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Effect of Pineapple Leaves Extract (PLE) on Body Weight Gain and Histopathological Changes in Liver of Hypercholesterolemic Rats.

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ABSTRACT

The present research was undertaken to study the effects of oral intake of Pineapple (*Ananas comosus* L.) Leaves Extract (PLE) at three dosage (250, 500 and 750 mg/kg b. wt.) on hypercholesterolemic rats after 4 weeks of treatment on body weight gain% (BWG %), feed efficiency ratio (FER) and relative weight of liver. Histopathological examination of liver was also studied. Thirty male Wister Albino rats were distributed into five equal groups (6 rats each) as follows: group1: negative control group, group 2: positive control (hypercholesterolemia rats) group, groups 3, 4 and 5 fed. on high cholesterol and high fat diet (HCFD) and treated with orally doses of PLE of 250, 500 and 750mg/kg b. wt., respectively. The results showed that, the total content of phenolic compound of pineapple leaves extract was 22.28 mg/100 ml. oral intake of Pineapple *(Ananas comosus* L.) leaves extracts (PLE) at three dosage (250, 500 and 750mg/ kg) for four weeks to hypercholesterolemia rats reduces body weight gain and feed efficiency ratio. On the other hand, the decreased of liver weight compared to hypercholesterolemia male rats (positive control group) by 27.77 %, 34. 82 % and 64.63 % were observed at the three dosage (250, 500 or 750mg/ kg) respectively. Histopathological examination of liver sections of rats of PLE -treated groups showed amelioration of histological changes caused by high level of cholesterol in the positive control group.

Key words: Pineapple leaves, Pineapple *Ananas comosus* L., rats hypercholesterolemia, body weight gain% (BWG %), feed efficiency ratio (FER).

Introduction

Pineapple AC leaves extraction dictated the presence of carbohydrates, alkaloids, saponins, sterols/terpenes, flavanoids, tannins and phenolic compounds and proteins and amino acids. Presence of flavanoids, phytosterol, glycosides and phenols, phenolic compounds and tannins were very prominent. Most antioxidant activities of plant sources are derived from phenolic-type compounds (Kataki, 2010; and Kalpana *et al*., 2014).

It is reported that the extract of *Ananas comosus* leaf (EAL) is rich in phenolic acids, including p-coumaric acid, caffeic acid, 1-O-p-coumaroylglycerol, and 1-O- caffeoylglycerol. The most abundant and stable component of EAL is p-coumaric acid, which is the main form of phenolic component presented and excreted in vivo. Animal experiments have demonstrated that the lipid-lowering effect of p-coumaric acid manifests by protecting low-density lipoprotein cholesterol from oxidation. Therefore, it is used as a bioactive marker for quality control of EAL (Chai *et al*., 2013).

Ananas comosus (AC) possess a wide array of pharmacological properties such as antibacterial activity, antihyperlipidemic activity, Antidysuria activity, antitumor activity (Kalpana *et al*., 2014).In Thailand, AC was used as an indigenous medicinal plant for the treatment of dysuria. In China, AC cortexes served as alexiphamic, antitussive and antidiarrhea agents and AC leaves were usually used as antidyspepsia or antidiarrhea agents in Chinese Traditional Medicine (Xie *et al*., 2006). The cortex of the plant has been using as the medication in dysuria, auxipharic, antitussive and antidiarrheal agents (Islam *et al*., 2011).

Hypercholesterolemia is a major socioeconomic problem in common individuals as well as health professionals due to the strong correlation between cardiovascular diseases and lipid abnormalities (Venkadeswaran *al et*., 2014). Hyperlipidaemia is manifested as hypercholesterolemia and /or hypertriglycerolemia. However, hypercholesterolemia is the most common hyperlipidaemia (Vuyyuru *et*

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al., 2012). Hypercholesterolemia is a condition where there is an aberrantly elevated concentration of cholesterol in the blood (Bamimore, 2013).

Abreu *et al*. (2014) aimed to determine whether a hypercholesterolemia diet induced hepatic steatosis. They measured , body weight and food intake , Food efficiency, the levels of serum components activities of ALT, and AST, TC , TG , HDL, glucose, and total protein . Results showed that hypercholesterolemia diet did not affect body weight, but resulted in the accumulation of lipids in the liver, increased serum activities of ALT, AST, TC , TG.

Vuyyuru *et al*. (2012) evaluated the anti hyperlipidemic activity of high fat diet induced hyperlipidemic in rats. In high fat diet (HFD) induced hyperlipidemia rats treated with 600 mg/kg,400 mg/kg and 200 mg/kg hydro-alcoholic extract of *Ananas comosus* (HAAC), physical parameters like body weights, feed intake, organ weights, biochemical parameters like blood glucose, lipid profile were monitored. After 56 days significant reduction in body weights, TC, TG, LDL, VLDL and increased HDL compared to HFD control were shown. The results indicated that administration of HAAC at a dose of 600 mg/kg produced significant antihyperlipidemic activity in HFD induced hyperlipidemic rats. HAAC at a dose of 400mg/kg and 200mg/kg shows less effect than the 600mg/kg in reducing the lipid levels and body weights.

