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Research Article

ANTI-OBESITY ACTIVITY OF *ZIZIPHUS MAURITIANA* : A POTENT PANCREATIC LIPASE INHIBITOR

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ABSTRACT

The Anti-obesity activity of Ziziphus mauritiana Lam bark powder (ZMBP) on High Fat Diet (HFD) induced obesity in rats was studied. Obesity was induced in Wistar rats by feeding High Fat Diet (HFD) for 70 days. The rats were distributed in 5 groups (n=5) viz Normal Control**,** Obese Control, Obese rats with 250 mg/kg ZMBP , Obese rats with 500 mg/kg ZMBP, Obese rats with Sibutramine, 0.90 mg/kg treatment. The rats were dosed orally by gavage daily for a period of 90 days. At the end of 90 days treatment with ZMBP the obese rats showed 16.33 % reduction in body weight gain at 250 mg/kg dose, 17.38 % ($p<0.05$) reduction at 500 mg/kg dose and Sibutramine showed 5.52% reduction in body weight gain respectively when compared with the obese control group. The DEXA analysis at the end of 90 days of treatment showed 68.99 % ($p<0.01$) decrease in the Fat mass at 250 mg/kg dose and 72.84 % ($p<0.001$) decrease in the Fat mass at 500 mg/kg dose when compared with the obese control group. The pancreatic lipase activity in 250mg/kg and in 500 mg/kg reduced significantly ($p<0.001$) when compared with the obese control group.

Keywords: Obesity, Fat mass, Tissue triglycerides, Pancreatic lipase, Insulin resistance

INTRODUCTION

Obesity is considered an emergency health problem in all industrialized countries and in spite of the number of studies to prevent or treat obesity, its prevalence continues to rise[1]. Genetic predisposition, changes in life style and diet are among the various factors which lead to increase in incidence of obesity and related consequences such as cancer, aging, cardiovascular diseases and number of other pathological conditions including type 2 diabetes[2, 3].

Primarily, obesity is regarded as a disorder of lipid metabolism and the enzymes involved in this process could be selectively targeted to develop anti-obesity drugs[4]. Several anti-obesity drugs such as Orlistat reduces intestinal fat absorption by inhibiting pancreatic lipase whereas Sibutramine is a serotonin and norepinephrine reuptake inhibitor. Sibutramine and its active amine metabolites alter serotonergic and noradrenergic, but not dopaminergic, activity in brain areas that are involved in the control of appetite[5]. Recently, inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase (PL) is considered to be a novel approach for the treatment of obesity. Natural products provide a vast pool of PL inhibitors that may be developed into clinical products [6].

Ziziphus mauritiana / Ziziphus jujube are shrubs belong to family *Rhamnaceae* distributed in warm temperate zone from western Africa to India. Seeds and leaves of both these plants are used as folkloric medicine for treating hyperlipidemic and hyperglycemic conditions[7]. Thus, studies were undertaken to evaluate the anti-obesity activity of the bark of *Ziziphus mauritiana* shrub in HFD induced obese rats.

MATERIALS AND METHODS

Plant materials

Ziziphus mauritiana (ZM) bark was collected in the month of November 2010 from a full grown tree in the Solapur District of Maharashtra state, India and identified by Dr. P. G. Diwakar, Joint Director, Laboratory of Botanical Survey of India, Pune, India under the Voucher No. APT02 reported through Certificate No. BSI/WRC/Tech./2011. The bark was cleaned, dried and was grounded to a fine powder. This was used as test drug.

Animals

Male Wistar rats of age 6 to 8 weeks were procured from National Toxicology Centre, Pune, India. They were housed under standard conditions of temperature and relative humidity with 12 hr light/dark cycle. The animals were fed on standard commercial pelleted diet and water *ad libitum.* The Institutional Animal Ethical Committee approved the experimental protocols as per CPCSEA guidelines through the research project no. 10.

