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Comparative evaluation of anti-obesity effect through pancreatic lipase inhibition of methanolic extract of the bark of *Saraca asoca* and *Cynometra travancorica*

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ABSTRACT: *Saraca Asoca,* a member of the *Caesalpiniaceae* sub-family, is a native plant utilized extensively in ayurvedic medicine. This study compares the anti-obesity effect of methanolic extract of the bark of *Saraca asoca* and *Cynometra travancorica*, two common ingredients of many Indian traditional medicines. Plant material extracts were prepared using simple maceration technique. Adult 150-180 gm weighed Sprague dawley rats were used for high fat diet induced *in vivo* anti-obesity study for eight weeks. Weekly bodyweight was measured, and terminal serum lipid profile was estimated to assess the anti-obesity activity of standard drug orlistat (60 mg/kg) and extracts at a dose of 400 mg/kg. *In vitro* enzyme inhibition study was performed to assess the effect of standard or extracts on pancreatic lipase. Increase in bodyweight found corresponding with the normal weight gain for the control group whereas it is significantly increased with high fat diet fed group. While in the orlistat or extract treated found to have resisted significant changes in bodyweight ($p<0.001$). Terminal blood samples were collected from all animals and serum lipid profile were evaluated. An increase in the levels of TC, TG, and LDL of animals in high fat diet fed group when compared to normal control group. Whereas the increase lipid levels were reversed in all treated groups. HDL level found to have increased in standard and extract treated groups. *In vitro* enzyme inhibition study revealed the inhibitory potential of *S. asoka* (IC_{50} of 306.15µg/ml) and *C. travancorica* (IC_{50} of 301.94 µg/ml) on pancreatic lipase when compared with orlistat (IC_{50} of 262.17 µg/ml) ($p<0.05$). This study suggests that the methanolic extract of the bark of *S. asoca* and *C. travancorica* possess significant anti-obesity activity on high fat diet induced obesity rat model and *in-vitro* pancreatic lipase inhibition.
KEYWORDS: *Saraca asoca*; *Cynometra travancorica*; anti-obesity; pancreatic lipase inhibition.

1. INTRODUCTION

A metabolic disease called obesity is defined by an excessive build-up of body fat brought on by an individual's energy intake surpassing their energy expenditure [1]. The World Health Organization (WHO) now views obesity as a severe concern to world health because it stems from an energy imbalance. Health issues including dyslipidaemia, hypertension, fatty liver disease, diabetes mellitus, cancer, osteoarthritis, and asthma are associated to it [2,3]. More than 1.9 billion persons, aged 18 and older, were classified as overweight in 2014, with more than 31% of them being obese as per WHO [4]. WHO also forecast that by 2030, this number will increase to roughly 3.3 billion (about 1.7 times) [5]. Obesity increases the risk of metabolic syndrome, which in turn increases the risk of hypertension, type 2 diabetes, dyslipidaemia, cardiovascular disease (CVD), and stroke [6,7]. To date, orlistat (Xenical), a moderately effective medicine licenced by the FDA for the long-term treatment of obesity, works by inhibiting the pancreatic lipase enzyme and blocks the absorption of around 30% of dietary fat [8].

Pancreatic lipase (triacylglycerol acyl hydrolase), a major enzyme involved in the absorption of dietary triglycerides, is released by the pancreas and catalyses the digestion of dietary triglycerides [9]. Pancreatic lipase is one of several lipases that hydrolyses between 50 to 70 percent of all ingested fats [10]. It is well

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established that pancreatic lipase inhibition reduces the absorption of fat and helps control obesity [11]. Ser152, Asp176, and His263 amino acids sustain pancreatic lipase's hydrolysis activity; Ser152, in particular, is in charge of lipolysis activity [12]. The function of food digestion and absorption inhibitors, a method of reducing the number of calories consumed through gastrointestinal mechanisms without affecting any central systems, is among the most crucial strategies in the treatment of obesity. One of the most extensively explored methods for evaluating the potential effectiveness of natural items as anti-obesity treatments is the suppression of digestive enzymes [13].