Mohamad *et al*. (2015) evaluated the antioxidant effects of pineapple vinegar in reversing of paracetamol-induced liver damage in mice. Pineapple vinegar (0.08 and 2 mL/kg) and synthetic vinegar were used to treat paracetamol-induced liver damage in mice. Oral administration of pineapple vinegar at 2 mL/kg reduced serum enzyme biomarker levels, including AST, ALT, ALP, and TG after 7 days of paracetamol treatment. Liver antioxidant levels such as hepatic glutathione, SOD and lipid peroxidation were restored after the treatment. Oral administration of pineapple vinegar at 0.08 and 2 mL/kg reduced serum enzyme biomarker levels, restored liver antioxidant levels, reduced inflammatory factor expressions, and down regulated liver cytochrome P450 protein expression in paracetamol-induced liver damage in mice.

The aim of present study to investigate the effects of oral intake of Pineapple (*Ananas comosus* L.) Leaves Extract (PLE) at three dosage (250, 500 and 750 mg/kg b. wt.) on hypercholesterolemia rats after 4 weeks of treatment on body weight gain% (BWG %), feed efficiency ratio (FER) and relative weight of liver. Moreover, the histopathological examination of liver was also studied.

Materials and Methods

Materials:

Pineapple (Ananas comosus L.*) leaves:*

Fresh pineapple leaves (*Ananas comosus* L.) used in this study was purchased from a local market, Jeddah, Kingdom of Saudi Arabia

Cholesterol (white crystalline powder) Bile acid and phenolic compounds were purchased from Sigma-Chemical Company st. Lowis, USA.

Animals:

A total number of thirty male albino rats of Wistar strain weighed 180-200g each, were obtained from the experimental Animal Unit of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia.

Methods:

Preparation of the Basal Diet:

The basal diet for rats was prepared using AIN-93 according to (Reeves *et al*., 1993). the basal diet consists of the following: Protein (Casein) 20%; Sucrose 10%;Corn Oil 4%; Choline Chloride 0.2%; Vitamin mixture 1%; Salt mixture 3.5%; Fibers (Cellulose) 5% and the remainder is Corn Starch up to 100%.

Induction of hypercholesterolemia:

Induction of hypercholesterolemia was induced by feeding the rats on basal diet plus 1% (w/w) cholesterol according to (Alissa *et al*., 2004), 0.2% (w/w) bile salts according to (Lamb *et al*., 1999) and 20% (w/w) saturated fat (Santillo *et al*., 1996) for four weeks.

Preparation of pineapple leaves extract PLE:

The leaves of pineapples was separated, cleaned and dried in oven at 40°C, then powdered in a grinder, then stored in an airtight container at 5°C until further use (Kalpana *et al*., 2014).

Total Phenolic Content in PLE were determined and identified by High-performance liquid chromatography (HPLC) according to the method reported by Mattila *et al.* (2000).

Experimental Design:

The experiment was performed on thirty male mature Wistar rats. Animals were distributed randomly into five equal groups, six rats each. Rats were housed in standard plastic cages at a room temperature maintained at 24± 2 °C, with fixed 12-hour lighting system. All rats were allowed to free access to basal diet and water for one week before starting the experiment for acclimatization. After acclimatization period, the rats were allocated in to the following groups:

Group (1): Rats were fed on the basal diet only, kept as a negative control group (Con -ve) and received oral gavages' of distilled water, for eight weeks.

The other four groups (n=24) rats were fed on experimental diet for four weeks. After this period, blood samples were taken for measuring total cholesterol level. Rats with blood cholesterol level ≥ 200 mg /dl were considered to be hypercholesterolemia (Iqbal *et al*., 2011). These rats were distributed in to the following groups:-.

Group (2): Rats were fed on experimental diet only, kept as a positive control group (Con. + ve) and received oral gavages of distilled water for four weeks.

Group (3): Rats were fed on experimental diet and orally PLE in a dose of 250 mg/kg body weight (b. wt.) for four weeks.

Group (4): Rats were fed on experimental diet and orally PLE in a dose of 500 mg/kg body weight (b. wt.) for four weeks.

Group (5): Rats were fed on experimental diet and orally PLE in a dose of 750 mg/kg body weight (b. wt.) for four weeks.

Biological evaluation:

Determination of feed intake (FI), body weight gain percent (BWG %) and feed efficiency ratio (FER):

Daily feed intake (FI) per group was calculated throughout the experimental period (8 weeks).The biological values of different diets were carried out by determination of body weight gain percent (BWG %). Feed efficiency ratio (FER), according to the method of (Chapman *et al*., 1959).

