CHAPTER 5

PHARMACOLOGICAL EVALUATION OF THE PHF FOR ANTIOBESITY AND IN VIVO ANTIOXIDANT POTENTIAL

5.1 Objective

To evaluate the PHF for its antiobesity and in vivo antioxidant potential in male and female Wistar rats

5.2 Introduction

Obesity is rising year-on-year and has presently become a global epidemic. Central obesity culminates in metabolic complications (Jung et al., 2014; O’Neill and O’Driscoll, 2015). Energy imbalance is a major factor contributing to adiposity and its associated disorders (Birari et al., 2010 and Strader et al., 1998). In recent years natural products have been tremendously used as efficient therapeutic procedure to manage obesity (Han et al., 2005).

5.2.1 High Fat Diet and obesity

High Fat Diet (HFD) - induced obesity elevates lipid profile (Balzan et al., 2013) and induces hyperplasia in parametrial fat (Lemonnier, 1972). Animal models such as mice, rats, etc. have been successfully used in antiobesity studies to simulate human obesity. HFD induced obesity in animal models makes it possible to study the potential therapeutic effects of various medicinal plants either individually or in combinations.
5.2.2 Obesity, oxidative stress, inflammation and NAFLD

HFD induced obesity is associated with chronic oxidative stress, notably in liver, heart and kidney (Noeman et al., 2011); as a result of atherosclerosis and non-alcoholic hepatic steatosis (Ye and Keller, 2010). The redox imbalance needs to be adequately neutralized by antioxidants (Peairs and Abbey, 2013 and Valdecantos et al., 2009). Obesity induced oxidative stress can instigate and perpetuate inflammation (Peairs and Abbey, 2013). Depletion of catalase, glutathione peroxidase and glutathione reductase causes malfunction of tissues and elevated inflammatory responses that promote occurrence of harmful obesity-related clinical responses (Keaney et al., 2003).

During inflammation the adipocytokines such as Interleukins (IL-1, 10, 18 etc.) and TNF-α increases in adipose tissues which are capable of regulating appetite (Savini et al., 2013). Hypertrophy of adipocytes results in apoptosis triggering adipose tissue inflammation (Ouchi et al., 2011; Gonzalez-Castejon and Rodriguez- Casado 2011 and Suganami et al., 2007). Since obesity is a chronic inflammatory disease, one of the major complications associated with it is an impaired antioxidant defence system. Moreover, redox-inflammatory processes, together with viscera adiposity initiate disorder of liver function which contributes to dyslipidemic conditions like hypercholesterolemia, hypertriglyceridermia (Bryan et al., 2013; Savini et al., 2013). Glutathione (GSH) participates in the cellular defense system against oxidative stress by reducing various cellular molecules such as deoxyribose nucleic acid proteins and lipids (Circu and Aw, 2012). It is also a substrate for reduction of hydrogen peroxide and organic peroxides by Glutathione Peroxidase (GPx). Assessment of enhanced rates of free radical formation in tissues is routinely accomplished by measuring the accumulation of more stable secondary by-products of redox reactions and/or diminution of small-molecule antioxidants such as glutathione.
High Fat Diet (HFD) and effects of fat accumulation have been reported to implicate in the pathogenesis of Non Alcoholic Fatty Liver Disease (NAFLD) and increased production of Reactive Oxygen Species (ROS); two major reasons for morbidity (Wang et al., 2008; Deng et al., 2005; Day and James, 1998). NAFLD is characterised by abnormal lipid accumulation accompanied by inflammation of hepatocytes (Cerpa-Cruz et al., 2013). NAFLD represents different states such as fatty liver (steatosis), Non Alcoholic Steato Hepatitis (NASH), latter being characterised inflammatory changes. NASH is associated with obesity, dyslipidemia and diabetes (Madan et al., 2006; Anstee and Goldin, 2006; Adams et al., 2005 and Browning et al., 2004).

5.2.3 Natural therapy- promising alternative

There are various drugs such as L-Carnitine, Orlistat, Sibutramine, etc., currently being used in the management of obesity. But due to their untoward side effects on long term use, more attention has being paid towards the search for newer and safer remedies, particularly from herbal sources (Mohamed et al., 2014) with good therapeutic window effects. There is an urgent need for safe and efficient remedies for management and treatment of obesity. Herbal medicine is a major component in all traditional medicine systems, and a common element in Siddha, Ayurvedic, Homeopathic, Naturopathic, traditional Chinese, African, Korean and Native American medicine. Since natural drugs assure safety complemented with effectiveness, many advanced researches have been undertaken on validation of various forms of preparations.

Our study is focussed on the antiobesity and antioxidant effects of a new combination of PHF consisting of four medicinal plants such as *Phyllanthus emblica*. L (fruits), *Curcuma longa*. L (rhizome), *Macrotyloma uniflorum*. L (seeds), *Plumbago zeylanica*. L (roots) and preparation of the formulation was done as mentioned in detail in the previous section (section 3.4.2.).
5.3 Materials and Methods

5.3.1 Animal studies

5.3.1.1 Experimental protocol

Thirty, each of male and female Wistar rats (100-150g) were purchased from M/s.Sri Venkateshwara Enterprises, Bangalore. They were maintained under standard conditions (temperature: 22±2°C, relative humidity of 60±5% and 12 hours light/dark cycle). They had free access to water *ad libitum* and were adapted to standard pellet diet for a week. The experimental protocol was approved and ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC) (IAEC/KU/BT/12/021).

5.3.1.2 Acute oral toxicity

Acute oral toxicity of the PHF was carried out as per OECD test guidelines 423 for testing in male and female Wistar rats. The animals were administered the PHF with a maximum dose of 200mg/kg, *per orally*. They were then observed for mortality and clinical signs of toxicity and general behaviour for a period of four hours and thereafter twice a day for the next thirteen days. The Lethal Dose (LD$_{50}$) value of PHF and its classification was performed in accordance with Globally Harmonised System (GHS) of classification and labelling of chemicals, 2013 and OECD 423, (2001).

5.3.1.3 Experimental diets

The untreated control group were fed with normal pellet chow diet supplied by M/s.VRK Solutions, Bangalore. The HFD group was fed with a modified HFD (Srinivasan *et al.*, 2005). Composition of modified HFD is shown in Table 5.1.
The commercially available rat chow pellets were nutritionally analysed and found to consist of protein 24%, fat 7%, carbohydrate 50% and a calorific value of 3.8 kcal/g. Whereas, our High Fat Diet (HFD) prepared for induction of obesity contained 5.5 kcal/g (protein 33%, fat 36%, and carbohydrate 24%; 55% kcal from fat content) (Table 5.1).

Table 5.1: Composition of (High Fat Diet) HFD

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Pellet Chow Diet (NPCD)</td>
<td>333</td>
</tr>
<tr>
<td>Lard</td>
<td>302</td>
</tr>
<tr>
<td>Casein</td>
<td>222</td>
</tr>
<tr>
<td>Corn starch</td>
<td>88</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin and mineral mix</td>
<td>60</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>g%</th>
<th>kcal%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>33</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>36</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>24</td>
</tr>
<tr>
<td>Calories (kcal/g)</td>
<td>5.5</td>
</tr>
</tbody>
</table>

5.3.1.4 Induction of obesity and grouping

After seven days of acclimatisation, each of the thirty female and male Wistar rats were randomly assigned into two groups: Group I rats (n=6) were fed with commercial/normal pellet chow diet and Group II animals (n=24) were fed High Fat Diet (HFD) for a period of 6 weeks.
5.3.1.4.1 Treatments

On Day 43, Group II animals were reallocated into four groups i.e. Groups II, III, IV and V. Group II (n=6) were fed with only HFD. The three treatment groups III, IV and V (each n=6) were simultaneously fed with HFD and different treatment regimen as mentioned in Table 5.2. The treatments were given for a period of four weeks.

Table 5.2: Grouping of animals for the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Untreated Control (commercial pellet chow diet)+ normal saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>High Fat Diet (HFD) + normal saline</td>
</tr>
<tr>
<td>Group III</td>
<td>HFD+ STD (L-Carnitine, 250 mg/kg, <em>per oral</em> route)</td>
</tr>
<tr>
<td>Group IV</td>
<td>HFD + PHF (200 mg/kg, <em>per oral</em> route)</td>
</tr>
<tr>
<td>Group V</td>
<td>HFD+ PHF (400 mg/kg, <em>per oral</em> route)</td>
</tr>
</tbody>
</table>

5.3.1.5 Food consumption and calorie intake

The food intake for each rat per day was calculated by subtracting the amount of food left over in each cage from the measured amount of food provided at the previous day (g/day/rat) and the mean of food consumption per rat was considered by dividing the amount of food eaten in a week by 7. The average of food consumptions was represented in g/day/rat. Determinations were made at the beginning of the experiment, once a week, after the fattening period, and at the end of the experimental period.