Saraca asoca (S. asoca) is the oldest holy plants, also known as *Saraca indica* and belonging to the *Caesalpiniaceae* family, is found all throughout the Indian subcontinent [14]. *S. asoca's* stem bark extracts have been shown to include secondary metabolites that are believed to be responsible for their medicinal effects. These include flavonoids, terpenoids, lignins, phenolic compounds, tannins, and more [15-18]. The pharmacological properties of the plant's many sections, including its antihyperglycemic, antipyretic, antibacterial, anthelmintic, and other activities, are widely documented in the literature [19-21]. Our previous studies evaluated and compared the anti-estrogenic, anti-inflammatory, anti-oxidant and toxicological evaluation of methanolic bark extract of the same plant [22-24]. *S. asoca* is the source of the traditional medication Asoka Aristha, which is used to treat menorrhagia [25]. The ayurvedic medication industry in Kerala, India, consumes roughly 105 tonnes of "Asokam" annually as a result of its many different applications [26]. The intensive use of this tree has almost completely devastated its natural habitat. This tree is scarce, hence other nearby or unrelated species' bark has been used in its place. It is typical to employ replacements from other plants of *Caesalpiniaceae* species, especially *Cynometra travancorica (C. travancorica)*. The botanical wealth of secondary metabolites found in the genus *Cynometra* has been linked to the biological functions and therapeutic applications mentioned for the medicinal plants incorporating it [27]. Uncertainty exists regarding the medicinal potential of *C. travancorica*. Therefore, this study was conducted to evaluate the anti-obesity and suppression of pancreatic lipase activity of *C. travancorica* and compare with that of *S. asoca*.

2. RESULTS

2.1. Effect of extracts on body weight

All of the experimental animals' initial body weights were nearly similar at the onset of the study, whereas at the end of two months, the HFD treated group showed significant increase in body weight compared to the control group (Figure 1). The final body weight and body weight gain in the treatment groups were significantly decreased by 16.21 % in the orlistat and extract treated groups showed 13.10 and 14.16 % reduction respectively.