At the end of the experimental period, all rats were fasted overnight then sacrificed. Blood samples were immediately collected from the retro orbital plexus with capillary tubes under mild ether anesthesia, into clean dried centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 15 minutes. Clear serum samples were carefully separated using Pasteur pipettes, and frozen at - 20°C until biochemical analysis (Margoni *et al*., 2011). The liver were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were washed with cold saline solution and dried between two filter papers then weighed and they saved for the histopathological examination. Calculation of the relative organs weight was done according to the following equation:

$$\text{Organ Relative Weight} = \frac{\text{Organ weight}}{\text{Animal final weight}} \times 100.$$

Livers were kept in 10% neutral buffered formalin pending for the histopathological examination.

Histopathological examination:

Specimens from the halves of liver were taken immediately after weighed the organs of the rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, and dehydrated in ascending concentration of ethanol (70, 80 and 90%), then cleared in xylene, and stained with Hematoxylin and Eosin (H&E) and examined microscopically according to (Bancroft and Gamble , 2008).

Statistical analysis:

Statistical analysis was done by using (SPSS) Statistical Package for the Social Sciences for Windows, version 22 (SPSS Inc., Chicago, IL, USA). Collected data was presented as mean± standard error (SE). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to (Armitage and Berry, 1987). All differences were consider significant if $P < 0.05$.

Results and Discussion

The quantification of total phenolic content of pineapple leaves extract (mg/100ml sample) were presented in Table 1, it was recorded 22.28 mg/100 ml.

Table 1: Total phenolic content of pineapple leaves extract PLE

Sample	Total phenolic compounds (mg/100ml)
PLE	22.28± 1.54

Mean ± SE of triplicate measurement.

Effect of pineapple leaves extract PLE on initial body weight, final body weight and body weight gain percent (BWG %) in hypercholesterolemia male rats:

The initial body weight, final body weight and body weight gain percent (BWG %) of hypercholesterolemia male rats treated with PLE at three doses levels (250, 500 and 750 mg/kg b. wt.) are presented in Table 2.

Table 2: Effect of oral intake of different doses from pineapple leaves extract PLE on the initial body weight, final body weight and body weight gain %(BWG %) in hypercholesterolemia male rats

Parameters / Groups	Initial weight (g)	Final weight (g)	Body weight gain (%)
Group (1) Negative control	193.61±4.84 [a]	369.83±5.15[b]	90.50±6.73[b]
Group (2) Positive control	191.24±1.26 [a]	402.50±3.70[a]	110.33±3.20[a]
Group (3) PLE 250 mg / kg.b.w.	194.96±5.69 [a]	359.50±15.03[c]	84.81±4.84[d]
Group (4) PLE 500 mg /kg.b.w.	195.23±4.26 [a]	366.33±5.94[b]	87.66±2.23 [c]
Group (5) PLE 750 mg /kg.b.w.	196.41±1.61 [a]	368.33±5.28[b]	87.75±4.01 [c]

Data are presented as means ± standard error, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Values with similar or partially similar superscripts are non-significant.

Data recorded in Table 2. showed that there were no significant differences in initial body weight between all experimental groups. A significant (P< 0.05) increase was observed in the final body weight of hypercholesterolemia rats (positive control group), compared to the normal rats (negative control group) by 8.83% as shown in Table 4.3 and Figure 4.3. Oral intake of PLE in doses of 250, 500 and 750 mg/kg b. wt., significantly (P< 0.05) decreased the final body weight when compared to the hypercholesterolemia rats (positive control group) by 10.68, 8.98 and 8.48 % respectively.

Concerning body weight gain percent, the results showed that there was significant increase in the hypercholesterolemia rats (positive control group) when compared to normal rats (negative control group) by 21.91 %. Oral intake of PLE in doses of 250, 500 and 750 mg/kg b. wt., significantly (P < 0.05) decreased the BWG % by 23.13, 20.54 and 20.46 % respectively, when compared to hypercholesterolemia rats (positive control group). These findings were in agreement with those obtained by Nwozo *et al*. (2011) and Osfor *et al* . 2013). The increase in the body weight of hypercholesterolemia rats might be because of increased amount of food and caloric intake with increased fat accumulation or storage in tissues.(Xie *et al*.(2014).