Chemicals

The Porcine Pancreatic Lipase, P-Nitro Phenyl Palmitate-pNPP were obtained from Sigma Aldrich, USA. All other chemicals were of analytical grade. High fat diet was obtained from VRK Nutritional Solutions, India. Standard drug Sibutramine was obtained from German Remedies, India. Chemicals required for PCR studies were obtained from Applied Biosystem, USA.

Acute toxicity Study

The acute oral toxicity test of ZM bark powder (ZMBP) was determined prior to the efficacy study as per the OECD (Organization for Economic Co-operation and Development) 423 Guidelines. Female Wistar rats were administered the ZMBP as a single dose of 2000 mg/kg body weight. The treated animals were observed for 14 days for mortality, clinical signs and symptoms.

Subacute toxicity study

The Subacute (28 days) oral toxicity study of ZMBP was conducted as per OECD 407 Guidelines to determine the dose that would be used for the efficacy study. Twenty four male rats were distributed in four groups (n=6). Normal control rats were treated with water at a dose of 10.0 ml/kg. ZMBP at a dose of 250, 500 and 1000 mg/kg was administered orally by gavage to the 2nd, 3rd, and 4th group rats respectively every day for 28 days. The treated animals were observed for 28 days for mortality, clinical signs and symptoms. Weekly body weight and food consumption data were monitored. At the end of 28 days blood was withdrawn to perform haematological and biochemical parameters. Histopathology of liver,kidney and heart were performed with the standard procedure[8].

Estimations

Haematological estimations were carried out using Mindray 2800 analyser, (China) and Biochemical parameters were carried out using SERI BSA 3000, (France). Blood glucose was estimated using Accu-check, Roche Diagnostic, GbH, (Germany). Fat analysis was

done using DEXA ®SABRE™ X ray Bone Densitometer, Orthometrix, Inc, (USA). MRI scanning of the animals was carried out using SIEMENS Syngo Fast View, (Germany).

Induction of obesity

The obesity was induced by feeding high fat diet (HFD) for 70 days[9]. The animals had free access to HFD (VRK Nutritional Solutions, India) and water. The composition of HFD was casein 30%, cholesterol 10%, ground nut oil 14%, corn starch 41%, vitamin/mineral 05% (protein 24%, carbohydrate 41% and fat 24%,) . The animals were screened for induction of obesity by analyzing parameters for obesity such as body weight, anthropometrical parameters[10] serum triglycerides, serum cholesterol, glucose tolerance test, insulin resistance test, DEXA analysis and MRI Scan.

Efficacy study

The obese animals were grouped randomly into 4 groups with 5 rats in each. One group of normal (lean) animals was also used as control in this study.

Group 1: normal (lean) control, Group 2: obese control, Group 3: obese rats administered orally 250 mg/kg ZMBP daily, Group 4: obese rats dosed with 500 mg/kg ZMBP daily, Group 5: obese rats dosed with, 0.90 mg/kg.

The rats were dosed orally daily for a period of 90 days. Food consumption and body weights were recorded every week for each animal. The insulin resistance test was performed on 0 day and at the end of the 90th day. Haematological and biochemical parameters including body fat analysis were carried out on 0 and 90th day. MRI scanning was carried out in the representative animals (2 nos.) in each group. The anthropometrical parameters were measured as per method described by Novelli et al., 2007[11]..

Lipid profile

On day 0 and at the end of 90th day, blood was drawn through retro-orbital plexus of the rats. Serum Cholesterol, HDL-cholesterol, triglycerides were determined by using standard commercial kits.

Faecal fat analysis

The faeces were collected from individual animal on day 0, day 30, day 60 and day 90 respectively. The fecal matter was dried in the oven at 70ºC in an oven for 1 h. The lipid extraction was carried out with 2.0 ml of chloroform: methanol (2:1) for 30 min at 60ºC. It was filtered and the volume was made to 4.0 ml with the extracting solvent. The fecal matter was resuspended in 2.0 ml of the solvent and kept for 30 min at 60ºC. After extraction it was filtered. The extracted solvent fractions were pooled together and evaporated to dryness. The lipid analysis was done gravimetrically. The fecal lipid was calculated as % weight of fecal matter [12].