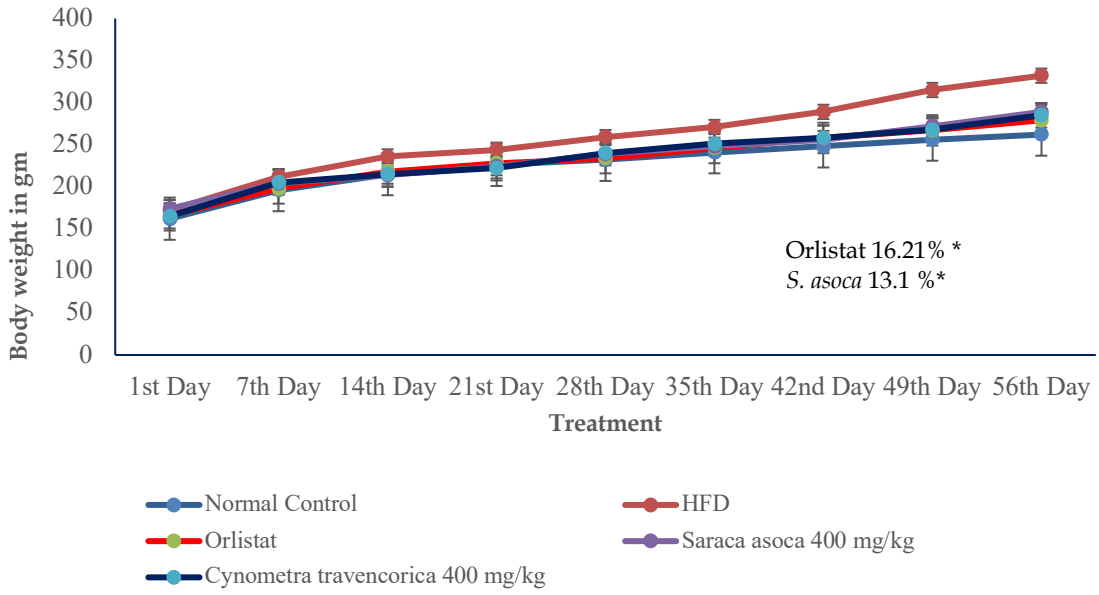

Figure 1. Body weight of rats fed with HFD for two months. All values are expressed as Mean ± SEM, (*n*=6) and *p < 0.001.

2.2. Effect of extracts on biochemical parameters

As mentioned in Table 1, it has been observed that rats fed with HFD consecutively for 56 days resulted in a marked increase in the level of lipid profile, characterised by elevated levels of TC, TG, LDL – C, HDL – C, AST and ALT when compared to the normal control. An increased level of LDL – C indicates hypercholesterolemia. However, treatment with extracts for 56 days reversed the hyperlipidaemic effect produced by HFD significantly. Similar results were obtained with standard drug orlistat.

Table 1. Effect of each extract on biochemical parameters and glucose level in obese rats. All values are expressed as Mean ± SEM, (n=6) and *p < 0.05.

Biochemical Parameters	Control (without HFD)	HFD	Standard orlistat	*S. asoca* (400mg/kg)	*C. travancorica* (400mg/kg)
TC (mg/dl)	71.26±0.47	120.3±1.07*	101.6±0.88	105.6±1.48*	104±1.38*
TG (mg/dl)	74±0.96	153.83±1.62*	73±0.69	75.63±1.24*	78.3±1.6*
HDL-C (mg/dl)	32±0.71	29.5±0.71	34.43±0.63	40.56±1.15*	38.75±1.29*
LDL-C (mg/dl)	23.46±0.45	76.73±2.12	45.53±0.65	51.26±1.28*	54.07±1.4*
Glucose (mg/dl)	122.36±1.55	144±0.53	123.43±1.23	126.16±0.6	128.98±0.7
AST (U/L)	53.26±1.04	98±1.06	60.5±1.32	62.5±0.98*	60.54±1.9*
ALT(U/L)	23.33±1.54	72.16±1.49*	29.6±0.88	32.5±0.42*	30.4±0.66*

2.3. Effect of extracts on pancreatic lipase inhibition assay

The hyperlipidemic activity of each extract were investigated by assaying the inhibition of pancreatic lipase. The inhibition of pancreatic lipase is very important for treating diet induced hyperlipidemia. Both extracts inhibited pancreatic lipase in a dose dependend manner. The percentage of inhibitory activity on enzyme using each extract was nearly same as that of the standard. *S. asoca* and *C. travancorica* of concentration 400 µg/ml showed 52.65±2.1 and 67.12±1.9 percentage of inhibition respectively. In the current study, *S. asoca* inhibited pancreatic lipase activity with IC_{50} of 306.15µg/ml and *C. travancorica* with IC_{50} of 301.94 µg/ml compared to orlistat with IC_{50} of 262.17 µg/ml (Figure 2).

