) in blood glucose levels and Oral administration of Metformin (200 mg/kg) with SMF (128 mT/hr/day) exposed group shows a significant decrease ($p<0.05$) in blood glucose.

Figure 2: Effect of Static Magnetic Field and Metformin on fasting blood glucose level in control and experimental rats

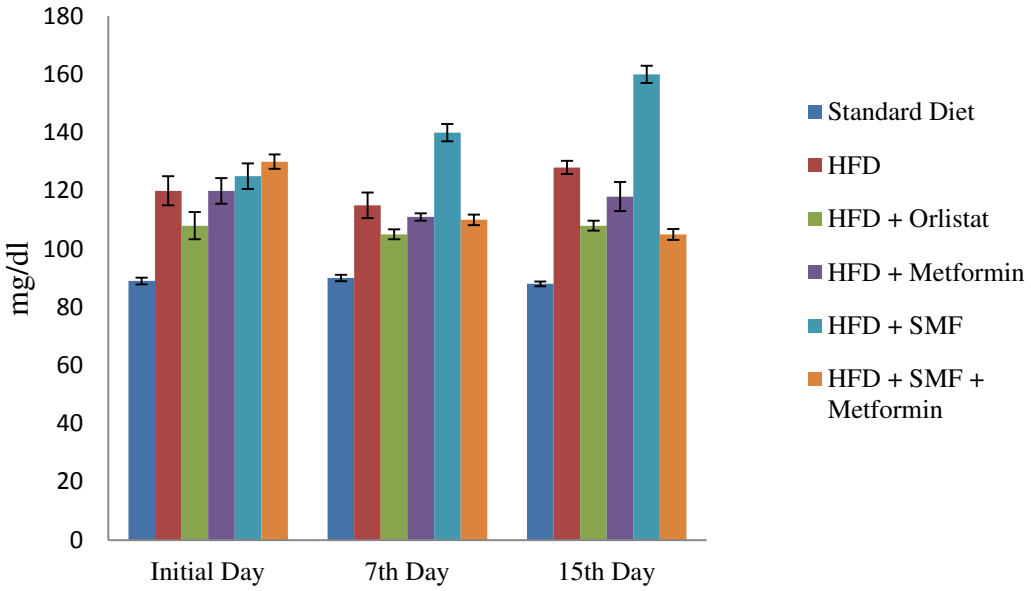


Table 5: Effect of Static Magnetic Field and Metformin on body weight (gm) changes in control and experimental rats

Groups	Initial Day	3rd Day	5th Day	7th Day	9th Day	11th Day	13th Day	15th Day
Standard Diet	160±3.54	155±5.00	160±3.54	165±3.54	165.4±3.20	170.40±3.74	175±3.54	180±4.40
HFD Overweight Rat	255±1.84	260±1.58	270±5.38	280±3.86	280.0±4.40	285.00±6.36	290±6.70	295±5.33
HFD + Orlistat (40 mg/kg)	260±3.54	255±1.84	240±1.84*	230±1.84**	235.0±1.87**	240.00±1.84*	240±1.70*	235±1.87**
HFD + Metformin (200 mg/kg)	270±5.38	275±3.52	269±6.00	265±5.24	265.0±5.78	269.00±6.00	260±5.24	260±5.47
HFD + SMF (128 mT/hr/day)	265±5.00	260±5.24	260±5.24	258±1.22*	255.0±5.00*	250.00±4.78*	255±5.00*	255±5.00*
HFD + SMF (128 mT/hr/day) + Metformin (200 mg/kg)	258±1.38	263±1.55	258±1.38*	255±5.00*	250.0±4.78*	250.00±5.09*	245±1.84**	243±2.93**

The Data Represented as mean ±SEM (n=5)

*P<0.05, **P<0.01 and ***P<0.001 Vs Induced Control

Table 5 and Figure 3 depicts the body weight changes of different groups of rats during the experimental period. HFD induced over weight rats showed that significant increase in body weight throughout the experimental period when compared to SD fed rats. Administration of Orlistat (40 mg/kg), SMF (128 mT/hr/day), SMF (128 mT/hr/day) with Metformin (200 mg/kg) shows decrease in body weight. While oral administration of Metformin (200 mg/kg) shows no significant change in body weight. On comparing the weight loss of SMF (128 mT/hr/day) treated animals the group SMF with Metformin (200 mg/kg) animals were found to be significant ($p<0.05$, $p<0.01$).

Figure 3: Effect of Static Magnetic Field and Metformin on body weight changes in control and experimental rats