Daily calorie intake (kJ/g) was calculated according to Zhao *et al.*, (2005) based on the formula:
Daily calorie intake (kJ/g) = daily food intake (g) x unit calorie (kJ/g).

Food Efficiency Ratio (FER) was calculated according to Cho et al., 2013, based on the formula:

\[
\text{FER} = \frac{\text{Increased body weight (g) [Final body weight (F2) - Initial body weight (F1)]}}{\text{Total food intake (g)}}
\]

Weight gain per caloric consumption = Final body weight (F2) - Initial body weight (F1) (g) / calorie value intake (kcal)

All the measurements were done after anaesthetisation of rats by inhalation of diethyl ether.

5.3.1.6 Anthropometric parameters measured

5.3.1.6.1 Body weight

The body weight (g) was recorded on Day 1 and weekly once using an electronic balance. At the end of the 10th week, blood was collected by retro-orbital puncture from overnight fasted rats after ether anaesthesia.

Percentage difference of body weight was calculated according to the formula:

\[
\frac{(\text{Final body weight} - \text{Initial body weight})}{\text{Initial body weight}} \times 100
\]
5.3.1.6.2 Body Mass Index (BMI)

The BMI was calculated by dividing the weight (g) by the length (cm²). The length of the rats was measured between nose and anus, leaving the tail region (Novelli et al., 2007).

5.3.1.6.3 Lee’s Index

Lee’s index was calculated as the ratio between cubic root of body weight and the Naso Anal Length (NAL) of the animal \[ \frac{3\sqrt{\text{body weight} (g)}}{\text{NAL} (cm)} \] (Novelli et al., 2007).

5.3.1.6.4 Abdominal Circumference (AC)

Abdominal Circumference (AC) was measured (cm) (Novelli et al., 2007) i.e., the girth at the level of the anterior abdomen, as an indicator of abdominal fat using a measuring tape.

5.3.1.7 Body temperature

The change in body temperature before and after drug administration was recorded on Day 70 using rectal tele thermometer at 30, 60, 90 and 120 minutes with a contact time of 1 minute. This experiment was carried out after measuring the body weight.
5.3.1.8 Spontaneous locomotor and exploratory behaviour

5.3.1.8.1 Locomotor activity

The effects of L-Carnitine and PHF on spontaneous locomotor activity were measured automatically by using Actophotometer, (Medicraft actophotometer, model No: 600-40, S. No: PA-0149, India). The beam breaks that were caused due to movement of rats were arbitrarily taken as units of activity; activity was recorded for 10 minutes. Basal activity score for all animals were noted before administration of the drugs. All the rats were re-tested for activity scores 30 minutes after administration of L-Carnitine and PHF (200 and 400 mg/kg) (Chirdrawar et al., 2011) (Figure 5.1).

Figure 5.1: Actophotometer

The effects of L-Carnitine and PHF on spontaneous locomotor activity were measured automatically by using Actophotometer, (Medicraft actophotometer, model No: 600-40, S. No: PA-0149, India). The beam breaks that were caused due to movement of rats were arbitrarily taken as units of activity; activity was recorded for 10 minutes. Basal activity score for all animals were noted before administration of the drugs. All the rats were re-tested for activity scores 30 minutes after administration of L-Carnitine and PHF (200 and 400 mg/kg) (Chirdrawar et al., 2011) (Figure 5.1).
5.3.1.8.2 Open field test

![Open field apparatus](image)

**Figure 5.2: Open field apparatus**

On Day 70, locomotor activity was recorded using open field behaviour test apparatus, 30 minutes after feeding of normal and HFD diet to group I and II and administration of L-Carnitine and PHF (groups III, IV and V). The apparatus consisted of a square wooden arena of 30 cm x 30 cm, 16 squares and wall with a height of 15 cm. Open field test was performed by placing the rat in the centre circle. Movements with respect to number of times of crossing (ambulatory activity), rearing (standing on rear paws) and grooming (rubbing face and repetitive licks directed to the body) were monitored visually for 5 minutes in a dim lit room (Chirdrawar *et al.*, 2011) (Figure 5.2).

5.3.1.9 Organ and Fat pad weights

After the animals were euthanised by ether overdose, they were sacrificed by cervical dislocation. Different organs (heart, liver, kidneys) and fat pads (mesenteric, epididymal and uterine fat pads) were removed. The samples were washed in ice cold saline followed by pH 7.4 buffer and blotted with filter paper. The samples were weighed on digital balance and weight was recorded in grams (g).
The relative organ weight was calculated using the following equation (Sahgal et al., 2010):

\[
\text{Relative organ weight (\%)} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100
\]

The adiposity index that is used to quantitatively measure the total fat composition, was calculated by the following equation (Gregoire et al., 2002):

\[
\text{Adiposity Index (\%)} = \frac{\sum \text{Fat p.a.d}}{\text{Body weight}} \times 100
\]

5.3.1.10 Biochemical analysis

5.3.1.10.1 Serum collection

On 0\textsuperscript{th} day and after the period of induction of obesity, animals were given mild anaesthesia. Blood was withdrawn via retro orbital sinus route and collected in sterile eppendorf tubes. The blood samples were also collected by cardiac puncture at the end of the experimental period after euthanizing the animals. To obtain a clear, non-haemolysed supernatant serum, the blood samples were centrifuged at 10,000 rpm for 10 minutes at room temperature. The serum was transferred into eppendorf tubes using clean dry disposable plastic syringes to be stored at -20°C.

5.3.1.10.2 Serum lipid profile and phospholipids measurements

The serum collected during the pre-treatment and post-treatment periods, were analysed with commercially available assay kits (M/s.Span Diagnostics, Surat, India) for determination of the levels of glucose (Glucose Oxidase-
Peroxidase- GOD/POD method) and lipid profile levels for High Density Lipoprotein Cholesterol (HDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), Total Cholesterol (TC), and Triglycerides (TG).

LDL-C was calculated using Friedewald’s equation (Friedewald et al., 1972):

\[
\text{LDL-C} = \text{[TC-\{HDL-C+ (TG/5)\}]}
\]

VLDL-C levels was calculated by subtracting the sum of HDL-C and LDL-C concentrations from TC (Morita et al., 1997)

Atherogenic Index (AI) was calculated as log (TG/HDL-C) (Dobiasova and Frohlich 2001) and Coronary Risk Index (CRI) as ratio of TC and HDL-C (Alladi and Shanmugasundaram 1989), respectively.

5.3.1.10.3 Analysis of kidney, liver function tests, cardiac marker enzymes

The following tests for enzyme activity respective of the organs were done in serum using commercial kits (M/s. Span Diagnostics).

a. **Liver function tests**: Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT).

b. **Kidney function tests**: Creatinine, Urea.

c. **Cardiac marker enzymes**: Creatinine Phosphokinase-MB isoenzyme (CK-MB), Lactate dehydrogenase (LDH).
5.3.1.10.4 Liver tissue preparation and biochemical analysis

5.3.1.10.4.1 Liver lipid content

Tissue lipids were extracted as supernatant from liver samples according to Folch et al., (1951) and the TC and TG levels were determined using commercial assay kits (M/s.Span Diagnostics, Surat, India).

5.3.1.10.4.2 Measurement of oxidative stress, LPO

A portion of each liver was taken from all the groups and tissue homogenate (10% w/v) was prepared with 0.025 M Tris-HCl buffer, (pH 7.5). After centrifugation at 3500 rpm for 10 min, the clear supernatant was used to measure the following hepatic markers of oxidative stress.

Thio Barbituric Acid Reactive Substances (TBARS) by the method of Okhawa et al., (1979). The levels of Reactive Oxygen Species (ROS) that are controlled by antioxidant enzymes like SOD (Super Oxide Dismutase) (Kakkar et al., 1984, CAT (Catalase) (Sinha, 1972), GPx (Glutathione Peroxidase) (Rotruck et al., 1973), GR (Glutathione Reductase) (Beutler et al., 1984) and GST (Glutathione -S- Transferase) (Habig et al., 1974) were estimated. The non-enzymatic scavengers such as reduced Glutathione (GSH) (Moron et al., 1979), Vitamin C (Omaye et al., 1979) and Vitamin E (Desai 1984) were determined. Total Protein was determined by method of Lowry et al., (1951).