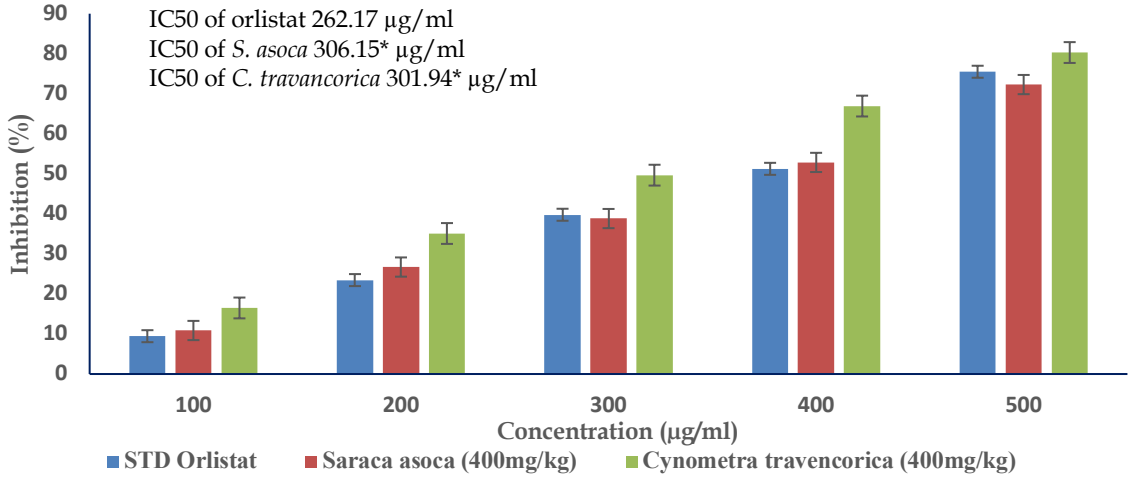

Figure 2. Inhibition of pancreatic lipase by each extract. Orlistat was used as positive control. All values are expressed as Mean ± SEM, (n=6), *p < 0.05.

2.4. Histopathology of liver

Normal rat liver sample did not exhibit any cellular ageing or necrosis (Figure 3A). Whereas, rats given HFD revealed severe vascular congestion, fatty deposition, and foamy hepatocyte degeneration in their liver sections (Figure 3B), while rats received standard drug treatment had normal hepatocytes, although there was some degree of oedema (Figure 3C). Rats given with extracts revealed restored normal hepatocytes in their liver sections (Figure 3D, 3E).