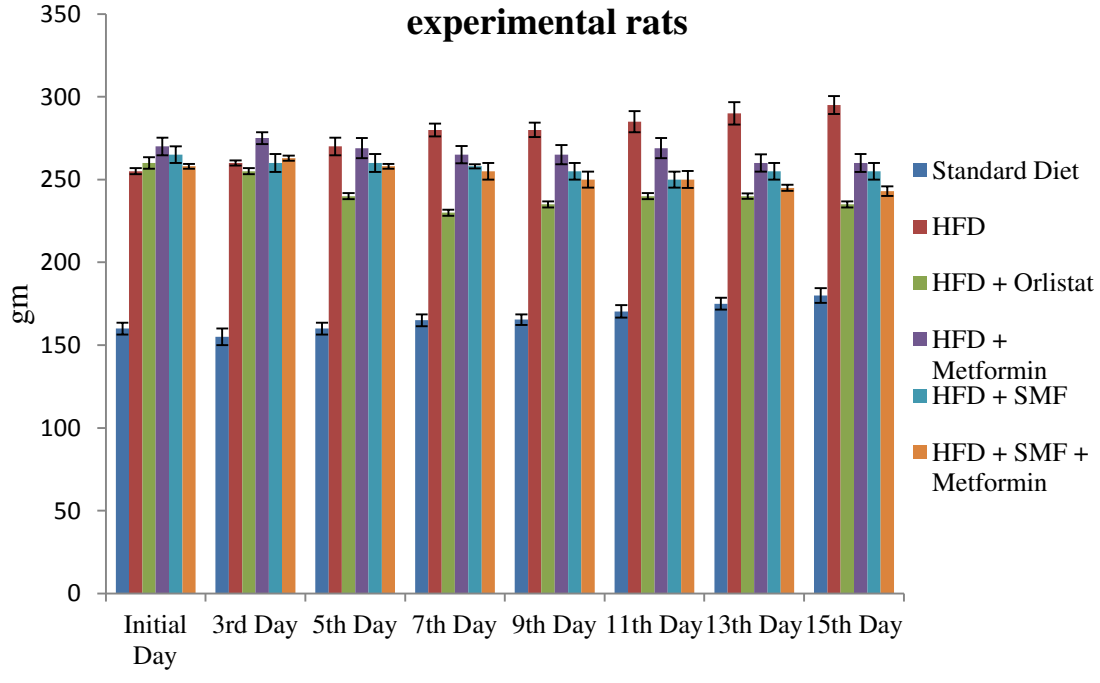


Table 6: Effect of Static Magnetic Field and Metformin on food and water intake and body temperature in control and experimental rats

Groups	Food intake (gm)	Water intake (ml)	Body temperature (◦C)
Standard Diet	85.69±0.81	170.00±1.31	33.20±0.002
HFD Overweight Rat	122.08±1.31	175.00±1.44	33.61±0.007
HFD + Orlistat (40 mg/kg)	110.00±1.86	172.00±1.44	33.34±0.007
HFD + Metformin (200 mg/kg)	117.46±9.07	175.85±1.63	33.52±0.007
HFD + SMF (128 mT/hr/day)	119.92±2.36	172.85±1.63	33.43±0.010
HFD + SMF(128 mT/hr/day) + Metformin (200 mg/kg)	115.15±2.23	171.85±1.63	33.40±0.008

The Data Represented as mean ±SEM (n=5)

*P<0.05, **P<0.01 and ***P<0.001 Vs Induced Control

The food and water intake, body temperature were measured in normal and experimental rats of treatment are given in Table 6 and Figure 4. HFD induced overweight rats showed no significant change in food and water intake, body temperature on compared to SD fed rats. Oral administration of Orlistat (40 mg/kg) and Metformin (200 mg/kg), SMF (128 mT/hr/day) exposed group and Oral administration of Metformin (200 mg/kg) with SMF (128 mT/hr/day) exposed group also shows no significant change in food and water intake, body temperature on compared to HFD overweight rat.

Figure 4: Effect of Static Magnetic Field and Metformin on food and water intake and body temperature in control and experimental rats

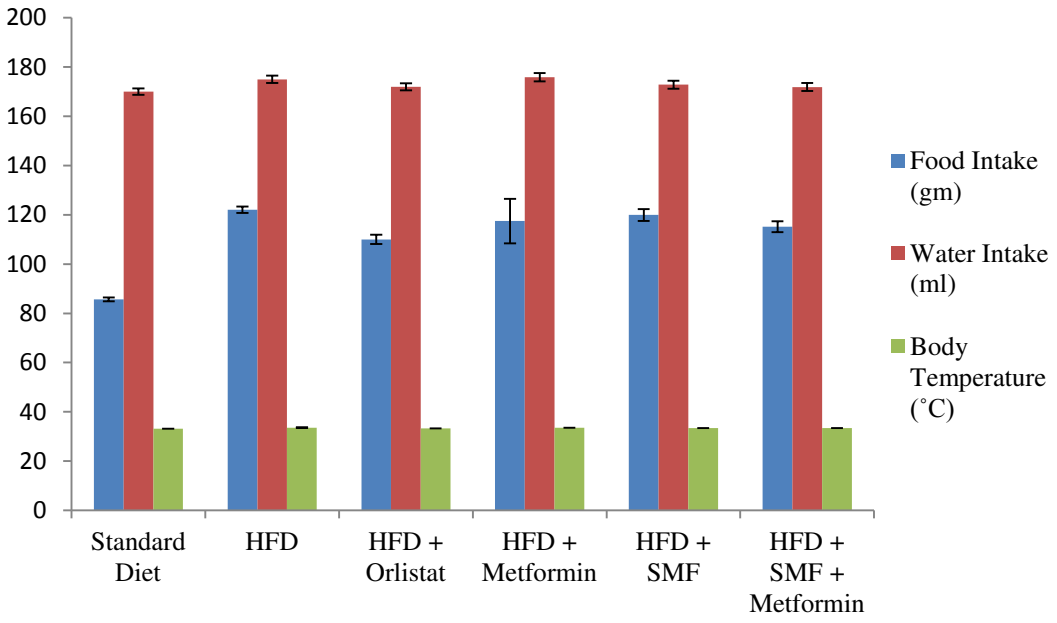


Table 7:Effect of Static magnetic field and Metformin on locomotor activity in control and experimental rats