5.3.1.10.5 Estimation of serotonin in brain tissue homogenates

Levels of serotonin of brain homogenates was determined by the method of Schlumf et al., (1974).
5.3.1.11 **Histopathological studies**

Permanent histopathological preparations using routine methods of Bancroft and Stevens (1982) were made. Liver, heart and adipose tissues were fixed in 10% neutral buffered formalin (10ml formalin in 100 ml normal saline). The tissues were subsequently dehydrated in upgraded concentrations of ethyl alcohol and water. The tissues were cleansed in xylene, impregnated and embedded in paraffin wax. Several sections of 3-6 μm were cut using a microtome (Bright Instruments Co.Ltd., U.K.) and stained with hematoxylin and eosin dye (H&E). The architecture was observed at low power (10X). The changes due to liver injury were observed under high power (40X).

5.3.1.12 **Statistical analysis**

The results are expressed as mean ± S D (6 numbers of rats each in 5 groups). Mean differences between the groups were analysed by Analysis of Variance (ANOVA) followed by Bonferroni’s post test using Graph Pad Prism 5.01 software. P values, *p< 0.05, †p<0.01, ‡p<0.001 were considered significant between group II and treatment groups III, IV and V. *p<0.001 between group I and II.

5.4 **Results**

5.4.1 **Acute toxicity studies**

The rats were administered with a maximum dose of PHF (2000 mg/kg body weight). The lethal dose (LD50) of 2 g/kg body weight was fixed. The results of the acute toxicity study showed no mortality and toxicity symptoms in rats. This was observed till the end of the study. The gross behavioural parameters such as alertness, grooming, touch response, pain response etc. were normal (Table 5.3) and showed no significant changes in food and water intake.
Since there were no gross pathological findings in the experimental animals, in accordance with the GHS Classification and Labelling of chemicals, our PHF was classified as Category-5. Hence, one tenth (200 mg/kg) and one fifth (400 mg/kg) of the LD$_{50}$ was selected for the study.

5.4.2 Effects of L-Carnitine and PHF on food and calorie intake in male and female Wistar rats

Food intake was significantly increased (p<0.001) in HFD group compared to the untreated control group. The percentage gain in food consumption during the first week was non-significant in male rats (p> 0.05, 0.4 %) but was significant in female rats (p<0.001, 7.1%). The percentage gain was however significant from the second week to the sixth week in both the sexes (p<0.01, p<0.001, males- 6.9 % to 16.6 %; females- 8.7 % to 15.3 %).

Table 5.3: Gross behavioural outcomes observed in Wistar rats after the administration of PHF

<table>
<thead>
<tr>
<th>S. No</th>
<th>Response</th>
<th>Head</th>
<th>Body</th>
<th>Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Alertness</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2.</td>
<td>Grooming</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>3.</td>
<td>Touch Response</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4.</td>
<td>Pain response</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>5.</td>
<td>Tremors</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>
6. **Convulsion**  Absent  Absent  Absent  Absent  Absent  Absent  Absent

7. **Righting reflux**  Normal  Normal  Normal  Normal  Normal  Normal  Normal

8. **Gripping strength**  Normal  Normal  Normal  Normal  Normal  Normal  Normal

9. **Pinna reflux**  Present  Present  Present  Present  Present  Present  Present

10. **Corneal reflux**  Present  Present  Present  Present  Present  Present  Present

11. **Writhing**  Absent  Absent  Absent  Absent  Absent  Absent  Absent

12. **Pupils**  Normal  Normal  Normal  Normal  Normal  Normal  Normal

13. **Urination**  Normal  Normal  Normal  Normal  Normal  Normal  Normal

Treatments with L-Carnitine and PHF (200 mg/kg and 400 mg/kg) by oral route for 4 weeks significantly reduced (p<0.05, p<0.01, p<0.001) food intake by 12.8 %, 9.5 % and 9.7 % in male rats and by 12.9%, 12.3% and 12.5 % in female rats respectively at the end of the study (Table 5.4). The normal pellet chow diet of a calorie value of 380 kcal/100g and 550 kcal/100g from HFD was continuously given to female and male Wistar rats during the experimental period of 10 weeks. Figures 5.3, 5.4 (i, ii) show the changes in food consumption on feeding with HFD and subsequent treatment effects of L-Carnitine and PHF in both the sexes of Wistar rats.

Daily calorie intake and Food Efficiency Ratio (FER) (Table 5.4) increased significantly in HFD rats of both the sexes (p< 0.05). Whereas, subsequent treatment with L-Carnitine and PHF showed significant reduction (p<0.05) in daily calorie intake and FER.
5.4.3 Effects of L-Carnitine and PHF on body weight in male and female Wistar rats

The body weights of HFD induced rats (group II) increased significantly (p< 0.001) compared to untreated control rats (group I) during the HFD induction period of six weeks. The body weight gain in the first week was non-significant (p> 0.05, male rats- 0.09% and female rats- 2.31%) whereas, body weight gain was significant from the second week to sixth week (p<0.001, males- 3.9% to 15%; females- 5.1% to 16.7%). A 19.5% and 21.7% increase in body weight was observed in male and female HFD group respectively as compared to the untreated rats (group I).

Treatments with L-Carnitine and PHF (200 and 400 mg/kg) by oral route for four weeks significantly reduced (p< 0.001) body weight gain by 55%, 56.5% and 56.4% in male rats and by 45.6%, 46.9%, 46.2% in female rats respectively. However, there were no significant differences in body weight gain between the two doses of PHF i.e. 200 and 400 mg/kg treatments i.e. even the lower dose was effective in achieving significant weight loss. Overall, the above results indicated strong antiobesity effects of the PHF on HFD induced obese rats similar to L-Carnitine (Tables 5.4, 5.5). Figures 5.5, 5.6 (i, ii) show body weight changes in all the groups.
Figure 5.3 i, ii: Effects of L-Carnitine and PHF on food intake in male Wistar rats

Figure 5.4 i, ii: Effects of L-Carnitine and PHF on food intake in female Wistar rats

All values are expressed as mean ± S D (n=6 rats/group). *p< 0.001 compared between group II with Group I and $p< 0.001, \#p< 0.01, \@p< 0.05 when compared between Group II and treatment groups III, IV, V using ANOVA followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral); Group V- HFD+ PHF (400 mg/kg, per oral);The above results indicate that food intake significantly reduced in treatment groups III, IV and V in both the sexes.
### Table 5.4: Percentage change in food intake, weight gain/calorie consumption, daily calorie intake (kJ/g), FER during the experimental period in male and female Wistar rats

<table>
<thead>
<tr>
<th>Gps</th>
<th>Percentage difference in food intake (%)</th>
<th>Weight gain/calorie consumption</th>
<th>Daily calorie intake (kJ/g)</th>
<th>Food Efficiency Ratio (g/ kcal x 10²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>14</td>
<td>12</td>
<td>F</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>-8.2</td>
<td>-8.7</td>
<td></td>
<td>0.401</td>
</tr>
<tr>
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<td></td>
<td>15</td>
<td>F</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.90</td>
<td>±0.45</td>
<td></td>
<td>0.81</td>
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<td>III</td>
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<td></td>
<td>F</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>-12.8</td>
<td>-12.9</td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>14</td>
<td>F</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>-9.56</td>
<td>-12.3</td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>15</td>
<td>F</td>
<td>12</td>
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<tr>
<td></td>
<td>-9.7</td>
<td>±0.37</td>
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<td>0.76</td>
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</tbody>
</table>

All values are expressed as mean ± S D, (n=6 rats/group). *p<0.001 as compared between treatment groups III, IV, V with Group II (ANOVA, followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral); Group V- HFD+ PHF (400 mg/kg, per oral); The above parameters showed significant decrease in the treatment groups III, IV and V in both the sexes.
Figure 5.5 i, ii: Effects of L-Carnitine and PHF on body weight in male Wistar rats

All values are expressed as mean ± S D (n=6 rats/group). *p< 0.001, †p< 0.05 compared between group II with Group I and ‡p< 0.001, §p< 0.01 when compared between Group II and treatment groups III, IV, V using ANOVA followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral); Group V- HFD+ PHF (400 mg/kg, per oral); The above results showed that body weight significantly reduced in the treatment groups III, IV and V in both the sexes.