Effect of pineapple leaves extract PLE on feed intake and feed efficiency ratio (FER) and liver relative weight on hypercholesterolemia male rats:

Effect of oral intake of different doses (250, 500 and 750 mg/kg b. wt.) from pineapple leaves extract PLE on feed intake , feed efficiency ratio (FER)and liver relative weigh in hypercholesterolemia male rats are illustrated in Table 3. Feed intake was significantly (P< 0.05) increased in the hypercholesterolemia rats (positive control group), compared to normal rats (negative control group) by 4.18 %.Oral intake of PLE at three dosage levels 250, 500 and 750 mg/kg b. wt., decreased feed intake as compared to hypercholesterolemia rats (positive control group) by 21.09, 13.88 and 17.35 % respectively. It is clear from Table 3. that FER in hypercholesterolemia rat (Positive control group) significantly (P< 0.05) increased when compared to normal rats (negative control group) by 42.3 %. Significant (P< 0.05) decreases were observed in rats orally with PLE in doses of 250,500 and 750 mg/kg b. wt., as compared to the positive control group by 26.35, 21.62 and 21.6 % respectively These findings are in agreement with the previous results by Ma *et al* . (2007) and Cho *et al*. (2010) who suggested that both caffeic acid and chlorogenic acid significantly lowered body weight and visceral fat mass. In addition, the significant reduction might be because of estrogenic constituents that indirectly increase thyroid hormone levels and shift lipid metabolism towards its catabolic side (Basch *et al*. (2003).

Table 3: Effect of oral intake of different doses from pineapple leaves extract PLE on the feed intake and feed efficiency ratio (FER) and liver relative weight in hypercholesterolemia male rats:

Parameters / Groups	Mean of daily feed intake (g/rat/d)	Feed efficiency ratio (FER)	Relative liver weight
Group (1) Negative control	28.21±.29^{b}	1.04± .05^{b}	2.87±.18^{d}
Group (2) Positive control	29.39±.18^{a}	1.48± .03^{a}	4.68±.32^{a}
Group (3) PLE 250 mg / kg.b.w.	23.19±.18^{c}	1.09± .11^{b}	3.38±.10^{c}
Group (4) PLE 500 mg / kg.b.w.	25.31±.17^{c}	1.16± .09^{c}	3.05±.10^{d}
Group (5) PLE 750 mg / kg.b.w.	24.29±.17^{c}	1.16± .078^{c}	3.73±.09^{b}

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Values with similar or partially similar superscripts are non-significant.

Effect of oral intake of different doses from pineapple leaves extract PLE on liver relative weight in hypercholesterolemia male rats are presented in 3. Concerning the liver relative weight of rats, the results

showed that hypercholesterolemia male rats had a significant (P< 0.05) increase in the relative weights of liver as compared to normal rats (negative control group) by 63.06.%

Oral intake of different doses from PLE (250, 500 and 750 mg/ kg b. wt.,) significantly (P< 0.05) decreased of liver weight compared to hypercholesterolemia male rats (positive control group) by 27.77 %, 34. 82 % and 64.63 % respectively

Our findings were in accordance with those results obtained by Matos *et al*. (2005) who reported that the increase in liver weight of hypercholesterolemia male rats could be a consequence of the higher fat content (fat/liver).Meng *et al*. (2006), and Xie *et al*. (2014) reported that the phenol content of pineapple leaf significantly attenuated the increase in liver lipid accumulation in HFD fed mice. Thus, enhanced fat oxidation metabolism may contribute to the effects of PLP.

The increase in the body weight of hypercholesterolemia rats might be because of increased amount of food and caloric intake with increased fat accumulation or storage in tissues.(Xie *et al*.(2014).

Histopathological Examination of Liver:

(Fig1.a-c) of negative control group showed that liver has ill defined liver Lobules that can be identified by the presence of central veins (arrows) and portal areas (Fig 1.a) . Hepatocyte are arranged as cords around the central vein (CV) .They have rounded central euchromatic (active) nuclei and lightly stained (pink) acidophilic cytoplasm with (blue) basophilic spots represented rough endoplasmic reticulum responsible for protein synthesis. Hepatocytes are separated by blood sinusoids that are lined by simple epithelial cell (Fig1.b). Portal area contains branches of hepatic artery (HA) bile duct (BD) and portal vein (PV).They are surrounded by loose corrective tissue with few cells (Fig 1.c).