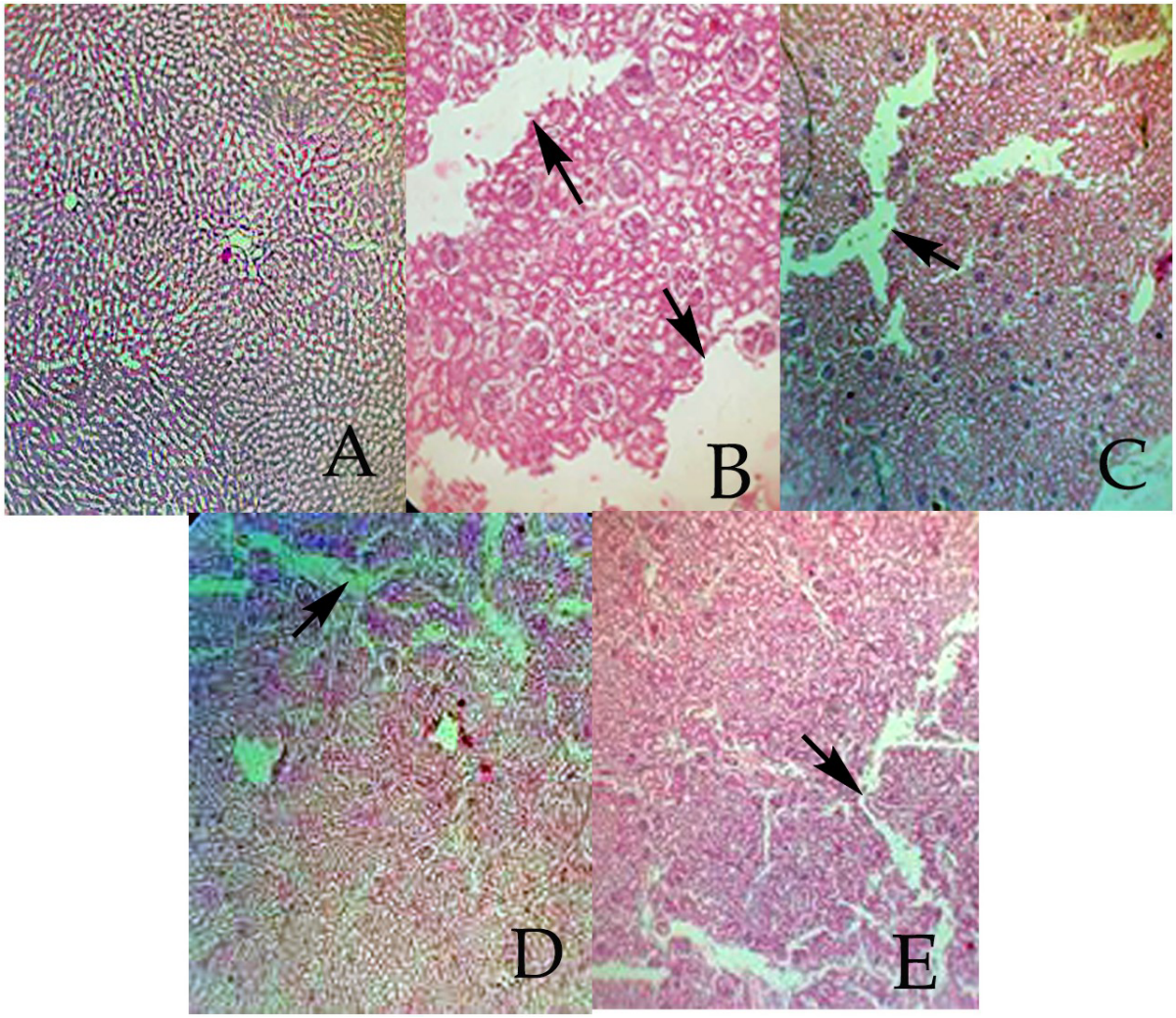

Figure 3. Histology of liver samples. A- histological sample of rat liver of control group which shows normal cell structure. B- histological sample of rat liver, treated with High Fat Diet (HFD) which shows significant cellular degeneration and inflammation. C- histological sample of rat liver treated with the standard drug orlistst (60 mg/kg), which shows reversal of cellular degeneration and inflammation. D and E groups, treated with *S. asoca* (400 mg/kg), E- *C. travancorica* (400 mg/kg) respectively, show considerable improvement in cellular degeneration and inflammation.

3. DISCUSSION

Numerous natural items, including green tea, tea catechins, etc., have been researched for their potential to reduce hyperlipidemia and prevent obesity [28-30]. TGs, which make up the majority of dietary fats, are hydrolyzed by pancreatic lipase, a crucial enzyme in the breakdown of fat, into free fatty acids and monoglycerides [31]. Triglycerides release free fatty acids into the bloodstream, where they are carried to the liver and adipose tissue, causing lipid accumulation and the onset of obesity. Pancreatic lipase inhibition decreases fat absorption and digestion [32,33]. This study examined the effects of a methanolic bark extract of *S. asoca* and *C. travancorica* on pancreatic lipase activity, obesity, and hyperlipidemia in rats that had been given a high-fat diet (HFD) to induce obesity and hyperlipidemia. The most prevalent obesity model that closely resembles real obesity is HFD induced obesity. Humans who consume HFD develop central abdominal

adiposity, the most prevalent kind of obesity and a major risk factor for developing diabetes and cardiovascular problems.

Our earlier research on *C. travancorica* extract demonstrated that 400 mg/kg was both toxicologically safe and had strong antiestrogenic activity coupled with antiinflammatory properties [22]. Treatments with *S. asoca* and *C. travancorica* at the aforementioned dose significantly decreased body weight, the production of fat, and liver tissue inflammation. This study demonstrated that rats treated to HFD for two months saw a considerable rise in body weight, confirming the condition of obesity. Despite the fact that there was a considerable variation in body weight between the high-fat and standard diet groups, there was no discernible difference in the amount of food consumed daily by the animals. In contrast, when HFD rats are treated with both extract, their body weights are noticeably reduced in comparison to the HFD-administered rats. The outcome also shows that supplementing with extracts at a dose of 400 mg/kg can assist to maintain current body weight by avoiding weight gain.