Figure 5.6 i, ii: Effects of L-Carnitine and PHF on body weight in female Wistar rats

All values are expressed as mean ± S D (n=6 rats/group). *p< 0.001, †p< 0.05 compared between group II with Group I and ‡p< 0.001, §p< 0.01 when compared between Group II and treatment groups III, IV, V using ANOVA followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral); Group V- HFD+ PHF (400 mg/kg, per oral); The above results showed that body weight significantly reduced in the treatment groups III, IV and V in both the sexes.
Table 5.5: Percentage of body weight gain during the experimental period in male and female Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight gain (g)</th>
<th>Percentage of body weight gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>I</td>
<td>69.18</td>
<td>61.42</td>
</tr>
<tr>
<td>II</td>
<td>108.30</td>
<td>97.16*</td>
</tr>
<tr>
<td>III</td>
<td>89.05#</td>
<td>79.00#</td>
</tr>
<tr>
<td>IV</td>
<td>91.38#</td>
<td>81.30#</td>
</tr>
<tr>
<td>V</td>
<td>91.21#</td>
<td>80.30#</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S D (n=6 rats/group). *p< 0.001, compared between group II with Group I and *p< 0.001 when compared between Group II and treatment groups III, IV, V using ANOVA followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral); Group V- HFD+ PHF (400 mg/kg, per oral); The above results indicated that there was significant reduction in body weight gain in the treatment groups III, IV and V.

5.4.4 Effects of L-Carnitine and PHF on BMI, Abdominal Circumference (AC) in male and female Wistar rats

5.4.4 i: Effects on AC- The AC of HFD induced rats (group II) increased significantly (p< 0.05, males: 16.7% and females: 21%) than the untreated control group (group I). The percentage gain in AC was significant (p< 0.001) in the treatment groups (males: Group III- 5.7%; IV- 9.1%; V- 8.4% and females: III- 4.2%; IV- 6.5%; V- 5.5%) as compared to the gain in the HFD group. The percentage reduction in AC of treatment groups for both the sexes was significant (p<0.001, males: III- 9.43%, IV- 6.5%, V- 7.1%; females: III- 13.9%, IV- 12.0%, V- 12.8%) compared to HFD group. The above results indicated no
significant differences between the two doses of PHF i.e. 200 and 400 mg/kg treatments and that the lower dose also showed equivalent antiobesity effects compared to L-Carnitine (Table 5.6). Figure 5.7 i shows changes in AC in all the groups of rats.

5.4.4 ii: Effects on BMI- The BMI of HFD fed rats (group II) increased significantly (p< 0.05, male: 49% and female: 60%) than the untreated control group (group I). The percentage gain in BMI was significant (p< 0.05) in the treatment groups (males: Group III- 32%; IV- 33%; V- 33.4% and females: Group III- 30.1%; IV- 30.3%; V- 30 %) compared to the gain in group II. The percentage reduction in BMI in treatment groups for both the sexes III, IV, V was significant (p<0.05, males- 11.5%, 10.5%, 10.6%; females- 10.7%, 10.6%, 10.7% respectively) compared to HFD group (Table 5.6). Figure 5.7 ii shows changes in BMI in all the groups of rats.

Figure 5.7 i, ii: Changes in Abdominal Circumference (AC) and BMI observed in male and female Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). a, b, c, d, e, p< 0.001 compared between group II with Group I and f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, x, y, z, p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); It was observed that Abdominal Circumference and BMI significantly reduced in the treatment groups III, IV and V.
Table 5.6: Percentage increase/ decrease in AC and BMI in male and female Wistar rats

<table>
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<tr>
<th>Groups</th>
<th>AC (%)</th>
<th>BMI (%)</th>
<th>Lee’s index</th>
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<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>I</td>
<td>-14.34</td>
<td>-12.44</td>
<td>-33.00</td>
</tr>
<tr>
<td>II</td>
<td>16.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>III</td>
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<tr>
<td>V</td>
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<td>-12.8&lt;sup&gt;#&lt;/sup&gt;</td>
<td>-10.6&lt;sup&gt;@&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S D, (n=6 rats/group). *p< 0.001 compared between treatment groups III, IV, V with Group I and *p< 0.001, *p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); Significant reduction in AC and BMI and Lee’s index was observed in the treatment groups III, IV and V.

5.4.5 Effects of L-Carnitine and PHF on body temperature (thermogenic effects) in male and female Wistar rats

Treatments with a dose of 250 mg/kg of L-Carnitine and low and high doses of PHF showed significant rise in body temperature after 1 and 2 hours compared to untreated control and HFD fed rats of both the sexes. The temperature rise was observed after 60 minutes of treatment compared to body temperature change observed in group II rats (HFD control group) and untreated rats (Group I). Figure 5.8 i, ii presents changes in body temperature after 1 hour of administration of normal diet, HFD, different treatments.
Figure 5.8 i: Effects of L-Carnitine and PHF on body temperature (thermogenic effects) in male Wistar rats

Figure 5.8 ii: Effect of L-Carnitine and PHF on body temperature (thermogenic effects) in female Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). \(^*p< 0.05, ^{#}p<0.01, ^{#}p<0.001\) compared between treatment groups III, IV, V with Group II (ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II-High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral); Group V- HFD+ PHF (400 mg/kg, per oral); Significant increase in body temperature was observed in the treatment groups III, IV and V.

5.4.6 Effects of L-Carnitine and PHF on spontaneous locomotor activity in male and female Wistar rats

Open field test for measuring the exploratory behavior was carried out in all the groups of rats (Figures 5.9 i, ii). Rats maintained as HFD control group
(Group II) showed a significant decrease (p< 0.001) in rearing activity and ambulatory as compared to untreated control rats. Treatments with L-Carnitine and PHF showed a significant increase (p< 0.05, p<0.01, p<0.001) in ambulatory and rearing activity that were altered due to HFD induced obesity.

The closed field test for studying the locomotor activity was carried in all the groups of rats (Figures 5.10 i, ii). The number of times the photocell beam interrupted by group II rats were significantly lesser than measured in group I rats. Whereas, the photocell beam interruption showed more significance (p< 0.001) in the treatment groups (III, IV and V) when compared to the HFD fed group (II). The above results indicated that the rats maintained on HFD (Group II) diet showed impaired locomotor activity and this impairment was significantly reduced (p<0.05, p<0.01, p<0.001) upon treatment with L-Carnitine and PHF.

5.4.7 Effects of L-Carnitine and PHF on organ weights (liver, heart, and kidney) and fat pads (mesenteric, epididymal, uterine)

Rats of both the sexes fed with HFD showed a significant increase (p<0.001) in relative weight of heart and liver compared to untreated control group. Treatments with PHF and L-Carnitine showed a significant decrease (p<0.05, p<0.01, p<0.001) in heart and liver weights compared to HFD group.

The relative weight of the epididymal (male) and uterine adipose tissue (female) were significantly lowered (p<0.01, p<0.001 respectively) in the treatment groups compared to the HFD group. Overall, the supplementation with PHF resulted in significantly relative lower weights compared to that of HFD group.
Figure 5.9 i, ii: Effects of L-Carnitine and PHF on exploratory behaviour in male and female Wistar rats.

Figure 5.10 i, ii: Effects of L-Carnitine and PHF on locomotor activity observed in treated and untreated male and female Wistar rats.

All values are expressed as mean ± S D, (n=6 rats/group). a$p< 0.001$ compared between treatment groups III, IV, V with Group I and b$p< 0.001$, c$p< 0.01$, d$p< 0.05$ compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral). Activity of the animals which is expressed in terms of ambulation and rearing was observed to be increased after the administration of PHF and L-Carnitine.
However, there were no significant changes (p>0.05) observed in relative weights of kidneys in all the groups of the both the sexes. Fat pad weights were expressed as relative weight (Figure 5.11 i, ii, iii) (Table 5.7, 5.8). However there were no significant changes in the weight of spleen in the both the sexes of rats.

5.4.8 Effects of L-Carnitine and PHF on biochemical parameters in male and female Wistar rats

5.4.8.1 Serum glucose

There was a significant increase in serum glucose levels in groups of both the sexes of HFD group (Figure 5.12 i-iv) (p< 0.001) compared to the untreated control group. The treatment groups i.e., L-Carnitine administered (group III), PHF 200 mg/kg (group IV) and PHF 400 mg/kg (group V) showed significant reduction (p<0.05) in glucose concentration during the experimental period.