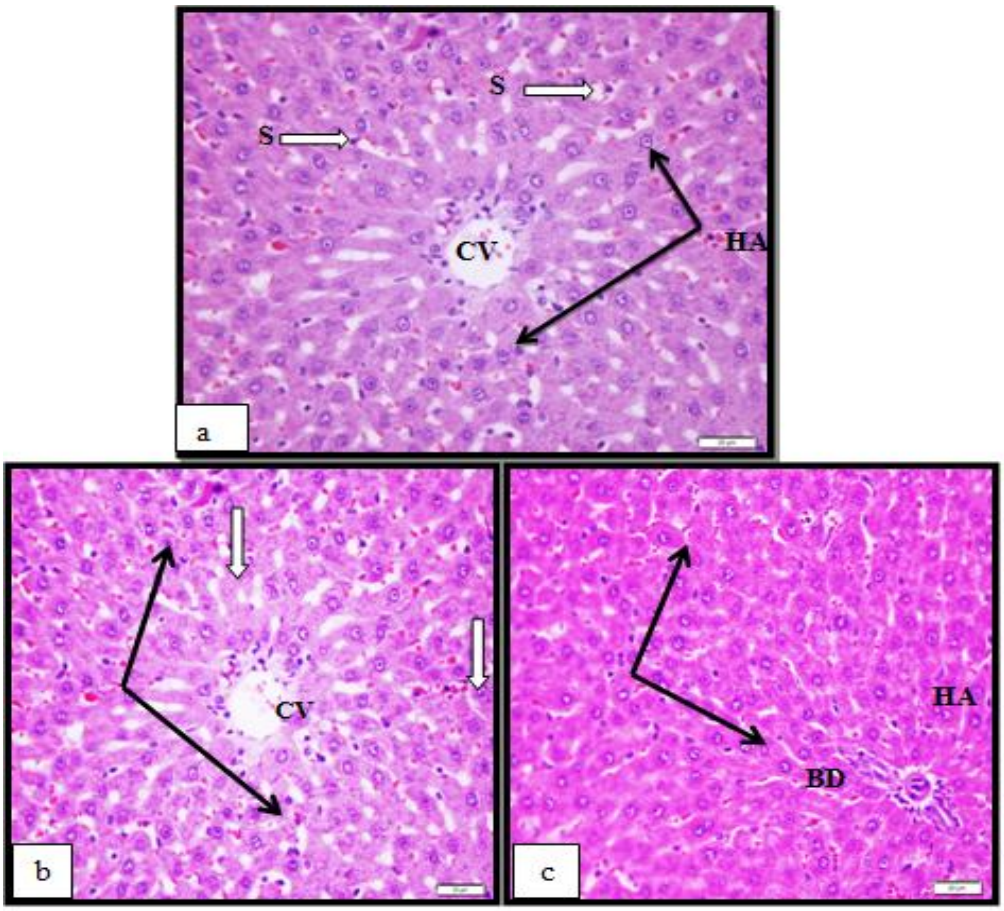

Fig. 1: (a-c)Sections from (negative control)control rat liver showing: a. Low power showing central veins (black arrows) and portal areas (white arrow) .b. High power to show central vein (CV) , hepatocyte cell cords (thin black arrows) separated by thin blood sinusoids (white arrows) . The cells have light stained cytoplasm, with basophilic spots (rough endoplasmic reticulum) and rounded nuclei .c. Portal region showing branches of hepatic artery (HA) and bile duct (BD) (H&E stainx 400).

Histopathological examination of liver of positive control showed slight individual variation regard to the effect of hypercholesterolemia on liver parenchyma. Most samples showed lipid deposition within hepatocytes (Fig 2.a-d). Lipid droplets vary in size from tiny small droplets (Fig 2.a-c) to large ones that occupy the whole cell (Fig 2.d).Some animals showed degeneration of hepatocytes with inflammatory changes and Hemorrhage (Fig 2.c).