Groups	Locomotors activity 14th Day
Standard Diet	150.00±3.62
HFD Overweight Rat	89.00±1.76
HFD + Orlistat (40 mg/kg)	130.00±1.70**
HFD + Metformin (200 mg/kg)	95.00±4.66
HFD + SMF (128 mT/hr/day)	98.00±1.41
HFD + SMF (128 mT/hr/day) + Metformin (200 mg/kg)	115.00±2.98**

The Data Represented as mean ±SEM (n=5)

*P<0.05, **P<0.01 and ***P<0.001 Vs Induced Control

The locomotor activity is measured in normal and experimental rats of treatment are given in Table 7 and Figure 5. HFD induced overweight rats showed significant decrease (P<0.05) in locomotor activity on compared to SD fed rats. Oral administration of Orlistat (40 mg/kg) and Oral administration of Metformin (200 mg/kg) with SMF (128 mT/hr/day) exposed group shows significant increase (P<0.01) in locomotor activity while Oral administration of Metformin (200 mg/kg) and SMF (128 mT/hr/day) exposed group showed no significant change in locomotor activty on compared to HFD overweight rat.

Figure 5: Effect of Static Magnetic Field and Metformin on locomotor activity in control and experimental rats

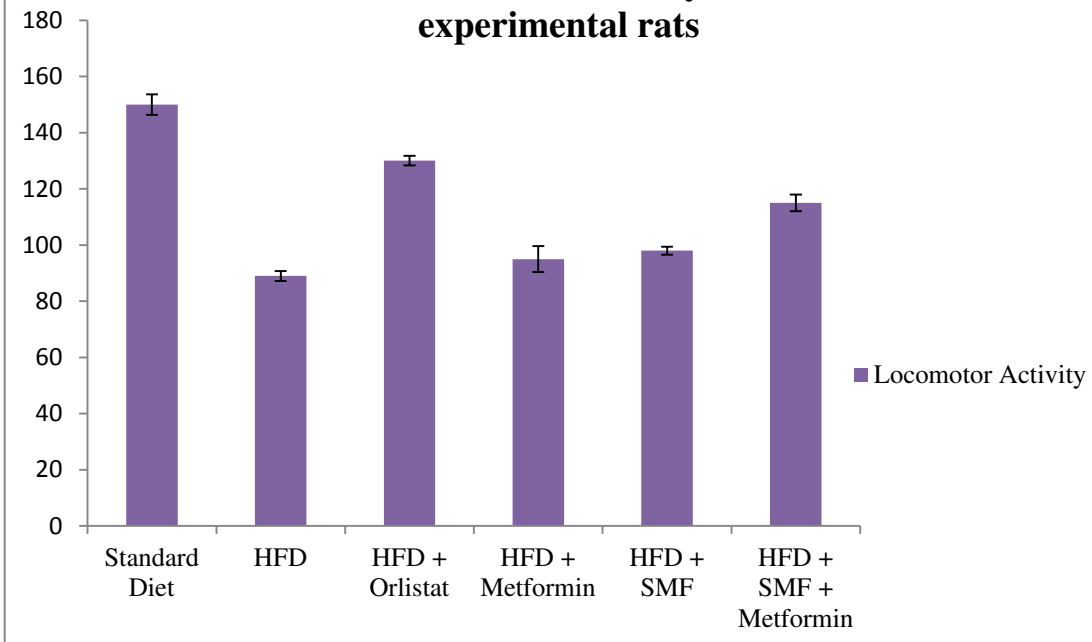


Table 8: Effect of Static Magnetic Field and Metformin on liver function parameters (IU/L) in control and experimental rats.

GROUPS	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Standard Diet	40.00±0.61	37.00±1.04	125.00±0.69
HFD Overweight Rat	52.00±0.40	40.00±0.65	160.00±1.34
HFD + Orlistat (40 mg/kg)	42.00±0.61	38.00±1.72	129.00±0.89**
HFD + Metformin (200 mg/kg)	49.98±0.34	42.00±0.67	140.00±1.68*
HFD + SMF (128 mT/hr/day)	49.00±0.35	39.00±0.53	134.00±0.92**
HFD + SMF (128 mT/hr/day) + Metformin (200 mg/kg)	45.00±1.40	38.50±1.71	132.00±0.48**

The Data Represented as mean ±SEM (n=5)

*P<0.05, **P<0.01 and ***P<0.001 Vs Induced Control

The above table 8 and figure 6 depicts the Serum levels of SGOT, SGPT, and ALP. The liver enzyme level found to increases in the HFD induced overweight rats. But on treatment with the standard Orlistat (40 mg/kg) the value of the enzyme level reduced to marked level. On treatment with SMF (128 mT/hr/day) with Metformin (200 mg/kg) the level of the enzyme reduced significantly (P<0.05, p<0.01) when compared to individual treatment of Metformin (200 mg/kg) and SMF (128 mT/hr/day). The levels of these found to be normal in group I normal control. But it

found to be increased in HFD induced overweight group (II). On comparison with HFD Overweight rats the levels of ALP, found to decrease significantly ($p<0.01$) in SMF (128 mT/hr/day) with Metformin (200 mg/kg) group.