5.4.8.2 Effects of L-Carnitine and PHF on serum lipid profile and phospholipids in male and female Wistar rats

Concentrations of serum lipids in different groups are shown in Figures 5.13 i, ii. HFD rats of both the sexes showed elevated levels of TG, TC, LDL-C and VLDL-C as compared to untreated control rats (p<0.001). The treatment groups showed significantly lowered levels TG, TC, LDL-C and VLDL-C in both the sexes as compared to the HFD group (p<0.05, p<0.01, p<0.001). HDL was significantly increased in L-Carnitine 250 mg/kg (males: +92%; females: +102%; PHF 200 mg/kg (males: +45%; females: +85% and PHF 400 mg/kg (males: +53%; females: +90% compared to HFD group (males: -17%; females: -57%. The protection % was higher males: 35%, 22%, 31%; females: 56%, 47% and 49% respectively in the treatment groups compared to untreated control.
Coronary risk index was significantly reduced in the treatment groups III, IV and V as compared to untreated control (Table 5.9).

Table 5.7: Percentage change in organ/tissue weight during the experimental period in male and female Wistar rats

| Gps | Male rats |  | Female rats |  |
|-----|-----------|  |-------------|  |
|     | % increase |  | % decrease |  |
|     | Liver (g) | Epididymal fat (g) | Mesentric fat (g) | Liver (g) | Uterine fat (g) | Mesentric fat (g) |
| II  | 32.3a | 922a | 219a | 45a | 198a | 550a |
| III | 10.4a | 35.4# | -14.3# | -41# | 21.3# | -55.2# |
| IV  | -8.3# | 33.1# | -13.0# | -19.5# | 15.1# | -58.2# |
| V   | -9.3# | 31.3# | -13.0# | -13.7# | 15.2# | -53.8# |

Table 5.8: Relative organ weight (%) and adiposity index (%) measured after the experimental period in male and female Wistar rats

| Groups | Relative liver weight (%) |  | Adiposity index (%) |  |
|--------|--------------------------|  |---------------------|  |
|        | M | F | M | F |
| I      | 0.028 ± 0.2 | 0.026±0.9 | 1.28±1.2 | 0.66±0.3 |
| II     | 0.031 ±0.3a | 0.030±0.2a | 2.99±1.1a | 2.89±0.9a |
| III    | 0.030 ±1.0# | 0.029±1.3# | 2.58±1.3# | 1.78±0.8# |
| IV     | 0.031 ±1.2# | 0.028±1.4# | 2.63±0.3# | 1.81±0.7# |
| V      | 0.031± 0.9# | 0.027±0.8# | 2.62±0.8# | 1.80±0.3# |

All values are expressed as mean ± S D, (n=6 rats/group). a_p< 0.001 compared between treatment groups III, IV, V with Group I and 5_p< 0.001, 6_p< 0.01, 7_p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); The above indices of obesity such as organ/tissue weight, their relative weight and adiposity index was found to be significantly decreased in the treatment groups III, IV and V.
Figure 5.11 i, ii, iii: Effects of L-Carnitine and PHF on organ weights (liver, heart) and fat pads (mesenteric, epididymal, uterine)

All values are expressed as mean ± S D, (n=6 rats/group). *p < 0.05 compared between treatment groups III, IV, V with Group I and "p < 0.01, #p < 0.01, @p < 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); Significant reduction in the weight of organ/ tissues was observed in treatment groups III, IV and V.
Figure 5.12 i-iv: Effects of L-Carnitine and PHF on glucose concentration in female and male Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). $^a$p< 0.001 compared between treatment groups III, IV, V with Group I and $^b$p< 0.001, $^c$p< 0.01, $^d$p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); Significant increase in levels of serum glucose was observed after the HFD induction; Significant decrease in levels of serum glucose was observed after the treatment with PHF and L-Carnitine.

In contrast, treatments with L-Carnitine and PHF significantly increased HDL-C concentrations in both the sexes. In addition, L-Carnitine and PHF (200 and 400 mg/kg) treatments in both HFD induced male and female rats caused
significant decrease (p<0.05) in atherogenic index (Figure 5.14 i) and coronary risk indices (p<0.05, Figure 5.14 ii). The protection % in HFD was calculated with respect to percentage difference between the HFD and the treatment groups, which showed more than 100 % reduction.

The concentrations of phospholipids and free fatty acids were significantly elevated in HFD group compared to untreated control rats (p< 0.05). However, treatments with L-Carnitine 250 mg/kg, PHF 200 mg/kg and PHF 400 mg/kg in both male and female rats showed significant reduction compared to HFD group (Figure 5.15 i, ii).

Table 5.9: Percentage change in serum lipid profile levels in male and female Wistar rats

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<tr>
<th>Gps</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>% Protection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
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<td>146</td>
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<td>57</td>
</tr>
<tr>
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<td>-36#</td>
<td>92#</td>
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</tr>
<tr>
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<td>-17#</td>
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<td>45#</td>
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</tr>
<tr>
<td>V</td>
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<td>-24#</td>
<td>-18#</td>
<td>-50#</td>
<td>53#</td>
<td>90#</td>
</tr>
</tbody>
</table>

Units for TG, TC, HDL, LDL and VLDL expressed as mg/dl ; Values expressed as %.

All values are expressed as mean ± S D, (n=6 rats/group). #p<0.001 as compared between treatment groups III, IV, V with Group II (ANOVA, followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II-High Fat Diet (HFD) + normal saline; Group III- HFD + STD (L-Carnitine, 250 mg/kg, per oral route); Group IV- HFD + PHF (200 mg/kg, per oral); Group V- HFD+ PHF (400 mg/kg, per oral); Significant decrease in TG, TC, LDL and VLDL increase in HDL was observed in the treatment groups III, IV and V.
Figure 5.13 i, ii: Effects of L-Carnitine and PHF on serum lipid profile in male and female Wistar rats.

All values are expressed as mean ± S D, (n=6 rats/group). *p< 0.001 compared between treatment groups III, IV, V with Group I and §p< 0.001, $p< 0.01, @p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral route); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); Significant decrease in TG, TC, LDL and VLDL increase in HDL was observed in the treatment groups III, IV and V.
Figure 5.14 i, ii: Atherogenic index in treated and untreated male and female Wistar animals.

Figure 5.15 i, ii: Effects of L-Carnitine and PHF on free fatty acids and phospholipids in male and female Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). a p< 0.001 compared between treatment groups III, IV, V with Group I and $ p< 0.001, \# p< 0.01, @ p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); Significant decrease in decrease in AI and CRI was observed in the treatment groups III, IV and V of female rats compared to male rats.
5.4.8.3 Effects of L-Carnitine and PHF on hepatic and faecal lipids in male and female Wistar rats

Figure 5.16 i shows changes in hepatic TC and TG content in all groups. HFD fed group showed significant increase (p<0.001) in the lipid contents compared to group I. Significant decrease (p<0.05, p<0.01, p<0.001) in hepatic TC and TG content was observed in treatment groups (III, IV and V) compared to the group II.

Faecal lipid content analysis was done and showed significant changes. There was a significant elevation (p<0.05, p<0.01, p<0.001) in the lipid content in the groups III, IV and V compared to group I (Figure 5.16 ii) compared to HFD group. The results indicate there was significant elimination of lipid contents through the faeces.

**Figure 5.16 i, ii: Effects of L-Carnitine and PHF on hepatic and faecal lipids in male and female Wistar rats**

All values are expressed as mean ± S D, (n=6 rats/group). *p< 0.001 compared between treatment groups III, IV, V with Group I and †p< 0.001, ‡p< 0.01, §p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, *per oral*); Group IV- HFD + PHF (200 mg/kg, *per oral route*); Group V- HFD+ PHF (400 mg/kg, *per oral*); Significant increase and decrease in faecal and hepatic lipid levels respectively was observed in the treatment groups III, IV and V.
5.4.8.4 Effects of L-Carnitine and PHF on serum and hepatic markers of oxidative stress in male and female Wistar rats

The HFD group showed a significant (p< 0.001) decrease in serum and hepatic GST, GPx, GR, SOD, and CAT, GSH, Vitamin C and E levels compared to untreated group. L-Carnitine treatment at 250 mg/kg dose and PHF treatments at 200 and 400 mg/kg doses resulted in significant (p< 0.05, p<0.01, p<0.001) increase in the levels of both the enzymic and non enzymic antioxidant levels (Figures 5.17, 5.18, Tables 5.10, 5.11 shows the levels of enzymic and non enzymic antioxidants in both the sexes of Wistar rats).

