CHAPTER - 4

Determination of Anti-obesity and Anti-hyperlipidemic Activities of Extracts in High Fat Diet Induced Obese Rat Model
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4.1 INTRODUCTION

In the last two decades, despite significant improvement in public education and pharmacologic management, obesity is increasing in alarmingly high rates. Indians are also reported for obesity and their consequences. There is a constant rise in obesity related deaths each year (Mokdad et al., 2004). Major health consequences associated with overweight and obesity are dyslipidemia, coronary artery disease, type-2 diabetes, reproductive and gastrointestinal cancers, sleep apnea, stroke, fatty liver and osteoarthritis (Padwal et al., 2003). Recently, it has been found to promote prostate cancer, too (Ribeiro et al., 2012). Obese patients may lower the risk of cardiovascular diseases and type-2 diabetes by reducing only 5-10% weight (Avenell A et al., 2004; Goldstein DJ et al., 1992; Padwal RS et al., 2007).

The mechanism of high fat diet (HFD) induced obesity is still unclear, but long-term exposure to a HFD can increase body weight and adiposity in human and animals (Ji hyun et al., 2005). Additionally, obesity is the most important risk factor for complex and chronic liver disorders. These liver disorders begin as steatosis and may progress to steatohepatitis, cirrhosis, liver failure and hepatocellular carcinoma (Berrin and Elvan 2009). Hypercholesterolemia, elevated low density lipoprotein (LDL), and triglycerides all are associated with obesity (Shashikiran et al., 2004). Obesity is also linked to low levels of high density lipoprotein (HDL). Lipid abnormalities are very common in obese and are considered a major risk factor for development of atherosclerosis (Khurram et al., 2006). Hence it is very important to prevent and control early stages of hyperlipidemia, completely with drug therapies.

Orlistat, the drug used for the treatment of obesity, possesses several unpleasant adverse reactions including greasy stools, and fecal soiling that have been shown to compromise the patient compliance (Kopelman et al., 2007). And also, it needs supplementation of fat soluble vitamins in diet (Filippatos et al., 2008). These adverse reactions triggered a wealth of studies that searched for natural inhibitors of the pancreatic lipase (PL) with comparable efficacy to orlistat, but void of its side effects.

Despite the potential size of the market for antiobesity drugs and the imperative need for safe and efficient drugs, the current status for the development of such drugs is still unsatisfactory. Natural products have been the
foundation for the discovery of many important drugs. Consumption of edible plants could be a more effective method for the prevention or treatment of hyperlipidemia. Many edible plants present an exciting opportunity for the development of newer therapeutics for biologically active antihyperlipidemic agents from natural resources, especially the reduction of fat digestion and absorption (Dougall and Stewart, 2005; Kurihara et al., 2006; Matsumoto et al., 2010; Moller and Roos, 2009; Zhang et al., 2008). It is believed that they are able to delay postprandial hypertriacylglycerolemia and hypercholesterolemia in obese patients.

The high fat content of the diet leads to pronounced increase in the weight gain. Inclusion of cholesterol and cholic acid in the diet leads to a pronounced elevation in the serum total cholesterol levels (Monsen et al., 1972).

4.2 MATERIALS AND METHODS

4.2.1 Materials

Standard chow diet for rats was procured from Nutrivet Life Sciences, Pune; cholesterol and sodium cholate were purchased from SD Fine chemicals, Mumbai; Dalda ghee from Vanaspati Dalda. total Cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and blood glucose (BG) standard kits were purchased from Span diagnostics, Surat, Gujarat. Atorvastatin and orlistat were obtained from Cipla Ltd., Bangalore and Ranbaxy laboratories Ltd., Mumbai, respectively. All other chemicals used for the present study were of analytical grade and purchased from local vendors.

4.2.2 Preparation of Plant Extracts

Methanolic extracts of *H. rosa sinensis* (MEHR) and *T. anguina* (META) were prepared in 0.5% CMC at a final concentration of 500 mg/kg body weight followed by sonication for 30 mins to obtain a uniform suspension.
4.2.3 Preparation of High Fat Diet
HFD was prepared by mixing 30% of dalda ghee, cholesterol (2%) and sodium cholate (1%) with standard powdered chow diet of rats.