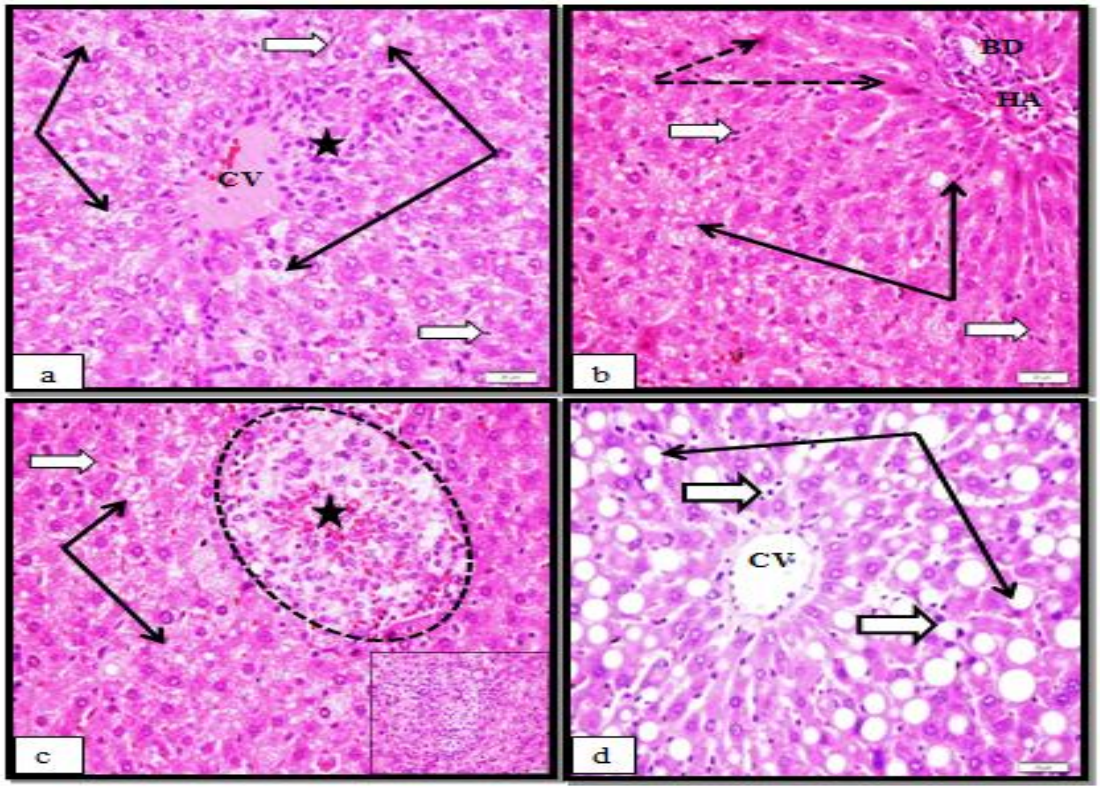

Fig. 2: (a-d) Sections of liver from different rats of (positive control)receiving hypercholesterolemia showing : a. Marked lipid deposition of large size within hepatocytes . b. Central vein (CV) region showing swollen hepatocytes (thin black arrows). with lipid deposition (rounded unstained vacuoles of various sizes) Blood sinusoids are ill defined and compressed . The nuclei are small .c. Region from other animal showing rounded area with degenerated hepatocytes (dotted circle) and hemorrhage (star). Also see swollen hepatocytes full with lipid droplets (black arrows) .d. Portal region showing branches of bile duct (BD) , hepatic artery (HA) and hepatocytes full with lipid droplets (black arrows) also there is dark shrunken (dotted arrows)degenerated hepatocytes (apoptotic cells) (H&E stainx 400).

Histopathological examination of rats' liver treated with 250 mg/kg b.wt. with PLE showed improvement of liver histological changed induced by hypercholesterolemia. Hepatocytes looked normal with absence of lipid deposition, degenerated hepatocytes or inflammatory changes observed in non-treated groups (Fig 3. a).

However, few animals where lipid profile was not return to normal there was no improvement and hepatocytes showed lipid droplets but less than non-treated group (Fig 3 .b-c).

Histopathological examination of rats group treated with 500 mg/kg b.wt. of PLE showed in (Fig 4 a , b).

In this group sections from all animals at both central vein regions(Fig 4 . a) and portal areas (Fig 4.b) showed normal hepatocytes with absence of lipid deposition .Central veins and blood sinusoids looked also normal .No inflammatory changes or degenerated cells were observed among hepatocytes or around portal areas (Fig 4).

In group of rat, treated with 750 mg/kg b.wt, the histopathological examination marked variations in response to administration PLE.

In animals showed improvement of lipid profile (rat1, rat2, rat6) where blood cholesterol ranged from 380, 386, 379 mg/dl to 140, 143, 149 mg/dl; the liver parenchyma (hepatocytes and sinusoids) looked normal and similar to that of control (Fig 5.a-c).