In case of SGOT and SGPT the value found to increase in HFD induced overweight group (II) on comparing with SD fed rats. On treatment with Orlistat (40 mg/kg) SGOT and SGPT levels in serum founds to decrease. Similarly the treatment with Metformin (200 mg/kg), SMF(128 mT/hr/day), SMF(128 mT/hr/day) with Metformin (200 mg/kg) also improves the level of SGOT and SGPT in test group (IV ,V an VI).

Figure 6: Effect of Static Magnetic Field and Metformin on liver function parameters in control and experimental rats

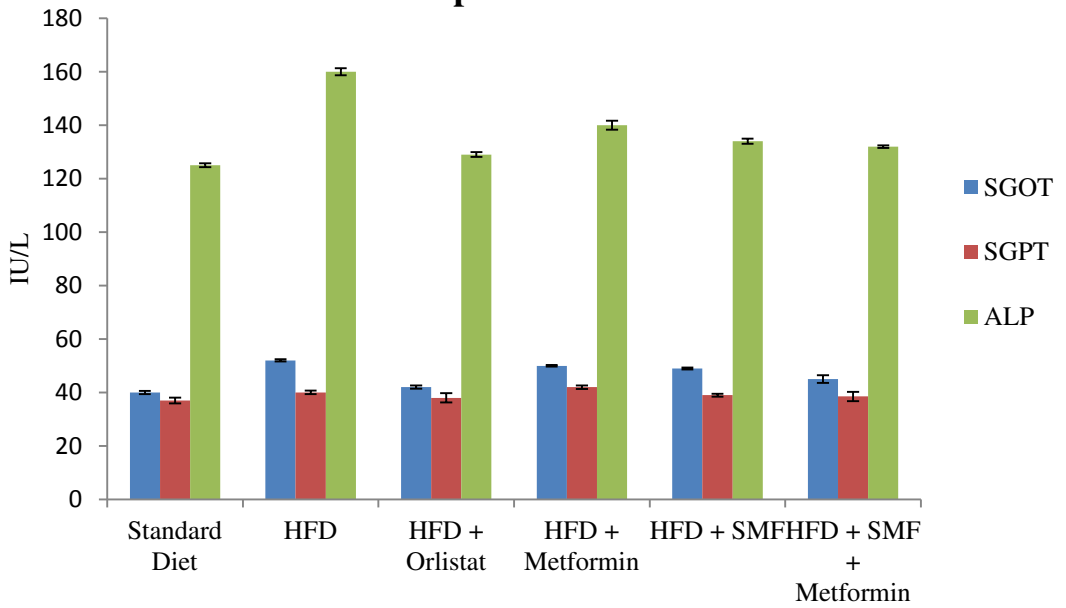


Table 9: Effect of Static Magnetic Field and Metformin on lipid profile (mg/dl) in control and experimental rats

Group	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Standard Diet	67.00±0.43	77.00±0.40	27.00±0.34	15.00±0.54	29.50±1.19
HFD Overweight Rat	77.00±0.40	190.00±0.53	37.00±0.38	19.00±0.80	28.00±0.63
HFD+Orlistat (40 mg/kg)	68.00±0.54	108.00±0.86	30.00±0.34	15.60±0.34	29.10±0.39
HFD+Metformin (200 mg/kg)	69.00±0.54	229.00±0.85*	35.00±0.57	18.50±0.80	29.20±1.09
HFD+SMF (128 mT/hr/day)	50.00±2.99*	107.00±0.86**	34.20±0.63	17.00±0.80	28.50±0.29
HFD+SMF (128 mT/hr/day) + Metformin (200 mg/kg)	67.00±2.16	152.00±0.70*	33.00±0.87	16.50±0.80	29.00±0.38

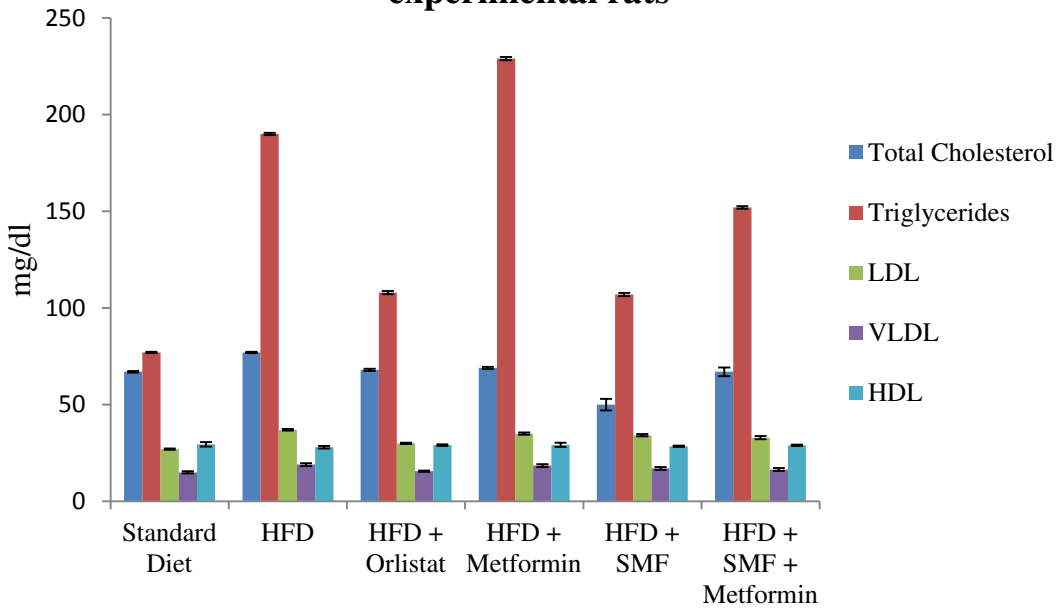
The Data Represented as mean ±SEM (n=5)