Organ weights

At the end of 90 days the animals were sacrificed and organs were dissected and weighed. The epididymal fat and the peritoneal fat tissues from the control, obese control, test drug treated and standard drug treated rats were compared to analyze the total fat deposition.

Tissue triglyceride (TG) levels

The liver, heart, epididymal fat pads and peritoneal fat pads were extracted in heptane: isopropanol (3:2) at 4ºC. The TG content was measured using a biochemical analyzer using commercial kit. The TG content was calculated as mg per gram of tissue[13].

Pancreatic lipase analysis

The pancreas from each animal was removed and the pancreatic lipase was analyzed by a method described by Shamsher et al. 2005[14]. A stock solution of p-nitro phenol was prepared in 0.05 M Tris buffer at pH 8.5. Different dilutions of p-nitro phenol were prepared and Standard curve was obtained which was read in a spectrophotometer at 410 nm. Pancreatic lipase from the pancreas tissue homogenate (100 mg of tissue used to prepare tissue homogenate) acts on p-nitro phenyl palmitate (pNPP) to release yellow coloured p-nitro phenol which was measured spectrophotometrically. The reaction was carried out at 45ºC for 20 min. The release of p-nitro phenol was measured spectrophotometrically at 410 nm. The blank was run with the same reaction mixture heated in boiling water bath for 10 min. Results were expressed as units/mg of protein.

Histopathology

Histopathology of liver, kidney and heart was performed with the standard procedure[15].

Gene expression

Total RNA was isolated from liver using the triazole reagent, according to the manufacturer's instructions. Total RNA was reverse transcribed into first-strand cDNA following the manufacturers procedure. The synthesized cDNA was used as a template for polymerase chain reaction (PCR) amplification. Real time PCR was performed using step one real time PCR system (ABI). The insulin receptor substrate-1(IRS-1), Adiponectin R_2 and PGC-1α SYBR Green primers were used for real time RT-PCR analysis.

A dissociation curve analysis of all primers showed a single peak. PCR were carried out for 45 cycles using the following conditions: denaturation at 95ºC for 45 sec, annealing at 62.7ºC for 30 sec, and elongation at 72ºC for 15 sec. Mean Ct of the gene of interest was calculated from duplicate measurements & normalized with the mean Ct of a control gene GAPDH[16].

Statistical analysis

Statistical analysis was performed by one way ANOVA, using Dunnett's multiple comparison test: prism card 5 graph pad software.

RESULTS

Acute toxicity study

The ZMBP was found to be safe at 2000 mg/kg as per the OECD 423 guidelines. No clinical signs of intoxication were observed in any animal till the end of the study and no mortality observed.

Subacute toxicity studies

The Subacute (28 days) oral toxicity studies with ZMBP showed 33.33 % mortality at a dose of 1000 mg/kg body weight. There was no mortality observed at dose of 250 and 500 mg/kg body weight of ZMBP. All the animals appeared normal and showed no clinical signs of intoxication. No change in the food consumption was observed. No statistically significant difference in the haematology and blood chemistry parameters were observed in the 250 and 500 mg/kg ZMBP dose groups as compared to the control group animals. The histopathology of liver, kidney and heart did not show any toxicity.

Induction of obesity

The rats were subjected to HFD for 70 days to induce obesity. The rats fed on HFD showed 42.77% ($p<0.01$) increase in body weights at the end of 70 days compared to the rats fed normal diet. The HFD fed rats did show insulin resistance and increase in body fat as revealed by MRI Scan (Figure 1a and 1b).