4.2.4 Animal Model
Adult albino wistar rats of either sex, weighing 150-170 g were procured from Bharat Serums and Vaccines Ltd., Thane, Mumbai. The animals were housed in standard environmental conditions with the temperature ranging between 22-25°C and animals were exposed to 12 h/12 h artificial light-dark cycle. The animals were placed in a propylene cages with stainless grill top and bedding of clean corn cob. Water and food (Nutrivet Life Sciences, Pune) were available ad libitum. The rats were acclimatized to the laboratory conditions for 10 days prior to initiation of the experiment. CPCSEA number for animals has been described in the chapter 2, section 2.2.3.

4.2.5 Experimental Design
After the acclimatization period, animals were randomly divided into six groups with six rats in each group.
Group I- Normal control
Group II- HFD control
Group III- Orlistat (30 mg/kg b.w.)
Group IV- Atorvastatin (30 mg/kg b.w.)
Group V- MEHR (500 mg/kg b.w.)
Group VI- META (500 mg/kg b.w.)

4.2.6 Induction of Obesity with Hyperlipidemia
Group I normal control group received standard chow diet, whereas Group II-VI received HFD. Animals in normal control group were fed with standard chow diet while the other groups were fed with HFD ad libitum, throughout the experiment. The measured amounts of diet was placed in the cage after weighing accurately. The rats were given HFD for 28 days to induce obesity with hyperlipidemia.
4.2.7 Treatment
Atorvastatin, orlistat, MEHR and META were prepared in 0.5% CMC to obtain their respective concentrations followed by sonication for 30 mins to obtain a uniform suspension. Treatments of all the groups were started from 29th day and continued for 10 days. The treatment groups III and IV were given standard drugs, orlistat and atorvastatin respectively at 30 mg/kg b.w by oral intubation. Treatment groups V and VI were given MEHR and META extracts at 500mg/kg b.w. by oral intubation. During the course of treatment, the treatment groups were continued to feed with HFD.

4.2.8 Analytical Methods
4.2.8.1 Determination of body weight and food intake
Throughout the 38 days of study the body weight and food intake of rats were monitored on a weekly basis. Food intake was measured as per cage basis.

4.2.8.2 Biochemical parameters
At the end of the study, i.e. on the 38th day, blood was collected by retro orbital plexus, under mild ether anesthesia after 8 hr fasting and allowed to clot for 30 minutes at room temperature. Serum was separated from the blood samples after centrifugation at 3000 rpm for 20 minutes and stored at -20 °C until further biochemical estimations were carried out. The Serum samples were analyzed spectrophotometrically for; lipid profile, that includes, TC, TG, LDL-C, HDL-C, VLDL-C; liver enzymes that includes AST and ALT and blood glucose using analytical kits as per manufacturer’s instructions.

4.2.8.3 Abdominal circumference
The abdominal circumference i.e., immediately anterior to the forefoot were determined, on the 38th day of the study, in all anaesthetized rats.

4.2.8.4 Organ weights
At the end of the study, animals were dissected, and their organs (liver, kidney, peritoneal fat, perirenal fat) were collected and weighed.
4.2.8.5 Liver and Fecal fat content
Feces were collected at the final day of the experiment and air-dried at 60°C for 12 h to a constant weight for fecal fat determination. Livers were collected at the end of the study after necropsy of all the rats. The lipids in the liver and feces were extracted and purified according to the method of Folch et al. (1957). Lipids were extracted with chloroform/methanol mixture (2:1, v/v). After extraction total lipids were determined gravimetrically.

4.2.9 Statistical Analysis
The results were expressed as mean ± SD (n=6). Statistical analysis was carried out using Graph Pad prism 5 software (version 4.03). The data was analyzed by one way ANOVA, followed by Dunnet’s multiple comparison test. All groups were compared with HFD control group and $P<0.05$ was considered statistically significant.

4.3 RESULTS
4.3.1 Body Weight
The body weights of rats are shown in Figure 4.1. After 28 days on HFD, there was a significant increase in body weights in Groups II to VI when compared to Group I animals, fed with the standard chow diet. The body weight gain was significantly suppressed with the treatment of atorvastatin, orlistat, MEHR and META, in the groups III- VI respectively, by the end of the study ($P<0.01$). The percentage reduction in body weight from day 28 to day 38 by the test extracts MEHR and META was 38.6% and 51.6% respectively, compared to orlistat and atorvastatin which showed 54% and 40% reduction in body weights of the rats respectively.
Figure 4.1: Effect of MEHR and META on body weights of rats fed with HFD. The values are mean ± S.D (n=6). Treatment groups were compared with HFD control (** P<0.01 and *** P<0.0001) and normal control with HFD control # P< 0.0001.