*P<0.05, **P<0.01 and ***P<0.001 Vs Induced Control

The levels of Total Cholesterol, Triglycerides, LDL and VLDL tend to rise in HFD induced overweight rats. The level reaches its maximum in HFD overweight rats group. But the condition was overcome in Orlistat (40 mg/kg), Metformin (200 mg/kg), SMF (128 mT/hr/day) and SMF (128 mT/hr/day) with Metformin (200 mg/kg) treated animals. The effect of decrease in lipid parameters was found to be significant ($p<0.01$) in the SMF (128 mT/hr/day) treated animals.

In case of HDL the value decreases in HFD overweight rats on comparing to Standard diet group. This effect was reversed in Orlistat (40 mg/kg), Metformin (200 mg/kg), SMF (128 mT/hr/day) and SMF (128 mT/hr/day) with Metformin (200 mg/kg) treated animals.

Figure 7: Effect of Static Magnetic Field and Metformin on lipid profile in control and experimental rats



SUMMARY AND DISCUSSION

The present thesis entitled "Combined Effect of Static Magnetic Field and Metformin on Weight Loss In Overweight Rats" deals with the exploration of pharmacological screening of Magnetic Field with Metformin. On past two decades Magnetic field is used for treating Bone disorders and Nerve disorders. The results obtained from using magnetic field by various researchers were shown in the table 3.1 and 3.2. The SMF exposure device was fabricated by using 3-D Ansys Maxwell engineering software, 2014 version. Approval was obtained from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC), proposal number NCP/IAEC/2016-2017-01.

Consumption of a diet rich in calories in the form of sugars and fats and its accumulation in most part of the body leads to excessive growth of adipose tissue, chronic inflammation, and metabolic perturbations. It can also lead to the development of hyperlipidemia, atherosclerosis and abnormal lipid metabolism (Onody *et al*., 2003). Epidemiological studies recommended that excess dietary energy intake is one of the frequent causes of obesity (Lee *et. al*., 2008 and Bhandari *et. al*., 2011). In the present study, the effects of SMF with Metformin on the alterations of glucose, liver parameter and lipid profile in high-fat diet (HFD) rats were investigated. Our earlier studies indicated that the rats fed with HFD showed significant increase in body weight, plasma glucose, insulin, and leptin, which leads to complications clinically (Sudhakara *et al*., 2014).

The animals were grouped into Group 1 (SD Control) Group 2 (HFD control), Group 3 (HFD + Orlistat 40 mg/kg/day/po for 2 weeks), Group 4 (HFD + Static Magnetic Field 128 mT/1h/day for 2 weeks), Group 5 (HFD + Metformin 200 mg/kg/day/po for 2 weeks), Group 6 (HFD + Static Magnetic Field 128 mT/1h/day with Metformin 200 mg/kg/day/po for 2 weeks).

In case of blood glucose level the drastic increase in glucose levels was due to the release of the hyperglycemic hormone (glucagon) or the inhibition of the hypoglycemic

hormone (insulin) as previously shown by Lahbib, *et al*., 2010 and also due to conformational change of insulin was observed by Elferchichi *et al*., 2011, this leads to decreasing the binding capacity of insulin. The elevated glucose level in blood was reversed by treatment with Combined SMF with Metformin. This may be possibly due to the insulin like effect of SMF with Metformin on peripheral tissues, either by promoting glucose uptake and metabolism or by inhibiting hepatic gluconeogenesis.

Treatment with SMF resulted in reduction in body weight indicating that SMF posess weight reducing property. The decreasing body weight might due to decrease in body fluid and protein content or other factor including hormonal changes (Lahbib, *et al*., 2010). In the present study, the result showed that no significant change in food and water intake and body temperature was observed on treatment with combined SMF and Metformin. Nevertheless, the body weight decrease could not be related to dehydration but probably due to hypoxia status known to alter the body weight (Amara *et. al*., 2006). Locomotor activity could be decreased in HFD induced rats were observed in this study. In addition to that, Increased locomotor activity were observed on treatment with combined SMF and Metformin. This may be due to decreasing in body weight with improving the functions of sciatic nerve. Previous study reported by Sekinz *et al*., 2006 shows alteration in sciatic nerve function on exposing SMF.

Serum liver biomarkers (SGOT and SGPT) are important criteria for the evaluation of liver toxicity. The amounts of enzymes that leak into the blood stream indicate severity of hepatic damage. In the present study, the serum liver function parameters such as SGOT, SGPT and ALP were increased significantly in HFD induced rats indicate that the hepatic injury was induced by HFD induced obesity. The increased serum levels of SGOT and SGPT were due to the damage in structural integrity of liver, since these enzymes are normally located in the cytoplasm and released into the circulation after cellular injury. Along with SGOT and SGPT, the other biomarkers for liver damage are ALP was also increased in HFD treated groups. The liver function parameters were significantly decreased after the post treatment of Metformin and its combination with SMF therapy only while increased levels were observed in SMF alone group (Sivakumar *et al*., 2016).