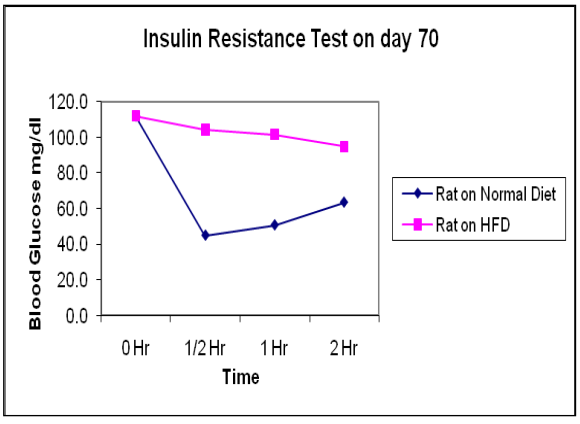


Figure 1a: Insulin resistance test on 70th day in normal and HFD induced obese rats

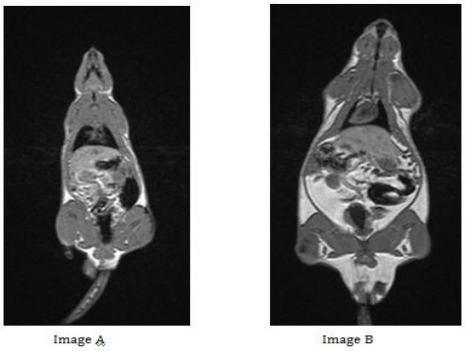


Figure 1b: MRI scan images A : Rat fed on normal diet for 70 days, B: Rat fed on HFD

Efficacy data

After 90 days of treatment with ZMBP, the obese rats showed 16.33% and 17.38% ($p<0.05$) reduction in body weight gain at 250 and 500 mg/kg respectively when compared with the obese control group. The rats treated with standard drug Sibutramine though showed 5.52% reduction in body weight gain, it was not significant in comparison with the obese control group (Table 1).

The DEXA analysis showed 68.99% ($p<0.01$) reduction in the fat mass at 250 mg/kg dose and 72.84% ($p<0.001$) reduction in the fat mass at 500 mg/kg dose after ZMBP treatment when compared with the obese control group (Table 1). The MRI Scanning of rats showed significant depletion in the body fat mass (Figure 2).

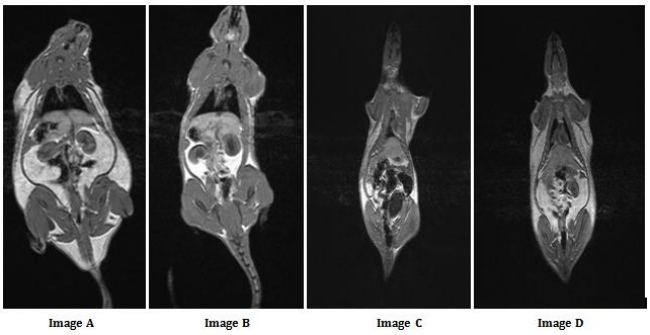


Figure 2: MRI Scan images at the end of 90 days A: Obese control, B: animal dosed with 250 mg/kg ZMBP, C: animal dosed with 500 mg/kg ZMBP, D: animal dosed with Sibutramine0.90 mg/kg ZMBP

No apparent changes in the food uptake were observed in the treated and untreated animals. At one hour 60.4% and 67.9% decrease in insulin resistance was observed as shown in the figure 3 in the 250 and 500 mg/kg dose groups respectively in comparison with obese control group. The standard drug group showed 56.4% decrease in insulin resistance at one hour when compared with obese control group.