4.3.2 Food Intake
As shown in Figure 4.2, food intake of all the groups that received HFD, was higher when compared with Group I that received standard chow diet. At the end of the experiment, the HFD control group consumed 81% more food than the normal control group. Whereas, the consumption of food by the treatment groups was lesser food than HFD control group. The percentage reduction in food intake was highest in META treated group i.e., 35.7%, followed by MEHR with 22.7%.
Figure 4.2: Effect of MEHR and META on body weights of rats fed with HFD. The values are represented as mean per cage.

4.3.3 Serum Lipid Parameters
The effect of administration of MEHR and META on serum lipid and protein components in rats is shown in Figure 4.3. It may be seen that the levels of TC, TG, LDL-C and VLDL-C in serum were significantly decreased in all the treatment groups III to VI (Figure 4.3 B-E) ($P<0.0001$). The percentage reduction in TC in META treated group was 74%, whereas MEHR showed reduction by 66% (Figure 4.3 B). MEHR lowered the serum TG and LDL-C levels by 79% and 80%; and META by 77% and 69%, respectively. Serum HDL-C levels were significantly increased with the treatment of only META i.e., 36% (Figure 4.3A).
Figure 4.3: Effect of MEHR and META on HDL(A), TC(B), LDL(C), VLDL(D) and TG(E) of rats fed with HFD. The values are represented as mean ± S.D. (n=6) Treatment groups were compared with HFD control (*P<0.05 and *** P<0.0001) and normal control with HFD control (#P<0.0001).
4.3.4 Serum Glucose, AST and ALT

Serum glucose levels were raised after induction of obesity in HFD group as compared to normal control (P<0.001) (Figure 4.4 A). Reduction in serum glucose levels could be seen in treatment groups by 45.4%, 58.6%, 54.4% and 43% in Groups III to VI, respectively (P<0.001). Serum AST levels were increased in HFD control group when compared with normal control (P<0.001) (Figure 4.4 B). AST levels were markedly increased after the META treatment by 42% when compared with HFD control (P<0.001). But no significant changes in AST levels were observed in other treatment groups. Likewise, ALT levels were significantly increased after the treatment with META by 182% when compared with HFD control (P<0.0001) (Figure 4.4 C). No significant changes were observed in other treatment groups.

**Figure 4.4:** Effect of MEHR and META on serum glucose(A), AST(B) and ALT(C) levels of rats fed with HFD. The values are represented as mean ± S.D (n=6). Treatment groups were compared with HFD control (*** P<0.0001) and normal control with HFD control # P< 0.0001.
4.3.5 Abdominal Circumference

Abdominal circumferences were significantly increased by 22\% in HFD control group when compared with normal control group ($P<0.01$, Figure 4.5). Whereas, MEHR and META treated group showed significant decrease in abdominal circumferences by 12\% and 20\% respectively, after treatment of 10 days ($P<0.05$). Also, atorvastatin and orlistat treated group showed significant reduction in abdominal circumference by 19\% and 16\% respectively ($P<0.01$).

![Abdominal Circumference Graph]

Figure 4.5: Effect of MEHR and META on abdominal circumference of rats fed with HFD. The values are represented as mean ± S.D (n=6). Treatment groups were compared with HFD control (** $P<0.01$ and *** $P<0.0001$) and normal control with HFD control # $P<0.0001$.

4.3.6 Organ Weights

Figure 4.6 (A-D) shows the organ weight of the experimental animals at the time of necropsy. Weight of Liver, peritoneal and perirenal fat pads of all the treatment groups were significantly changed at the end of the study ($P<0.05$). All the treatment groups showed significant reduction in liver, peritoneal fat pad and perirenal fat pad weights. The percentage reduction in peritoneal and perirenal fat pads were 51.8\%, 68.5\% respectively with MEHR and 41.5\%, 55.5\% respectively with META treated rats (Figure 4.6 C-D). Also, liver and kidney weights were lowered in both MEHR and META treated groups by 21.12\% and
16.6%, respectively (Figure 4.6 A-B). However, kidney weights were not affected in any of the groups.