The most common lipid abnormalities in overweight rats are hypertriglyceridemia and hypercholesterolemia. Administration of the SMF with Metformin decreases hypertriglyceridemia and hypercholesterolemia significantly ($P<0.01$). The observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis. HDL cholesterol level was significantly improved by the combined SMF with Metformin treatment.

CONCLUSION

This study entitled **"Combined effect of Static Magnetic Field and Metformin on weight loss in overweight rats"** shows a potential antiobesity activity and could protect the body against glucose, liver and lipid parameters related to obesity and its complications. Thus confirming its general use in medical practice to cure and manage body weight. There is a need for further investigation to evaluate the exact mechanism for biochemical activity in order to confirm which are responsible for antiobesity.

REFERENCES

Abdelmelek H, Molnar A, Servais S, Cottet-Emard JM, Pequignot JM, Favier R and Sakly M. Skeletal muscle HSP72 and norepinephrine response to static magnetic field in rat. *Journal of Neural Transmission*, 2006; 113: 821-827.

Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki T, Ravanat JL, Favier A, Sakly M and Ben Rhouma K. Effects of subchronic exposure to static magnetic field on testicular function in rats. *Archives of Medical Research*, 2006[a]; 37: 947-952.

Amara S, Abdelmelek H, Salem MB, Abidi R and Sakly M. Effects of static magnetic field exposure on haematological and biochemical parameters in rats. *Brazilian Archives of Biology and Technology*, 2006[b]; 49: 889-895.

Ammari M, Jeljeli M, Maaroufi K, Sakly M, Abdelmelek H and Roy V. Static magnetic field exposure affects behavior and learning in rats. *Electromagnetic Biology and Medicine*, 2008; 27: 185-196.

Belova NA and Acosta-Avalos D. Review article: The effect of extremely low frequency alternating magnetic field on the behaviour of animals in the presence of the geomagnetic field. *Journal of Biophysics*, 2015; 1-8.

Bhandari U, Kumar V, Khanna N and Panda BP. The effect of high fat diet induced obesity on cardiovascular toxicity in Wistar albino rats. *Human and Experimental Toxicology*, 2011; 30: 1313-1321.

Bhatia AL. Static magnetic field as biological modifier: a study on temperature dependent influence. *Indian Journal of Biochemistry and Biophysics*, 1999; 36: 361-364.

Camila G, Pereira LRL, Nelson IJ, Evandro Jose C, Almeida CAND, Dermeval DC and Queiroz RHC. Tolerability and effectiveness of Fluoxetine, Metformin and

Sibutramine in reducing anthropometric and metabolic parameters in obese patients. *Archives of Endocrinology and Metabolism*, 2006; 50: 1020-1025.

Cason AM, Denbleyker M, Ferrence K, Smith JC and Houpt TA. Sex and estrous cycle differences in the behavioral effects of high-strength static magnetic fields: role of ovarian steroids. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*, 2006; 290: 659-667.

Colbert AP, Wahbeh H, Harling N, Connelly E, Schiffke HC, Forsten C, Gregory WL, Markov MS, Souder JJ, Elmer P and King V. Static magnetic field therapy: A critical review of treatment parameters. *Evidence-Based Complementary and Alternative Medicine*, 2009; 6: 133-139.

Dixit P, Prakash T, Karki R and Kotresha D. Anti-Obese activity of *Butea monosperma* (Lam) bark extract in experimentally induced obese rats. *Indian Journal of Experimental Biology*, 2012; 50: 476-483.

Elferchichi M, Mercier J, Bourret A, Gross R, Lajoix AD, Belguith H, Abdelmelek H, Sakly M, and Lambert K, Is static magnetic field exposure a new model of metabolic alteration? comparison with zucker rats. *International Journal of Radiational Biology*, 2011; 87: 483-490.

Elferchichi M, Mercier J, Coisy-Quivy M , Metz L, Lajoix AD, Gross R, Belguith H, Abdelmelek H, Sakly M and Lambert K. Effects of exposure to a 128-mT static magnetic field on glucose and lipid metabolism in serum and skeletal muscle of rats. *Archives of Medical Research*, 2010; 41: 309-314.

Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 1972; 1: 499-502.

Ghodbane S, Amara S, Arnaud J, Garrel C, Faure H, Favier A, Sakly M and Abdelmelek H. Effect of selenium pre-treatment on plasma antioxidant vitamins a (retinol) and e (alpha-tocopherol) in static magnetic field-exposed rats. *Toxicology and Industrial Health*, 2011[a]; 27: 949-955.

Ghodbane S, Amara S, Garrel C, Arnaud J, Ducros V, Favier A, Sakly M and Abdelmelek H. Selenium supplementation ameliorates static magnetic field-induced disorders in antioxidant status in rat tissues. *Environmental Toxicology and Pharmacology Journal*, 2011[b]; 31: 100-106.

Golay A. Metformin and body weight. *International Journal of Obesity*, 2008; 32: 61-72.

Gorczynska E and Wegrzynowicz R. Glucose homeostasis in rats exposed to magnetic fields. *Investigative Radiology*, 1991; 26: 1095-1100.

High WB, Sikora J, Gurbil KU and Garwood M. Subchronic *in vivo* effects of a high static magnetic field (9.4 T) in rats. *Journal of Magnetic Resonance Imaging*, 2000; 12: 122-139.

Houpt TA, Carella L, Gonzalez D, Janowitz I, Mueller A, Mueller K, Neth B and Smith JC. Behavioral effects on rats of motion within a high static magnetic field. *Physiology and Behaviour*, 2011; 102: 338-346.