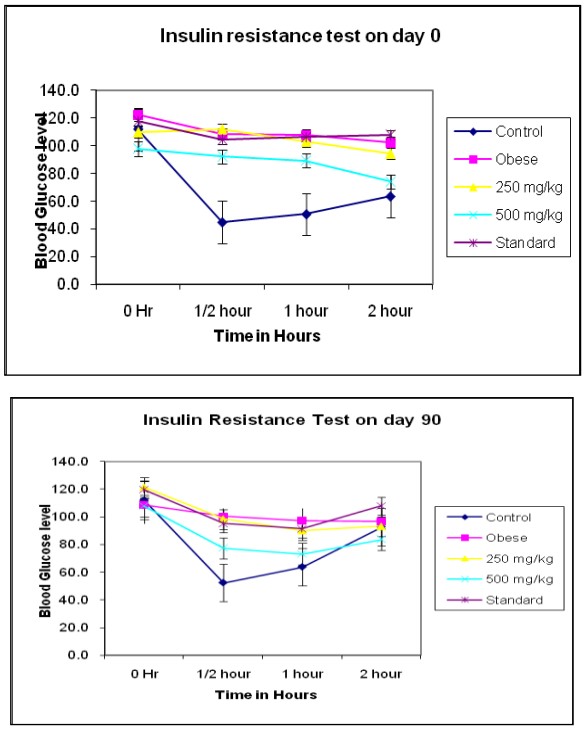


Figure 3: Insulin resistance test carried out after ZMBP administration for 90 days in obese rats

Fat analysis

ZMBP showed significant reduction in the weight of the peritoneal fat pad. Average peritoneal fat pad weights in obese rats treated with 250 mg/kg dose and 500 mg/kg dose group were recorded to be 9.82 ± 5.80 g ($p<0.01$) and 4.64 ± 2.54 g ($p<0.001$) respectively as compared to the 27.35 ± 35 g recorded in the obese control group animals. The standard drug treated animals showed no significant reduction in the peritoneal fat pad weights (18.88 ± 4.23 g). The ZMBP effect was minimal on the epididymal fat pad weights (Table 1).

Table 1: Body weight data and anthropometrical parameters in normal and HFD induced obese rats

Group	Body weight in grams	Thoracic Circumference in cms	Abdominal Circumference in cms	DEXA FAT in grams	Epididymal Fat tissue weight in grams	Peritoneal Fat in grams
Normal Control	381.1 ± 14.1	16.2 ± 1.3	18.4 ± 0.5	Not detected	3.6 ± 2.1	4.6 ± 2.4
Obese Control	518.4 ± 58.8	17.6 ± 1.9	21.8 ± 1.1	85.4 ± 30.4	6.3 ± 3.0	27.3± 3.5
250 mg/kg	433.7 ±19.3*	15.6 ± 1.1	18.4 ± 1.9*	26.5±20.4**	3.9 ± 1.1	9.8 ± 5.8**
500 mg/kg	428.3 ± 9.3*	17.5 ± 1.4	18.2 ± 2.8*	23.2±26.1***	4.1 ± 2.0	4.6 ±2.5***
Standard	489.8 ± 77.4	18.6 ± 2.1	21.1 ± 1.9	84.1 ± 16.7	9.0 ± 4.6	18.9 ± 4.2

Not Detected= Not detectable by DEXA analyzer *= p<0.05, **=p<0.01, ***=p<0.001

Haematology and biochemical parameters

There were no significant changes observed in the haematological parameters in all the groups when compared with the obese control group. However significant ($p<0.001$) decrease in the serum triglycerides was observed in the 250 mg/kg (93.80 ± 11.60 mg/dl) and in 500 mg/kg (78.96 ± 9.24 mg/dl) dose group when compared with the obese control group (128.80 ± 8.58 mg/dl). There was minimal reduction observed in the serum cholesterol levels (Table 2).

Tissue triglycerides

The treatment of ZMBP showed significant ($p<0.01$) decrease in the triglycerides deposition in the liver of 250 mg/kg (11.2 ± 4.6 mg/g and in 500 mg/kg (12.8 ± 1.3 mg/g) dose group when compared with the obese control group (18.9 ± 3.1 mg/g). The triglyceride content in peritoneal fat pad of 250 mg/kg (22.0 ± 3.4 mg/g) and 500 mg/kg (17.9 ± 2.7 mg/g) dose group animals were significantly reduced with $p<0.01$ and $p<0.001$ respectively as evident in the Table 2.

Pancreatic lipase activity

The pancreatic lipase activities in obese rats treated with 250 mg/kg ZMBP were (5.13 ± 0.71 U/mg of protein) and in rats treated with 500 mg/kg were (4.01 ± 0.86 U/mg of protein) ,thus showed significant reduction (p<0.001) in comparison with the obese control group (9.73 ± 2.39 U/mg of protein) (Table 2).