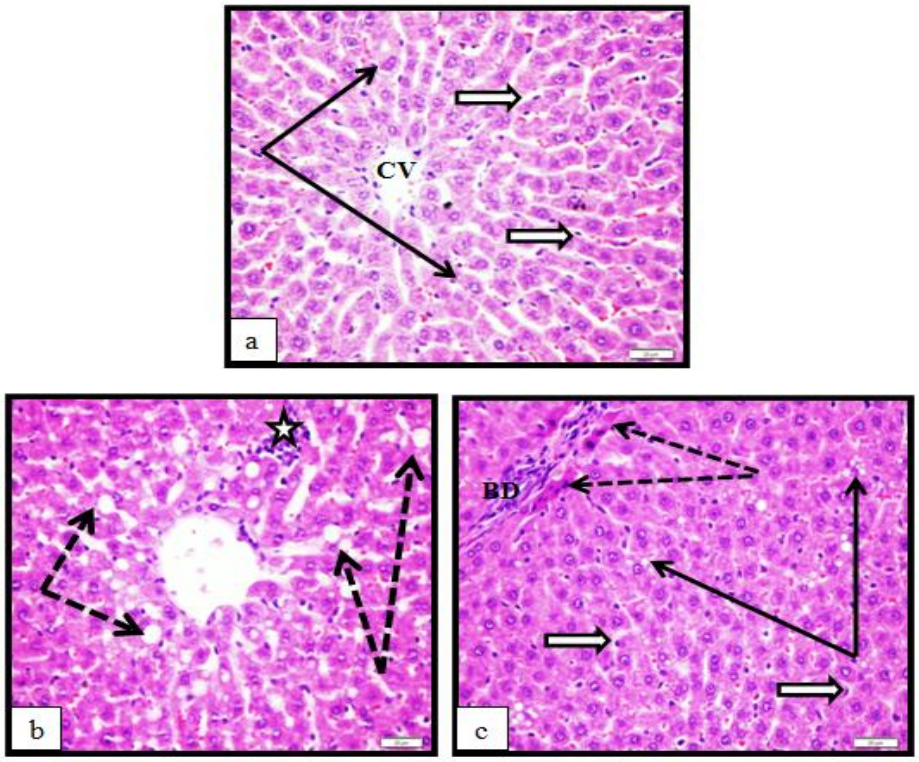

Fig. 3: (a-c)Section in the liver of hypercholesterolemia rat after treatment with 250 mg/ kg b. wt. of (PLE) showing : a. central vein regions with normal appearance of central vein (CV) and hepatocyte cell cords (black arrows) with no deposition of lipids or any features of cell damage (degeneration or apoptosis) blood sinusoids are also normal (white arrows). b. some animals still showed few large droplets in some hepatocytes (dotted arrows). And areas of cell degeneration (star). c. Portal region showing branches of bile duct (BD). Few hepatocyte cell cords (black arrows) showed few lipid droplets (black arrows). Blood sinusoids are normal (white arrows) (H&E stainx400).

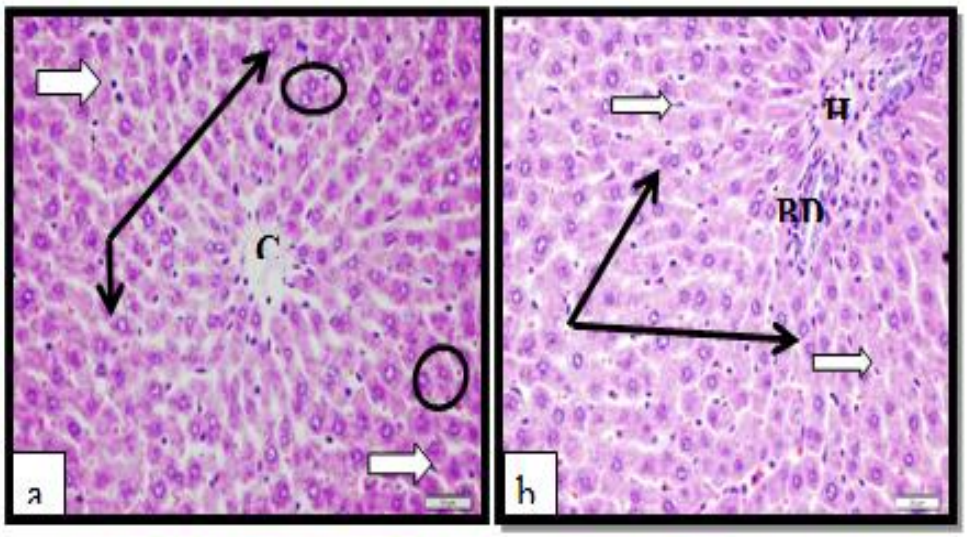

Fig. 4: (a-b) Section in the liver of hypercholesterolemia rat after treatment with 500 mg/ kg b. wt. of (PLE) showing : a. central vein region with normal appearance of central vein (CV) and hepatocyte cell cords (black arrows) with no deposition of lipids or any features of cell damage (degeneration or apoptosis) blood sinusoids are also normal (white arrows) .b. Portal region showing branches of bile duct (BD) hepatic artery (HA) also hepatocyte cell cords (black arrows) showed no deposition of lipids or any features of cell damage (black arrows) blood sinusoids are normal (white arrows) (H&E stainx 400).