5.4.8.5 Effect of L-carnitine and PHF on levels of hepatic enzymes and lipid peroxidation activity in male and female Wistar rats

The current results showed significant reduction (p< 0.001) in liver enzymes, AST and ALT in the HFD fed group compared to untreated control rats (Figure 5.19 ii). The Treatments with L-Carnitine and two doses of PHF (200 and 400 mg/kg) significantly reverted the levels to near normal values (p<0.05, p<0.01, p<0.001). There was no significant difference noted between the treatment groups (Groups III, IV, V).

Levels of TBARS were seen to be significantly increased (p< 0.001) in the HFD induced obese rats (Group II) compared to the untreated control group. Administration of PHF and L-Carnitine showed significant decrease (p< 0.05, p<0.01, p<0.001) in the levels and also no significant difference was observed in treatment effect between the PHF and L-Carnitine treatment groups (Figure 5.19 i).
Table 5.10: Effects of L-Carnitine and PHF on serum markers of oxidative stress (enzymic antioxidants) in male and female Wistar rats

<table>
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<tr>
<th>Gps</th>
<th>GST</th>
<th>GR</th>
<th>GPx</th>
<th>SOD</th>
<th>CAT</th>
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<td>F</td>
<td>M</td>
<td>F</td>
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</tr>
<tr>
<td>I</td>
<td>1.94±0.10</td>
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<td>IV</td>
<td>0.87±0.07</td>
<td>2.41±0.10</td>
<td>3.68±0.13</td>
<td>6.79±0.12</td>
<td>0.50±0.10</td>
</tr>
<tr>
<td>V</td>
<td>0.89±0.10</td>
<td>2.64±0.14</td>
<td>4.1±0.104</td>
<td>7.20±0.47</td>
<td>0.53±0.10</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S D, (n=6 rats/group). *p< 0.001 compared between group II with Group I and "p< 0.001, 5p< 0.01, 6p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); Significant increase of enzymic antioxidant levels was observed in the treatment groups.

SOD expressed 50% inhibition of NBT reduction in one minute; CAT expressed as µmoles of H2O2 decomposed /min/mg protein; GST expressed as µmoles of CDNB-GSH conjugate formed/min/mg protein; GR expressed as µmoles of glutathione consumed/min/mg protein; GPx expressed as µmoles of glutathione utilised/min/mg protein.
Figure 5.17: Effects of L-Carnitine and PHF on hepatic markers of oxidative stress (enzymic antioxidants) in liver tissues of male and female Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). *p< 0.001 compared between group II with Group I and **p< 0.001, ***p< 0.01, ****p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral); Significant increase of enzymic antioxidant levels was observed in the treatment groups.

SOD expressed 50% inhibition of NBT reduction in one minute; CAT expressed as μmoles of H2O2 decomposed /min/mg protein; GST expressed as μmoles of CDNB-GSH conjugate formed/min/mg protein; GR expressed as μmoles of glutathione consumed/min/mg protein; GPx expressed as μmoles of glutathione utilised/min/mg protein.
**Table 5.11**: Effects of L-Carnitine and PHF on serum markers of oxidative stress (non-enzymic antioxidants) in male and female Wistar rats

<table>
<thead>
<tr>
<th>Gps</th>
<th>Vit C M</th>
<th>Vit C F</th>
<th>Vit E M</th>
<th>Vit E F</th>
<th>GSH M</th>
<th>GSH F</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.23±0.04</td>
<td>1.38±0.04</td>
<td>0.95±0.01</td>
<td>0.94±0.03</td>
<td>1.55±0.04</td>
<td>1.57±0.01</td>
</tr>
<tr>
<td>II</td>
<td>0.59±0.02a</td>
<td>0.62±0.06a</td>
<td>0.51±0.01a</td>
<td>0.53±0.06a</td>
<td>0.44±0.04a</td>
<td>0.53±0.05a</td>
</tr>
<tr>
<td>III</td>
<td>1.18±0.006#</td>
<td>1.21±0.03#</td>
<td>0.87±0.01#</td>
<td>0.85±0.02#</td>
<td>1.25±0.01#</td>
<td>1.24±0.01#</td>
</tr>
<tr>
<td>IV</td>
<td>1.23±0.02#</td>
<td>1.24±0.02#</td>
<td>0.83±0.008#</td>
<td>0.83±0.03#</td>
<td>1.1±0.04#</td>
<td>1.07±0.15#</td>
</tr>
<tr>
<td>V</td>
<td>1.27±0.01#</td>
<td>1.28±0.04#</td>
<td>0.83±0.01#</td>
<td>0.84±0.02#</td>
<td>1.09±0.04#</td>
<td>0.98±0.09#</td>
</tr>
</tbody>
</table>

**Figure 5.18**: Effects of L-Carnitine and PHF on hepatic markers of oxidative stress (non-enzymic antioxidants) in liver tissues of male and female Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). *p< 0.001 compared between group II with Group I and *p< 0.01, *p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral). Significant increase of Vit C and E levels was observed in the treatment groups. Vit C, E expressed as IU/L & µg/gm tissue; GSH expressed as µg of GSH consumed / mg protein.
Figure 5.19 ii, iii: Effect of L-carnitine and PHF on levels of hepatic enzymes and lipid peroxidation activity in male and female Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). *p< 0.001 compared between group II with Group I and #p< 0.001, $p< 0.01, @p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Un treated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral). Significant decrease in AST, ALT and LPO levels was observed in the treatment groups. AST, ALT expressed as IU/L; LPO expressed as nmol of MDA/mg protein.

5.4.8.6 Effects of L-Carnitine and PHF on levels of cardiac biomarkers in male and female Wistar rats

The HFD group showed significant (p< 0.001) raised levels of serum LDH and CK-MB compared to the untreated control rats. Our findings indicated that HFD induced obese rats on treatment with L-Carnitine and PHF exhibited significant (p<0.05, p<0.01, p<0.001) decrease in LDH and CK-MB concentrations (Figure 5.20 i, ii). The above results indicated that relation of lipid peroxide formation and diagnostic myocardial enzymes (LDH and CK-MB).
5.4.8.7 Effects of L-Carnitine and PHF on brain serotonin levels in male and female Wistar rats

The results showed significant reduction (p< 0.001) in brain serotonin levels the HFD fed group compared to untreated control rats (Figure 5.21). The Treatments with L-Carnitine and two doses of PHF (200 and 400 mg/kg) significantly reverted the levels to near normal values (p<0.05, p<0.01, p<0.001). There was no significant difference (p> 0.05) noted between the treatment groups (Groups III, IV, V).

Figure 5.20: Effects of PHF and L-Carnitine on LDH and CK-MB levels in male and female Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). "p< 0.001 compared between groups II with Group I and "p< 0.001, "p< 0.01, "p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I- Untreated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral). Significant increase in LDH and CK-MB was observed.
Figure 5.21: Effects of L-Carnitine and PHF on brain serotonin levels in male and female Wistar rats

All values are expressed as mean ± S D, (n=6 rats/group). "p< 0.001 compared between groups II with Group I and "p< 0.001, "p< 0.01, "p< 0.05 compared between treatment groups III, IV, V with Group II using ANOVA, followed by Bonferroni’s multiple comparison test. Group I-Untrated Control (commercial pellet chow diet); Group II- High Fat Diet (HFD) + normal saline; Group III- HFD+ STD (L-Carnitine, 250 mg/kg, per oral); Group IV- HFD + PHF (200 mg/kg, per oral route); Group V- HFD+ PHF (400 mg/kg, per oral). Significant increase in LDH and CK- MB was observed.

5.4.8.8 Effects of L-Carnitine and PHF on renal function tests in male and female Wistar Rats

There were no significant changes in the concentrations of urea, creatinine and uric acid in all the groups.
5.4.8.9 Effects of L-Carnitine and PHF on histopathology

5.4.8.9.1 Adipose tissue

Histopathological examination of adipose tissue of untreated control showed adipocytes with regular integrity devoid of any lipid accumulation and inflammatory changes. However, in HFD group adipocytes were seen with remarkable increase in size, infiltration of inflammatory cells and edema [Figures 5.26 and 5.27 (i-v)].

5.4.8.9.2 Inflammation, necrosis and fatty changes in liver tissues

Figures 5.22, 5.23, 5.24, 5.25 (i-v) shows histopathological findings in liver tissues of all the groups. There were no pathological changes in untreated control liver tissues [Figures 5.22, 5.23, 5.24 and 5.25 (i)] which showed a normal morphology for the hepatocytes. The normal liver cells possessed distinct cell outline, prominent dark stained nuclei and eosinophilic cytoplasm. The hepatocytes were radially arranged like the spokes of a wheel forming sheets of 2 cells thickness. The lamina of the hepatic cells was well-defined and between the lamina (sheets) of hepatic cells, venous sinusoids radiated from the central vein towards the periphery.