Houpt TA, Cassell JA, Riccardi C, Kwon B and Smith JC. Suppression of drinking by exposure to a high-strength static magnetic field. *Physiology and Behaviour*, 2007; 90: 59-65.

Houpt TA, Pittman DW, Barranco JM, Brooks EH and Smith JC. Behavioral effects of high-strength static magnetic fields on rats. *Journal of Neuroscience*, 2003; 23: 1498-1505.

Ichioka S, Minegishi M, Iwasaka M, Shibata M, Nakatsuka T, Ando J and Ueno S. Skin temperature changes induced by strong static magnetic field exposure. *Bioelectromagnetics*, 2003; 24: 380-386.

ICNIRP (International Commission on Non-Ionizing Radiation Protection). Guidelines on limits of exposure to static magnetic fields. *Health Physics*, 2009; 96: 504-514.

Kanai S and Taniguchi N. Efficacy of static magnetic field for pain of adjuvant arthritis rats. *Advances in Bioscience and Biotechnology*, 2012; 03: 511-515.

Kang JG and Park CY. Anti-Obesity drugs: A review about their effects and safety. *Diabetes and Metabolism Journal*, 2012; 36: 13-25.

Kolin A. An Electromagnetic flowmeter: Principle of method and its applications to blood flow measurement. *Experimental Biology and Medicine*, 1936; 35: 53-56.

Lahbib A, Elferchichi M, Ghodbane S, Belguith H, Chater S, Sakly M and Abdelmelek H. Time-dependent effects of exposure to static magnetic field on glucose and lipid metabolism in rat. *General Physiology and Biophysics*, 2010; 29: 390-395.

Laszlo J and Gyires K. 3T Homogeneous static magnetic field of a clinical MR significantly inhibits pain in mice. *Life Sciences*, 2009; 84: 12-17.

Laszlo JF and Porszasz R. Exposure to static magnetic field delays induced preterm birth occurrence in mice. *American Journal of Obstetrics and Gynecology*, 2011; 205: 362.26-362.31.

Lee HS, Park HJ and Kim MK. Effect of Chlorella vulgaris on lipid metabolism in Wistar rats fed high-fat diet. *Nutrition Research and Practice*, 2008; 2: 204–210.

Lerchl A, Honaka KO and Reiter RJ. Pineal-gland "magnetosensitivity" to static magnetic fields is a consequence of induced electric currents (eddy Currents). *Journal of Pineal Research*, 1991; 10: 109-116.

Lewczuk B, Redlarski G, Gak A, ZioBkowska N, Gornowicz BP and Krawczuk M. Review article: Influence of electric, magnetic, and electromagnetic fields on the circadian system: current stage of knowledge. *Biomed Research International*, 2014; 1-13.

Lloret-Linares C, Greenfield JR and Czernichow S. Effect of weight-reducing agents on glycaemic parameters and progression to Type 2 diabetes: A review. *Diabetic Medicine*, 2008; 25: 1142-1150.

Lohmann KJ. Q&A : Animal behaviour: Magnetic-field perception. *Nature*, 2010; 464: 1140-1142.

Lopes-Virella MF, Stone P, Ellis S and Colwell JA. Cholesterol determination in high density lipoproteins separated by three different methods. *Clinical Chemistry,* 1977; 23: 882-884.

Malin SK and Kashyap SR. Effects of Metformin on weight loss: potential mechanisms. *Current Opinion* in *Endocrinology*, *Diabetes*, and *Obesity,* 2014; 21: 323-329.

Markov MS. Magnetic field therapy: A review. *Electromagnetic Biology and Medicine*, 2007; 26: 1-23.

Miryam E, Aida L, Samira M, Mohsen S and Hafedh A. Effects of acute exposure to static magnetic field on ionic composition of rat spinal cord. *General Physiology and Biophysics*, 2010; 29: 288-294.

Mitri J and Hamdy O. Diabetes medications and body weight. *Expert Opinion on Drug Safety*, 2009; 8: 573-584.

Modak T and Mukhopadhaya A. Effects of citral, a naturally occurring antiadipogenic molecule, on an energy-intense diet model of obesity. *Indian Journal of Pharmacology*, 2011; 43: 300-305.

Novikov VV, Novikov GV and Fesenko EE. Effect of weak combined static and extremely low-frequency alternating magnetic fields on tumor growth in mice inoculated with the ehrlich ascites carcinoma. *Bioelectromagnetics*, 2009; 30: 343-351.

Okano H and Ohkubo C. Elevated plasma nitric oxide metabolites in hypertension: synergistic vasodepressor effects of a static magnetic field and Nicardipine in spontaneously hypertensive rats. *Clinical Hemorheology and Microcirculation*, 2006; 34: 303-308.

Okano H and Ohkubo C. Exposure to a moderate intensity static magnetic field enhances the hypotensive effect of a calcium channel blocker in spontaneously hypertensive rats. *Bioelectromagnetics*, 2005; 26: 611-623.

Okazaki R, Ootsuyama A, Uchida S and Norimura T. Effects of a 4.7 T static magnetic field on fetal development in ICR mice. *Journal of Radiation Research*, 2001; 42: 273-283.

Onody A, Csonka C, Giricz Z and Ferdinandy P. Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. *Cardiovascular Research*, 2003; 58: 663–670.