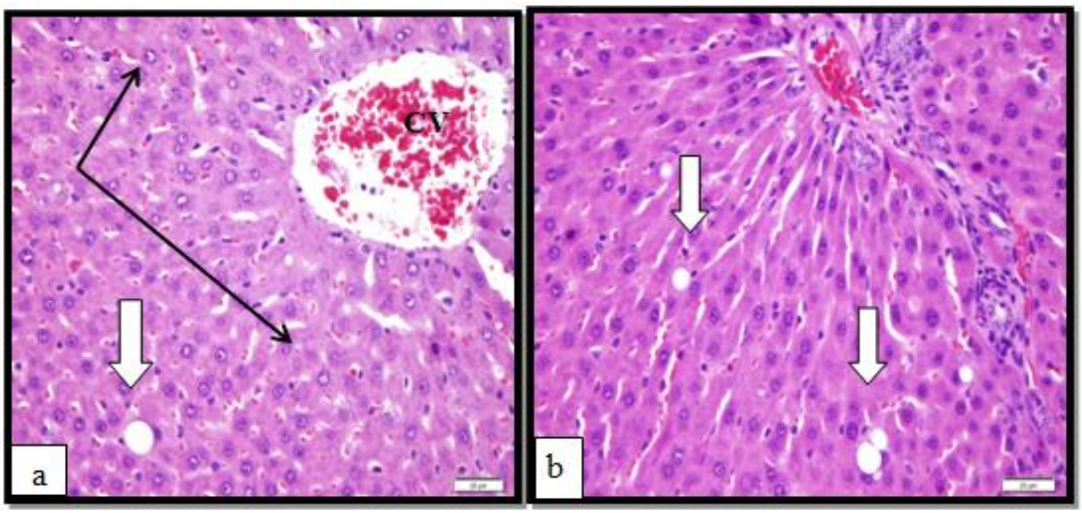

Fig. 5: (a-b)Section in the liver of hypercholesterolemia rat after treatment with 750 mg/ kg b. wt. of (PLE) rats that showed improvement in lipid profile showed : a. hepatocytes (black arrows) near central vein (CV) with absence of any vacuolation or degenerative changes seen in non - treated groups .b. Few scattered cells showed single lipid droplets (white arrows). Hepatocytes near portal region also showed normal structure with absence of any lipid deposition or inflammatory changes around portal vein (PV) or bile duct (BD) (H&E stainx4

In few animals where lipid profile still high and abnormal (rat3 , rat4, rat5) and blood cholesterol was 414 ,445 ,400 mg/dl to189 ,192 ,198 mg/dl ; the liver of such animals still showed histological deposition of lipids similar to what was observed in non-treated groups (Fig 6 .a-b). In conclusion, low and moderate doses of PLE were found to be more potent than high doses (750 mg/kg b.wt.) in controlling and maintain normal lipid profile in HFD fed animals . In such doses PLE was found to protect liver parenchyma from lipid deposition and their associated inflammatory reactions.

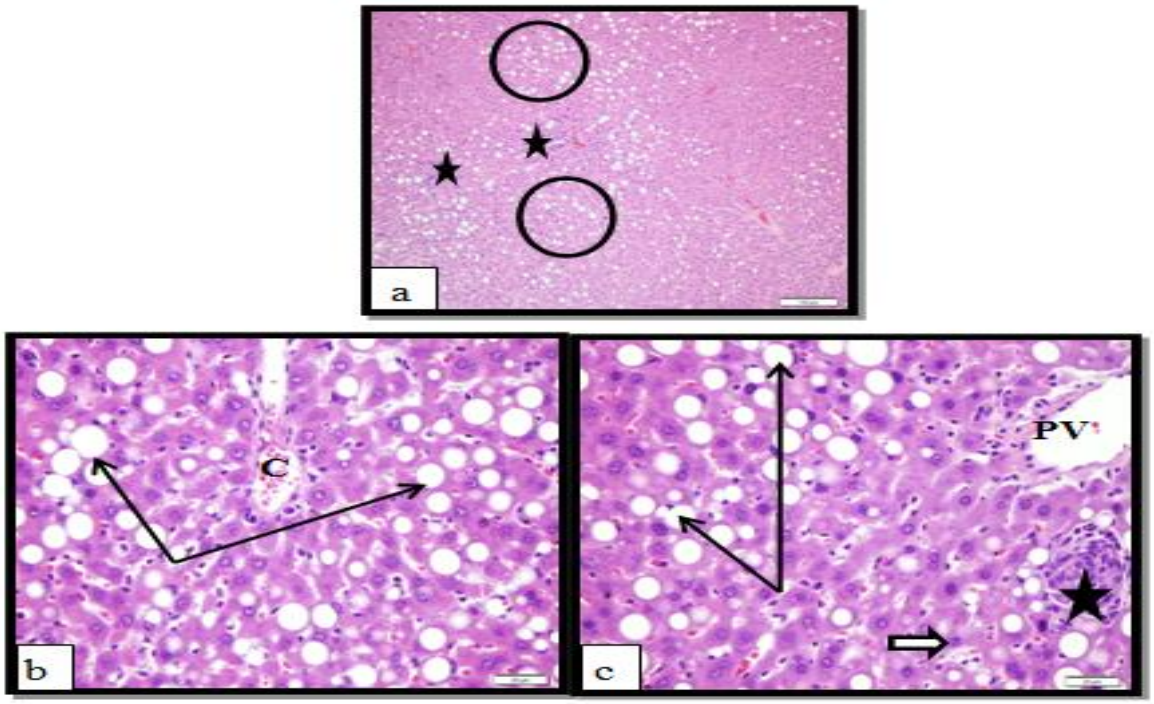

Fig. 6: (a-b) Section in the liver of hypercholesterolemia rat after treatment with 750 mg/ kg b. wt. of (PLE) which still showed high lipid profile showing : a. Marked deposition of lipid in most liver lobules (circles) with inflammatory foci (stars) .b. central vein region (CV) where hepatocyte cell cords (black arrows) showing deposition of lipids .c. Portal region showing part of portal vein (PV) and bile duct (BD) with inflammatory cells(star) also hepatocyte cell cords showed lipid deposition (black arrows) (H&E stainx400).

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