In HFD group [Figures 5.22, 5.23, 5.24 and 5.25 (ii)], fatty changes of hepatocytes were remarkable with significant accumulation of many lipid droplets in the hepatocytes and ballooning degeneration. HFD group showed noticeable damages such as lobular and portal tract inflammation, hepatocellular ballooning and necrosis accompanying the fatty liver or steatotic changes. Fatty liver changes observed in HFD group may be due to dyslipidemic changes as a result of obesity. This may be due to increased triglycerol content of liver due to elevated influx of NEFA into the liver. Distorted architecture of the central vein and nodule formation, portal fibrosis was observed.
On the contrary, L-Carnitine and PHF treatment groups showed mild and smaller degree of lipid accumulation and minimal damages which were characterized by large areas of normal architecture and less patches of necrotic hepatocytes and lesser pathological signs. Hence the microscopic images suggested that the PHF plays a role in preventing the progression of the lipidosis of hepatocytes [Figures 5.22, 5.23, 5.24 and 5.25 (iii, iv and v)].

5.4.8.9.3 Myocardial changes

The histopathological studies were carried out for confirming the severity of myocardial injury. It was observed that myocardial changes were prominent in male Wistar rats compared to female rats. In the HFD group (II), inflammatory cells were seen to infiltrate the myocytes. Changes such as necrosis and infiltration of leucocytes were also prominent. In treatment groups III, IV and V mild changes in inflammation, necrosis and restoration of normal architecture was observed [Figure 5.28 (i-v)].
Figures 5.22 (i- v): Histopathology of the fatty liver changes in male Wistar rats after treatment with PHF and L-Carnitine

i: photomicrographs of liver tissues of untreated control rats showing normal hepatocytes
ii: High Fat Diet fed rats shows distorted architecture with severe fatty liver changes.
iii: L-Carnitine 250 mg/kg treated rats shows liver tissues with normal hepatocytes.
iv: PHF 200 mg/kg treated shows liver tissues with moderate fatty liver changes.
v: PHF 400 mg/kg treated shows liver tissues with moderate fatty liver changes; magnification at 40 x.
Figures 5.23 (i-v): Histopathology of the fatty liver changes in female Wistar rats after treatment with PHF and L-Carnitine

i: photomicrographs of liver tissues of untreated control rats showing normal hepatocytes
ii: High Fat Diet fed rats shows distorted architecture with severe fatty liver changes.
iii: L-Carnitine 250 mg/kg treated rats shows liver tissues with normal hepatocytes.
iv: PHF 200 mg/kg treated shows liver tissues with moderate fatty liver changes.
v: PHF 400 mg/kg treated shows liver tissues with moderate fatty liver changes; magnification at 40 x.
Figures 5.24 i-v: Histopathology of the liver inflammatory changes in male Wistar rats after treatment with PHF and L-Carnitine

i: photomicrographs of liver tissues of untreated control rats showing normal hepatocytes ii: High Fat Diet fed rats shows distorted architecture with severe inflammation. iii: L-Carnitine 250 mg/kg treated rats shows liver tissues with normal hepatocytes with mild inflammation iv: PHF 200 mg/kg treated shows liver tissues with moderate inflammatory changes v: PHF 400 mg/kg treated shows liver tissues with moderate inflammatory changes; magnification at 40 x.
Figures 5.25 i-v: Histopathology of the liver inflammatory changes in female Wistar rats after treatment with PHF and L-Carnitine

i: photomicrographs of liver tissues of untreated control rats showing normal hepatocytes 
ii: High Fat Diet fed rats shows distorted architecture with severe inflammation 
iii: L-Carnitine 250 mg/kg treated rats shows liver tissues with normal hepatocytes with mild inflammation 
iv: PHF 200 mg/kg treated shows liver tissues with moderate inflammatory changes 
v: PHF 400 mg/kg treated shows liver tissues with moderate inflammatory changes; magnification at 40 x
Figure 5.26: Histopathological changes observed in the adipose tissue of female Wistar rats after treatment with PHF and L-Carnitine

i: Photomicrographs of adipose tissues of untreated control rats showing normal adipocytes. ii: HFD rats showing adipocytes of varying sizes (3-7 µm). iii: L-Carnitine 250 mg/kg treated rats showing normal adipocytes with mild inflammation iv: PHF 200 mg/kg treated rats showing varying sizes of adipocytes v: PHF 400 mg/kg showing normal adipocytes; magnification at 40 x.
Figure 5.27: Histopathological changes observed in the adipose tissue of female Wistar rats after treatment with PHF and L-Carnitine

i: Photomicrographs of adipose tissues of untreated control rats showing normal adipocytes. ii: HFD rats showing adipocytes of varying sizes (3-7 µm). iii: L-Carnitine 250 mg/kg treated rats showing normal adipocytes iv: PHF 200 mg/kg treated rats showing varying sizes of adipocytes with mild inflammation v: PHF 400 mg/kg showing normal adipocytes; magnification at 40 x.
Figure 5.28: Histopathology of myocardium in male Wistar rats after treatment with PHF and L-Carnitine

i: Photomicrographs of myocardial tissues of untreated control rats showing normal myocytes. ii: HFD rats showing myocardium with severe inflammation and edema iii: L- Carnitine 250 mg/kg treated rats showing normal myocytes iv: PHF 200 mg/kg treated rats showing myocardium with mild edema v: PHF 400 mg/kg showing normal myocytes; magnification at 40 x.
5.5 Discussions

In the present study, antiobesity and lipid lowering effects of PHF was assessed using HFD induced obesity model. Both the sexes of Wistar rats were selected for the study. Different animal models are reported to be used for obesity studies as they perfectly imitate obesity as seen in humans (Birari et al., 2010). Herbs used in combination can give a cumulative effect of the individual herbs and help in therapy (Moro et al., 2000).

5.5.1 Body weight

The current results showed that body weight increased significantly in the HFD group compared with the normal group (Figure 5.5, 5.6) which is associated with increased food intake and higher calorie intake. The phytoconstituents especially flavonoids have been reported for their appetite suppressing property (Chidrawar et al., 2011; Chidrawar et al., 2012). The qualitative analysis of the Poly Herbal formulation (PHF) that was done according to the standard methods (Harborne, 2002) indicated the presence of polyphenols (phenolic acids and flavonoids). Polyphenols are a major class of therapeutic agents known for the antioxidant activity with minimum adverse effects (Peairs and Abbey, 2013). Chemical characterization of major biologically active constituents of PHF by HPTLC showed the presence of polyphenols and flavonoids. According to Peluso, (2006) and Birari et al., (2010), plant flavonoids provide new leads for effective strategy in reducing the risk as well as for the treatment of obesity. Therefore, phytochemicals provide an effective alternative strategy for the treatment of obesity (Birari et al., 2010). Polyphenols, including flavonoids and tannins, show promising effects in tackling obesity via various mechanisms such as reduced proliferation of preadipocytes and inflammation, suppressed differentiation of adipocytes, increased lipolysis etc. (De La Garza et al., 2011; Gonzalez-Castejon and Rodriguez-Casado, 2011; Zhang and Huang, 2012).
5.5.2 BMI, Calorie intake and serotonin levels

BMI is one of the obesity indices that are effectively based on the relationship between height and weight. Previous workers have reported that lard based diet fed to rats resulted in obesity with characteristic visceral adiposity, dyslipidemia, hepatic steatosis etc. Three indicators of obesity including the percentage of body weight gain, BMI and AC were measured and were higher in group II. Our study showed that, following six weeks of administration of HFD group had higher BMI compared to the control group. The BMI decreased from 49.5% to 11% and from 21% to 11% in males and females respectively which shows a similar reduction in L-Carnitine treated group.

The total calorie intake was higher in group II. Obviously, this calorie intake was contributed by the calorie from the HFD. 1g of standard rat chow contributes to 3.8 kcal. Meanwhile, 1g of HFD yields 5.5 kcal. With increase in calorie intake, there is more deposition of lipid, which contributes to the development of obesity and metabolic syndrome.

Serotonergic agents help in achieving weight loss primarily by affecting appetite and satiety centres (hypothalamus). Drugs like Sibutramine show a similar action of reduced food intake (Hansen, 2012). In our study increased levels of serotonin of brain samples of treated groups were evident. Thus, PHF showed beneficial attributes such as reduced feed intake.