**Figure 4.6:** Effect of MEHR and META on liver(A), kidney(B), peritoneal fat(C) and perirenal fat(D) of rats fed with HFD. The values are represented as mean ± S.D (n=6). Treatment groups were compared with HFD control (*P<0.05, **P<0.001 and ***P<0.0001) and normal control with HFD control # P< 0.0001.
4.3.7 Fecal and Liver Fat Content

Hepatic accumulations of total lipids were significantly higher in rats fed with HFD in Group II, than those in Group I fed with standard chow diet ($P<0.05$, Figure 4.7 B). Whereas, liver lipid content was significantly decreased after treatment with orlistat, MEHR and META by 33.3%, 29.2% and 37.5% respectively when compared with HFD group ($P<0.01$). A tendency toward higher excretion of lipids was observed in the fecal fatty acid profiles of experimental groups, as compared to normal control (Figure 4.7 A). Specifically, orlistat and META treated groups showed an increase in total lipids in feces by 173.7% and 115.8% respectively when compared with HFD group ($P<0.001$).

**Figure 4.7:** Effect of MEHR and META on fecal and liver fat content of rats fed with HFD. The values are represented as mean±S.D (n=6). Treatment groups were compared with HFD control (*$P<0.05$, **$P<0.01$ and ***$P<0.001$) and normal control with HFD control # $P<0.0001$. 

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<th>Liver fat (g)</th>
<th>Fecal fat content (g)</th>
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<tr>
<td>I</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>II</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>III</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>IV</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>V</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>VI</td>
<td>0.1</td>
<td>0.2</td>
</tr>
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Group I- Control  
Group II- HFD control  
Group III- Atorvastatin  
Group IV- Orlistat  
Group V- MEHR  
Group VI- META
4.4 DISCUSSION

In this study, high fat diet induced rat model of obesity was used to check the antiobesity and antihyperlipidemic potential of MEHR and META. Rats are commonly used animal models for studying the adverse effects of obesity (Iossa et. al., 1999). Humans and rodents have a similar tendency to gain weight when exposed to a long term high calorie diet intake (Chicco et al., 1999). Hence, diet induced obesity model of rat possess excellent utility in predicting weight loss in man (Vickers et al., 2011).

Gain in body weight is considered as an index of obesity (Toplak et. al., 2000; Van der Ploeg, 2000). The body weight of all groups was increased during the study period, especially higher in case of HFD fed groups compared to that of the normal control group. The HFD promoted a significant increase in body weight over the six week period. MEHR and META treated group showed decline in body weight gain in 10 days. An evident justification for the decrease in body weight gain is the reduction in food intake especially amongst META treated group. Therefore, the observed loss of body weight in obese rats is possibly due, at least in part, to a non-significant reduced food intake.

The energy intake is an essential factor in the regulation of body weight (Chicco et al. 1999). Rats having a palatable HFD instead of a standard chow diet take in more calories per day than rats fed only with standard chow diet. Eventually, they become obese and exhibit a number of complications of obesity. The total energy intake was higher in HFD treated group than the normal control group due to increase in food intake. Thus, it confirms that obesity induced in HFD treated group was the result of energy intake exceeding energy expenditure (Acheson 2004). This is the source of the increase in body weight. Food intake of rats was lowered after the treatment with META. Hence, the weight loss effect of META may be attributed to its effects on promoting satiety (Godard et. al, 2010) and delaying absorption of nutrients during digestion, which have been frequently reported in literature (Cladodes, 1997).

HFD induced a profound buildup of energy in the form of body fat, including peritoneal and perirenal fat masses. In addition, weight of liver was also increased by accumulation of fats resulting in fatty liver in HFD group. With the similar tendency of hepatic lipid accumulation and deposit of perirenal, and
peritoneal fat in treated rats were inhibited by MEHR and META. Increase in weights of visceral organs can be considered as another index of obesity (Toplak et al., 2000; Van der Ploeg, 2000). The liver is the chief organ responsible for maintaining cholesterol homeostasis. Various studies show that diet rich in cholesterol increases the hepatic cholesterol content resulting in the increase of triglyceride synthesis (Fungwe et al., 1993; Liu et al., 1995). No significant differences were observed in weight of kidney of all the groups.