Parveen K and Uma B. Obesity pharmacotherapy: current status. *EXCLI Journal*, 2015; 14: 290-293.

Politanski P, Rajkowska E, Pawlaczyk-Quszczy_Nska M, Dudarewicz A, Wiktorek-Smagur A, Sliwi_Nska-Kowalska M and Zmy_Slony M. Static magnetic field affects oxidative stress in mouse cochlea. *International Journal of Occupational Medicine and Environmental Health*, 2010; 23: 377-384.

Pradeepa R , Anjana RM , Joshi SR, Bhansali A , Deepa M, Joshi PP, Dhandania VK *et al*., Prevalence of generalized and abdominal obesity in urban and rural India - the ICMR-INDIAB study (Phase-I) [ICMR-INDIAB-3]. *Indian Journal of Medical Research*, 2015; 142: 139-150.

Prina-Mello A, Farrell E, Prendergast PJ, Campbell V and Coey JM. Influence of strong static magnetic fields on primary cortical neurons. *Bioelectromagnetics*, 2006; 27: 35-42.

Priscilla H. Anti-Diabetes and Anti-Obesity Medications: Effects on weight in people with diabetes. *Diabetes Spectrum*, 2007; 20: 159-165.

Roeschlau P, Bernt E and Gruber WA. Enzymatic determination of total cholesterol in serum. *Clinical Biochemistry*, 1974; 12: 226-228.

Rosen AD. Studies on the effect of SMF on biological system. *Progress in Electromagnetics Research Symposium Proceedings*, 2010; 1171-1174.

Rukhsana A, Zakirul I, Moushumi S, Sohely NE, Suha J and Shamsun N. Anti-Obesity effect of mushroom *(ganoderma lucidum)* on experimentally induced obese rats. *Anwer Khan Modern Medical College Journal*, 2012; 3: 11-14.

Seifarth C, Schehler B and Schneider HJ. Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. *Experimental and Clinical Endocrinology & Diabetes*, 2013; 121: 27-31.

Sekino M, Tatsuoka H, Yamaguchi S, Eguchi Y and Ueno S. Effects of strong static magnetic fields on nerve excitation. *IEEE Transaction on Magnetics*, 2006; 42: 3584-3586.

Sivakumar A, Lavanya M, Alwin D and Venkataraman P. Effect of combination therapy of melatonin and Orlistat on high fat diet induced changes in lipid profiles and liver function parameters in serum of rats. *Obesity Medicine*, 2016; 2: 41-43.

Song MK, Bischoff DS, Uyemura K, Song AM and Yamaguchi DT. Prevention and treatment of obesity and diabetes and their related complications. *Journal of Molecular and Genetic Medicine*, 2014; 9: 1-13.

Sudhakara G, Mallaiah P, Rajendran R and Saralakumari D. Preventive effect of Caralluma fimbriata vs. Metformin against high-fat diet-induced alterations in lipid metabolism in Wistar rats. *Journal of Biomedicine and Pharmacotherapy*, 2016; 84: 215-223.

Sudhakara G, Mallaiah P, Srinivasulu N, Sasi Bhusana Rao B, Rajendran R and Saralakumari D, Beneficial effects of hydro-alcoholic extract of Caralluma fimbriata against high-fat diet induced insulin resistance and oxidative stress in Wistar male rats. *Journal of Physiology and Biochemistry*, 2014; 70: 311–320.

Sunyer PI and Xavier F. The obesity epidemic: pathophysiology and consequences of obesity. *Obesity Research*, 2002; 10: 97-104.

Suzuki Y, Ikehata M, Nakamura K, Nishioka M, Asanuma K, Koana T and Shimizu H. Induction of micronuclei in mice exposed to static magnetic fields. *Mutagenesis*, 2001; 16: 499-501.

Tietz NW. Clinical guide to laboratory tests. 2nd ed. W.B. Saunders Company, *Philadelphia*, 1990.

Tietz NW and Saunders WB. *Text book of clinical chemistry, Philadelphia*. 1997; 1388-1391.

Tsuji Y, Nakagawa M and Suzuki Y. Five-tesla static magnetic fields suppress food and water consumption and weight gain in mice. *Industrial Health*, 1996; 34: 347-357.

Von Diemen V, Trindade EN and Trindade MR. Experimental model to induce obesity in rats. *Acta Cirurgica Brasileira*, 2006; 21: 425-429.

Wilkinsons JH and Winsten S. Evaluation of a new system for the kinetic measurement of serum alkaline phosphatise. *Clinical Chemistry*, 1969; 15: 487-495.

Wolf PL, Williams D, Coplon N and Coulson AS. Low aspartate transaminase activity in serum of patients undergoing chronic hemodialysis. *Clinical chemistry*, 1972; 18: 567-568.

Yadollahpour A and Rashidi S. Therapeutic applications of electromagnetic fields in musculoskeletal disorders: a review of current techniques and mechanisms of action. *Biomedical and Pharmacology Journal*, 2014; 7: 23-32.

Yang PF, Hu LF, Wang Z, Ding C, Zhang W, Qian AR and Shang P. Inhibitory effects of moderate static magnetic field on leukemia. *IEEE Transaction on Magnetics*, 2009; 45: 2136-2139.

Yu S and Shang P. A review of bioeffects of static magnetic field on rodent models. *Progress in Biophysics and Molecular Biology*, 2014; 114: 14-28.

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