5.5.3 Implications of visceral adiposity

The visceral fat accumulation is associated with oxidative stress and metabolic syndrome (Fujita et al., 2006; Sankhla et al., 2012). HFD induced obese male and female rats weighed more (19 % and 20 % respectively) and accumulated more than 100 % of visceral fat. Consumption of the HFD (55 %
fat calories) led to obesity. One of the major reasons for HFD induced obesity is due to a positive energy balance leading to an increase in visceral fat deposition or abdominal obesity. This abdominal obesity particularly, a large increase in perirenal visceral adipose tissue mass, suggests that the excess energy leads to the buildup of adiposity. The administration of PHF and L-Carnitine resulted in attenuation of visceral fat accumulation a finding in accordance with Kim et al., 2008.

5.5.4 L-Carnitine, PHF-Counteraction - dyslipidemic condition

L-Carnitine, a quaternary amine (gamma-trimethylamino-hydroxybutyric acid) is found abundantly in liver and kidneys of humans. It is used as a dietary supplement by overweight and obese subjects that facilitate fatty acid transportation and its oxidation for lipolysis (Lee et al., 2006; Kalpana and Aruna, 2012). In animal models like C57BL/6J mice, L-Carnitine supplementation was found to reduce obesity in HFD fed group (Cha, 2008).

Treatments with PHF and L-Carnitine in HFD induced obese rats were able to improve serum lipid profile. It concludes that these treatments have a definite action on lipid metabolism. A diet containing cholesterol, higher energy density or higher calories is a major factor for the development of atherosclerosis. 1% decrease in HDL-C is associated with every 3-4% increase in the risk of heart disease. Cardiovascular risk is one of the serious complications caused as a result of obesity induced dyslipidemia.

In our present study, the lipid profile levels with respect to significant decrease in TC and LDL-C and enhanced levels of HDL-C was notable after the treatment with the PHF. The TGs are independently related to obesity induced cardiovascular complications. The decrease in TG concentrations was significant.

Atherogenic Index (AI) which is an indicator of atherosclerosis development was
significantly reduced in PHF treated groups. Decrease in levels of serum and hepatic lipids (TC, TGs), phospholipids, free fatty acids in PHF treatment groups as compared to HFD fed rats signifies that these changes can be attributed to the inhibition of lipid absorption in the intestine. The present findings are in accordance with that of Woo et al., (2008); González-Ortiz et al., (2008) and El-Metwally et al., (2003) who reported that L-Carnitine improves dyslipidemia. Dietary lipids in the form of chylomicrons are absorbed into the blood vascular system; TGs found in these chylomicrons are converted into fatty acids and glycerol by lipase enzyme which are eventually transported and stored in the liver and adipose tissue in the form of TG (Han et al., 2000; Han et al., 2005). The increase in rectal temperature, ambulatory and rearing activity was notable in PHF treatment groups IV and V of both the sexes and that may be attributed to the thermogenic property of the polyphenolic rich PHF extract. Thus the thermogenic action, regulation of lipid metabolism by decreased absorption of dietary fats may be because of the suppression of pancreas lipase activity which can be attributed to the PHF.

5.5.5 Obesity associated oxidative stress vs antioxidants

The current study examined the effects of PHF on oxidative stress and antioxidant enzyme in liver of HFD induced obese rats. Previous studies report that HFD feeding and its subsequent induction of obesity resulted in an increase in oxidative stress (Bhandari et al., 2013; Chaudhari et al., 2012; Wang et al., 2011; Fardet et al., 2008). GSH, one of the prominent antioxidants found in the liver, play an important role in protecting the cells from oxidative damage. GSH levels in the liver reflect the detoxification capacity of the organ. The evaluations of oxidative stress by antioxidant markers, SOD, GSH, and CAT have been established in animal models with obesity, dyslipidemia, diabetes etc. (Saiki et al., 2007).
The depleted status of antioxidant enzymes is represented particularly by GSH, SOD and catalase enzymes and the liver becomes susceptible to oxidative stress. This causes its impairment leading to damage of DNA, lipids and proteins and finally causing disruption of homeostasis (Schieber and Chandel, 2014). Our results showed significant increase in the enzymic antioxidant (p<0.05) enzyme (glutathione, SOD, catalase enzymes) and non–enzymic (GSH, vitamin C, and vitamin E) concentrations in treatment groups as compared to HFD group which showed significantly depleted antioxidant status. This indicated that Reactive Oxygen Species (ROS) were generated as a result of obesity induced oxidative stress and the antioxidant enzymes play a vital role in protecting the organism against the damaging effects of the free radicals (Schieber and Chandel, 2014).

The liver plays a central role in lipoprotein metabolism including enzymes and receptors involved in lipoprotein metabolism such as HMG Co A reductase and LDL receptors. HFD causes oxidative stress (enzymatic and non enzymatic) in rats which are indicated by low levels of SOD, CAT, GPx, GST and GSH in different organs such as heart, liver, kidney, etc. Antioxidants are known to effectively prevent this kind of cellular damage. The lipoprotein structure is maintained for the cellular uptake of serum lipids from the blood and oxidative state destructs this ability. Antioxidants such as vitamin E helps reduce oxidative damage enhancing uptake of lipid resulting in reducing TC levels. This is the reason for increased lipid peroxidation that exposed the animals to oxidative stress. TBARS is a good indicator of lipid peroxidation which is an autocatalytic, a common consequence of cell death. It was noted that on feeding with HFD for 6 weeks, addition of two doses of PHF (200 and 400 mg/kg) resulted in a significant reduction of liver cholesterol concentrations, while the PHF moderated this high fat diet induced increase without influencing food intake and growth. The antioxidant potential of PHF is strengthened by normalized levels of SOD, GSH, CAT which helps in combating the oxidative stress caused by high cholesterol diet.
Hepatotoxicity or liver injury is caused either by free radicals or due to depletion of endogenous pool of antioxidants e.g. GSH. Many authors have reported the important role of GSH in cellular functions. They can act directly or indirectly through enzymatic reactions to maintain the reduced state of the cells or tissues, and its severe depletion is reported to lead to liver injury (Yuan and Kaplowitz, 2009). We obtained significant depletion of enzymatic and non-enzymatic antioxidant levels in the liver after treatment with L-Carnitine and PHF comparing to HFD induced obese and normal rats. There was a significant increase in the activity of liver enzymes AST and ALT in HFD fed rats compared to the control rats. Liver is flooded with free fatty acids from the adipose tissue pool that causes inflammation within the liver cells leading to hepatic injury. The significant lowering effects on AST and ALT enzymes observed in our work also agree with the findings of Yapar et al., (2007). The above results support the view that oxidative stress takes place extensively in the liver during necrosis and this explains the protective effects of PHF on HFD are on par with induced hepatic injury [Figure 5.22, 5.23, 5.24, 5.25 (iv, v)]. Overall, the PHF was more efficient in increasing antioxidant levels and improvement of histological changes observed in liver and adipose tissues.

The findings of histopathological changes in HFD fed rats such as hepatic lipid accumulation and fatty degeneration are consistent with the results of serum lipid profile. Treatments with two doses of PHF showed considerable reduction in hepatic lipid accumulation as similar to the effect shown after treatment with L-Carnitine.

5.6 Conclusion

Obesity is associated with complications such as insulin resistance, dyslipidemia, cardiovascular disease (CVD). In this study it is concluded that HFD induced obesity was associated with increase in body weight, BMI, lipid
profile, AI, coronary risk index, relative organ and fat pad weights, liver enzymes, cardiac marker enzymes as well as hepatic oxidative stress. The obesity and its associated alterations in nutritional, anthropometric, biochemical parameters in this study could be ameliorated effectively by treatments with L-Carnitine and PHF. There was no significant difference between the treatment groups in antiobesity action studied with various parameters. The lower dose of PHF showed significant antiobesity effects such as lowering body weight, fat mass, triglycerides, etc., similar to the higher dose of PHF i.e. 400 mg/kg. However, slight sex-dependent changes between both the sexes were displayed. The male Wistar rats showed higher adiposity, dyslipidemic induced atherosclerotic risk factors compared to the female rats. This work demonstrated antiobesity, hypolipidemic, hepatoprotective and antioxidant effects of PHF which might be due to presence of polyphenolic constituents.

**Figure 5.29: Effect of HFD induced obesity in the male and female sexes of Wistar rats**

♂- male Wistar rats; ♀-female Wistar rats