Chronic dyslipidemia has been identified as a significant cause to atherosclerosis and other cardiovascular risks [34,35]. Each extract also decreased blood levels of TG, total cholesterol, and LDL cholesterol. The drop in serum lipid profiles suggested that each extract could slow down the transfer of lipids into the bloodstream, which would reduce fat buildup in tissues. These findings provide credence to the idea that by preventing intestinal absorption of dietary fat through inhibition of pancreatic lipase activity, the extracts may lessen the degree of obesity brought on by an HF diet. The histological analysis of the liver tissue in the current investigation revealed that the inflammation of the tissue was less severe in the HFD group than it was in the conventional group. ROS are primarily produced by lipid buildup in the liver and adipose tissue, which results in oxidative stress.

Histopathological tests were also conducted to support the results. According to the literature study, a high-fat diet, obesity brought on by it, and inappropriate lipid metabolism are all linked to inflammation, congestion, and nonalcoholic fatty liver disease (NAFLD), which in turn causes hepatic failure and raises blood levels of AST, ALT, and total bilirubin [36,37]. Our findings indicated that the aetiology of fatty liver or hepatic steatosis linked with obesity, as shown by expanding degeneration, may be significantly influenced by the consumption of high-fat diets. The findings of the current investigation proved that HFD damages hepatocellular tissue, as demonstrated by the substantial increase in serum enzyme (AST, ALT) activity and the exacerbated hepatic steatosis revealed in histological analyses of the liver. However, each extract's administration results in a brief drop in enzyme levels, indicating the extracts' ability to stop HFD's liver-damaging effects.

4. CONCLUSION

Most ayurvedic medications substitute species of the same origin. A broad range of quality control measurements is thus required to demonstrate the efficacy of such substitutions. This makes it impossible for Ayurveda to profit from its wealth, even by increasing its use. We have made an attempt to carry out preliminary biological justification for using *C. tavancorica* instead of *S. asoca* in pharmaceutical formulations. The findings of the current study confirm the traditional use of the methanolic extracts of *C. travancorica* and *S. asoca* for weight control. The results also concluded that plant extracts of *Caesalpiniaceae* family members exhibit inhibitory activities against pancreatic lipase *in vitro*. Further research is being done to identify the active ingredient in these plants as well as the mechanistic mechanism of each extract that aids in weight control.

5. MATERIALS AND METHODS

5.1. Sample collection

S. asoca, and *C. travancorica* were collected from different parts of the western ghats like Thrissur, Munnar, Wayanad, and Nelliyampathy areas and authenticated at, Pharmacognosy Division, Arya Vaidyasala, Kottakkal, Malappuram, Kerala, India. A sample of the dried plants were recorded in the Herbarium of the centre for medicinal plants research (CMPR), Arya Vaidyasala, Kottakkal (No.148363).

5.2. Sample preparation

The barks of *S. asoca* and *C. travancorica* were carefully gathered, diced into tiny pieces, cleansed with distilled water, dried in the shade, and then allowed to air dry for seven days at room temperature. About 150g powdered material was extracted with 500ml of methanol by soxhlation for 48 hours at 50°C. Periodically, the extracts were stirred and filtered. The filtrate produced an active solid residue after solvent evaporation

(Superfit Rotary Flash Evaporator- 40°C at 50 rpm), which was stored in a desiccator (Borosil 100mm Flenge 3082041) for further use. The percentage yield of the extracts, which was kept at 4°C, were found to be 13.56 ± 0.75% and 16.24 ± 0.65%.