Fecal fat

The decreased lipase activity in the ZMBP treated groups reflected with significant (p<0.05) increase in excretion of fecal fat as revealed in the Table 2.

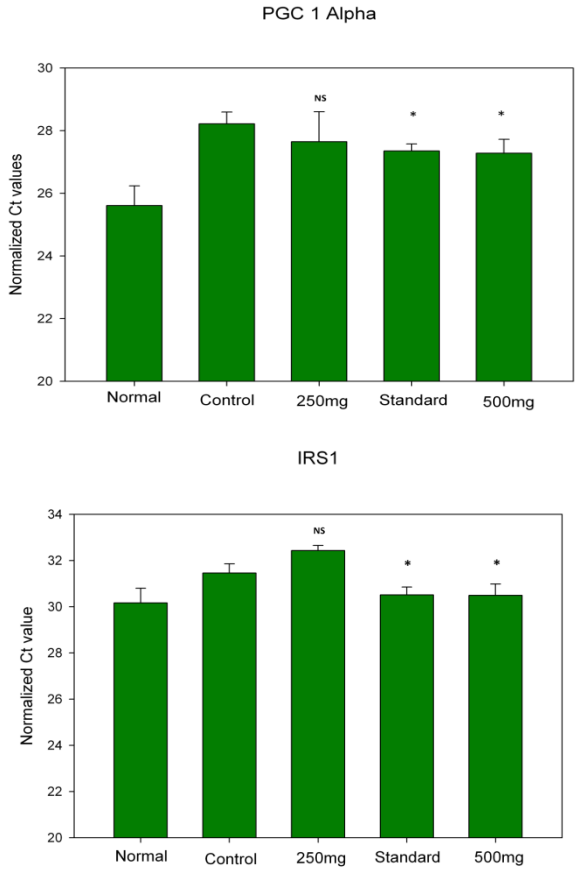
Table 2: Lipid profile and fecal fat analysis in normal and HFD induced obese rats after administration of ZMBP

Group	Serum TGmg/gm	Serum Cholesterol mg/dl	Liver TGmg/gm of tissue	Peritoneal FAT pad TGmg/gm of tissue	Epididymal Fat pad TG mg/gm of tissue	Faecal fat in %	Pancreatic lipase activity U/mg of tissue protein
Normal control	54.0 ± 08.3	55.2 ± 06.5	7.2 ± 0.7	28.7 ± 1.9	18.5 ± 1.8	3.7 ± 0.40	2.0 ± 0.63
Obese Control	128.8 ±08.6	144.2 ± 23.4	18.9 ± 3.1	36.8 ± 6.9	19.2 ± 3.0	4.4 ± 0.24	9.7 ± 2.4
250 mg/kg	93.8 ±11.6***	121.4 ± 04.3	11.2 ± 4.6**	22.0 ± 3.4**	16.5 ± 2.9	5.6 ± 0.50*	5.1 ± 0.7***
500 mg/kg	79.1 ±09.2***	130.0 ± 05.2	12.8 ± 1.3**	17.9 ± 2.7***	14.9 ± 3.8	6.2 ± 1.00*	4.0 ± 0.9***
Standard	127.8 ±04.2	129.2 ± 25.1	12.7 ± 2.4**	27.5 ± 9.9	18.5 ± 2.3	4.8 ± 0.50	7.5 ± 2.1

***= p<0.05, **=p<0.01, ***=p<0.001**

Gene expression

Also significant increase in the gene expression of PGC1α, IRS1 and adiponectin R2 were noted in liver tissue of 500 mg/kg dose group and in the standard drug dose group animals with p< 0.05 when compared with the obese control group (Figure 4).