The quantification of fat excreted in fecal matter is used as the primary assessment tool to examine the reduction of dietary fat absorption and to establish that a product has fat-binding properties (Gades and Stern, 2003; Hsu et al., 2006). Fecal excretions of total lipids were significantly higher in META and MEHR treated groups than those in HFD group. This suggests that, the intestinal absorption of lipids appeared to be more effectively inhibited by META and MEHR. But orlistat treated group showed maximum excretion of lipids in feces. The lowering of hepatic fat content and the elevation of fecal fat excretion in rats treated by MEHR and META indicates that hepatic lipid-lowering effect is probably related to a lower intestinal lipid absorption, resulting in an increase of hepatic bile acids biosynthesis, and finally leading to the decrease of hepatic lipid accumulations.

In the HFD rat model of obesity, high levels of serum TC and TG were reported. During HFD intake, liver can convert glucose into fatty acids, from which TGs are formed, which gets transported to the blood stream as VLDL-C and stored as fat in the adipose tissue (Colette et al. 2003). Delay in lipolysis of VLDL-C because of the competition for the site of lipoprotein lipase between VLDL-C from hepatic origin and chylomicrons from intestinal origin could lead to increase in TGs in the serum (Raveh et al. 2001). Treatment with MEHR and META caused significant decrease in TC, TG, VLDL-C, and LDL-C levels. This could be due to presence of phytosterols in the extracts, as they possess more affinity for micelles than cholesterol and reduce incorporation of cholesterol in micelles in the intestine (Ikeda and Sugano, 1998).

A significant increase in HDL-C level was observed in META treated group, a result in agreement with that of Kumar et al. (2009). Kumar et al., have reported *H. rosa sinensis* root extracts to have hypolipidemic effect on obese rats,
but it increases the HDL levels in rats. A low level of HDL is generally correlated with an increased risk of cardiovascular disease (Wilson et al., 1988). An increment in HDL levels by META could be attributed to presence of flavonoids and polyphenols in the extract as they are known to increase HDL levels (Daniel et al., 2003).

Herbal extracts rich in flavonoids and polyphenols have been reported to lower serum TC concentrations and LDL-C concentrations in humans (Arai et al., 2000; Koo and Noh, 2007; Tokunaga et al., 2002). This explains the effect of MEHR and META in lowering serum cholesterol levels in rats. A commonly suggested mechanism responsible for the lipid lowering effect of herbal proteins is the interference with circulation of lipids in the liver and intestines. This leads to an inhibition of hepatic secretion of lipids into circulation, which in turn is associated with hepatic lipoprotein production (Brandsch et al., 2010; Wahlqvist et al., 1999; Yang et al., 2011). Orlistat was also beneficial in lowering TC and LDL-C concentrations. This is consistent with the results of various earlier studies and is probably caused by both decrease in body weight and orlistat’s independent effect on cholesterol absorption (Hollander et al., 1998; Mittendorfer et al., 2000; Sjostrom et al., 1998)

The results showed that, HFD consumption leads to significant increase in serum glucose level in rats, which parallels with the previous studies (Zhang et al., 2008). Lowered hepatic uptake of glucose produced hyperlipidemia due to increased fat mobilization from the adipocytes and resistance to insulin. Impaired insulin action is linked with increase in lipid levels in blood. This leads to either storage of elevated lipids in tissues, e.g. muscle, liver, adipocytes or increased serum free fatty acids or triglycerides (Frayn, 2002). MEHR and META treatment significantly lowered serum blood glucose levels.

Polyphenols have been shown to modulate physiological and molecular pathways that are involved in energy metabolism, adiposity, and obesity. The process of development of fat cells, adipogenesis, is inhibited and the process of breakdown of fat cells is increased by plant polyphenols. This action proves the anti-obesity effect of polyphenols present in extracts (Raheem et al., 2013).

It was found that rats fed with high fat diet alone, developed a degree of hepatic steatosis or fatty liver when compared with normal control rats. Also serum AST and ATL levels were raised in HFD treated group when compared
with normal control group. The increase in liver enzymes levels and the formation of a fatty liver correlate with a significant increase of liver weights of HFD group. The treatments with MEHR resulted in prevention of hepatic fatty deposition in hepatocytes. However, significant elevation in serum AST and ALT levels were observed in META treated group. This could be due to presence of tannins or alkaloids in the extracts that are reported to be toxic when present in high amounts in herbal products (Mangan 1998).

Based on these broad observations, it was found that MEHR and META could effectively act as antiobesity and antihyperlipidemic agents. However, META treatment on HFD induced obesity resulted in detrimental effects on liver tissues. Whereas, MEHR counteracted the effects of HFD and normalized most of the biochemical parameters.
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