5.3. Animals and experimental protocol

The Wistar female rats weighing 80-100gm were purchased from the Small Animal Breeding Station (SABS), College of Veterinary, Agricultural University, Thrissur, Mannuthi, Kerala, and kept in a controlled environment in the animal house of Al Shifa College of Pharmacy (22-28°C temperature, 60-70% relative humidity, and 12 h dark/light cycle). Normal rat food (Sai Durga Feeds and Foods, Bangalore, India) as well as unlimited access to water *ad libitum* were given to the rats. The study was conducted with the approval of Institutional Animal Ethics Committee (IAEC; ACP/072/22) and all procedures were properly adhered to IAEC guidelines. The rats were fed either normal or HF diet for 8 weeks to induce obesity. The High Fat Diet (HFD) was prepared by mixing the control diet with 1.5% cholesterol, 20% palm oil and 0.25% cholic acid as described elsewhere [28]. The rats were randomly divided into five experimental groups as follows (n=6 rats/group): Group 1 (without high fat diet), Group 2 kept as high fat group, Group 3 treated with standard drug orlistat (60 mg/kg), Group 4 and 5 treated with extracts at a dose of 400 mg/kg.

5.4. Estimation of body weight

Using a digital weighing balance, the body weight (gm) was recorded on day one and then every week after that for 56 days. Additionally, for 56 days, the weekly food consumption measurements for each group were made.

5.5. Estimation of serum biochemical parameters

On the 56th day of the experiment, all animals were sacrificed by CO_2 chamber method. The blood samples were collected by cardiac puncture and allowed to stand for 30 min at 20 – 25 °C. The clear serum was separated at 2500 rpm for 10 min using a centrifuge. The levels of serum glucose, total cholesterol (TC), HDL cholesterol (HDL-C), TG, LDL cholesterol (LDL-C), Aspartate transaminase (AST) and Alanine transaminase (ALT) were determined by using fully Autoanalyzer (FUJI DRI-CHEM NX500i).

5.6. Pancreatic lipase inhibition assay

In accordance with a previously described technique, slightly modified *in vitro* pancreatic lipase test was conducted [29]. In brief, distilled water was used to dissolve porcine pancreatic lipase (Sigma-Aldrich; Merck KGaA) to a final concentration of 1 mg/ml. As the lipase substrate, 1% Triton X-100 was added to a stock solution of 1% (w/v) 4-nitrophenyl laurate (Sigma-Aldrich; Merck KGaA) in 5 mM sodium acetate (pH 5.0). The reaction was started by combining 80 ml of assay buffer, 30 ml of each extract and orlistat, and 4 nitrophenyl laurate. The mixture was then incubated at 37 °C for 2 hours before centrifugation at 23,00 x g for 2.5 min at 25 °C. At 400 nm, the absorbance was measured using a microplate reader. The results are expressed as percentage inhibition, and were calculated using the formula (A blank-B sample)/A blankX100, where A blank is the absorbance of the control and B sample is the absorbance of orlistat or each extract.

5.7. Histopathological analysis

On 56th day, rat liver tissues were dissected and fixed in 10% neutral-buffered formalin. After the liver tissue had been dehydrated, slices were taken out of the paraffin-embedded tissues. Deparaffinized, rehydrated, and fixed sections were stained with haematoxylin and eosin (H&E) dyes. Both a 10x and a 40x light microscope was used to view the slides. A photomicrograph was captured with a Motic camera (MOTICAM BTU10) and Moticonnect Image Plus 2.0 software to record any alterations in the structural architecture, portal or lobular inflammation, sinusoidal dilatation and congestion, swelling, degeneration, necrosis, and fatty change.

5.8. Statistical analysis

All data are presented as the mean ± the standard error of the mean. The data were compared using a one-way ANOVA and $p < 0.05$ was considered to indicate a statistically significant difference.

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Conflict of interest statement: The authors declare no conflict of interest in the manuscript.
Ethics Committee Approval: All the animal experiments related to the work is carried out from Al Shifa College of Pharmacy, approved by CPCSEA, Registration No. 1195/PO/Re/S/08/CPCSEA.

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