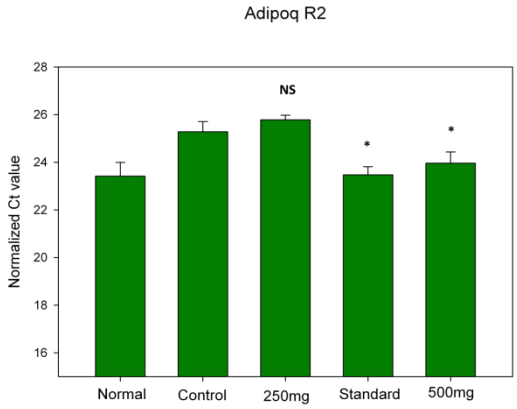


Figure 4: Gene expression in the Liver tissue of the rats sacrificed at the end of 90 days

DISCUSSION

Reduction of fat digestion through pancreatic lipase inhibition is now considered as a novel approach in treatment of obesity. Orlistat (Ro 18-0647), a hydrogenated derivative of lipastatin derived from *Streptomyces toxitricini* is a potent inhibitor of gastric, pancreatic and carboxylester lipases, and has proved to be effective for the treatment of human obesity[17]. Another antiobesity drug Sibutramine is believed to affect energy balance via reduction in food intake as well as by modulating energy expenditure [18].

Various studies have been conducted to study different activities of *Ziziphus mauritiana* plant. *Ziziphus mauritiana* extract showed anticancer, anti-inflammatory and antidiabetic activities [19, 20, 21]. A neo lignin isolated from *Ziziphus mauritiana* leaves found to increase the release of endogenous prostaglandin I_2 (the most potent natural inhibitor of platelet aggregation and a powerful vasodilator) from the rat aorta [22]. Very recently anti-obesity effect of *Ziziphus jujusba* leaf extract has been indicated in rats fed on high fat diet[23].

Present studies were therefore undertaken to determine anti-obesity activity of *Ziziphus mauritiana bark powder* in high fat diet (HFD) induced obese rats. HFD induced obese rats did show characteristic increase in body weights, body fat and insulin resistance. At the end of 90 day schedule of ZMBP administration, obese rats showed significant reduction in body weight gain over standard drug treatment. These results were comparable to the human studies reported earlier where there was 6.1% weight loss at 24th week[24]. DEXA analysis confirmed our results showing 68.99%

and 72.84% drop in fat mass at 250 and 500 mg/kg dose respectively. These findings were also visualized and supported by MRI scan. The peritoneal fat pad showed significant reduction in the weights, though epididymal fat pad showed reduction in weight, which was not statistically significant. Significant decrease in serum triglyceride levels were observed in ZMBP treated obese rats as compared to obese control group though the decrease in serum cholesterol levels found to be minimal. Concomitantly significant inhibition in pancreatic lipase activity was also observed at both dose levels. The anti-obesity activities of several medicinal herbs have been ascribed to increase fecal fat excretion via the inhibition of lipase activity [25] also evident in our studies. Thus reduction in body weight gain, loss of triglyceride content associated with increased fecal lipid excretion in ZMBP fed obese animals suggest an inhibitory mechanism in lipid absorption.

It has been demonstrated earlier that tannins from Banaba extracts induces glucose transport through activation of the insulin mediated signaling pathway in adipocytes [26]. In addition tannins inhibit adipocytes differentiation by inhibiting or altering the expression of key genes involved in the adipogenesis process[27]. ZMBP does contain large amount of tannins and also ZMBP fed obese animals did show significant increase in PGC1α, IRS1and adiponectin R2 gene expression. PGC1α responsible for increase energy expenditure by increasing mitochondrial biogenesis and respiration rates as well as the uptake and utilization of substrates for energy production[28]. Elevated insulin receptor substrate 1 expression reflects decrease in insulin resistance[29] also evident in ZMBP fed obese animals. Increase in the Adiponectin R2 expression is associated with reduction in adiposity and increases after weight reduction[30]. The histopathology of kidney, heart and liver showed no toxicity in ZMBP fed obese animals.

Thus our studies demonstrates the anti-obesity activity of *Ziziphus mauritiana* bark by reducing body weight gain , increasing fecal fat mass and decreasing insulin resistance in HFD fed obese rats. Polyphenolic compounds abundant in ZMBP may be responsible for the property of anti-obesity which further needs to be evaluated.

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Note

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