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HIGH-FAT DIET-INDUCED OBESITY IS ATTENUATED BY DATE SEED EXTRACT IN RAT MODEL

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ABSTRACT

Background: Obesity has been on the increase and is now an international health problem thus early must be control and prevent childhood obesity**.** ***Objective:*** This work was conducted to investigate the biochemical effect of date seed extract on lipid profile and hepatic antioxidant status in experimentally obese rats. ***Results:*** In this study 112 male albino Wister rats were involved and were divided randomly into fourth groups (28 rats in each group) and treated as following for 10 weeks: Group 1 served as control and received basal diet Group –II (Positive control obese group) rats were fed on high fat diet, Group III (date seed extract group): rats were fed on basal diet with administered orally date seed extract daily in a dose of 2 ml / kg b. wt. for 10 weeks Group IV rats were fed on high fat diet for 4 weeks followed by administration of date seed extract orally in a dose of 2 ml / kg b. wt. daily for 6 weeks. beside continuation feeding on HFD. The results showed that the daily oral administration of pits of date palm caused decrease in total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides levels, atherogenic indices, glucose and MDA level while caused an increase in HDL-cholesterol, reduced glutathione and superoxide dismutase activity. Concerning liver histopathology, it was found that oral administration of date seed extract decrease fat droplet gradually and retain liver to normal condition**.** ***Conclusion:*** On conclusion the date seeds have the potential to improve serum biochemical values, antioxidant status and normalized hepatic tissue in obese male rats.

INTRODUCTION

High fat diet (HFD) for more than two months results in weight gain, hyperlipidemia, hyperglycemia and oxidative stress **(1)**. High-fat diet resulted in hyperlipidemia in rats which could alter the related marker enzyme profiles in serum and liver tissue and progress to liver cirrhosis **(2)**. Continued high-fat feeding for long period lead to severe visceral obesity, diabetes or impaired glucose tolerance and lipoprotein abnormalities in rat and mice **(3)**. Egypt is the second important countries in date world production which produced 710000 tons, the Egyptian dates

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represented about 17% of the total world production [4]. Date seeds are potent antioxidants and strong free radical scavengers [5], they contain several antioxidant components including phenolic compounds, (phenolic acids, anthocyanins and flavonoids), The total phenolic content found in date seed was 48.64 mg/100 g, the phenolic acids detected in date seed were gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric acid and *o*-coumaric acid [6], selenium (coenzyme of GPX) vitamin c, oleic acid and carotenoids [6, 7], a recent study showed that the antioxidant of date seed extract (DSE) are approximately 27 fold of date fruit [6]. Date seeds contain high content of oleic and linoleic acid [8], which plays an important role for the prevention of the cardiovascular disease, oleic acid is long chain fatty acid that caused an increase in the high density lipoprotein (HDL) content in blood, and at the same time lowering the low density lipoprotein (LDL) content [9]. Aqueous extract of date seeds have hypoglycemic effect on T1DM in rats [10], also date seed extract- minimizes the diabetic toxic effects on the liver and kidneys of rats, compared to insulin administration as a single drug [11], extract of date seeds restored the normal functional status of the poisoned liver, and also to protect against carbon tetrachloride hepatotoxicity on the rat liver [7].

MATERIALS AND METHODS

A- Preparation and Extraction of date palm *(Phoenix Dactylifera* L.) seeds:

Seeds obtained from sukkary dates were purchased from super market Makro in Cairo city , the seeds were washed with tap water , left to dry , the seeds were milled in a heavy duty grinder to roasted and crushed , the crushed seed powder was added to distilled water to make a mixture of 50 gm / L the mixture was boiled until it becomes brownish in color for 30 minutes then finally filtered [12], The dose was 2 ml / kg , body weight administered to rats orally daily in all the experiments [12].

B- High fat diet (HFD)

Chemical analysis of HFD pellets and pellets ingredient were performed according to [13].

Table 1: Chemical and ingredient compositions of high fat diet

Ingredient	***(g%)***
Yellow corn	40.0
Wheat flour	30.0
Powder milk	10.0
Wheat bran	5.0
Corn oil	5.0
Berseem hay	8.5
Vitamins and minerals	1.0
Sodium chloride	0.5
Chemical	***(g%)***
Total protein	11.2
Ether extract	10.6
Ash	5.81

C. Animals:

Experiment was performed using 112 male rats, weighting 120 g. The Rats were randomly selected and transferred to an animal house having

standard conditions. Animals were quarantined and allowed to acclimate for a week prior to experiment. The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature and humidity-controlled room. Water and feed were supplied *ad libitum*.

D. Experimental conditions

Rats were involved and were divided randomly into four groups (28 rats in each group) and treated as following for 10 weeks: Group 1 served as control and received basal diet, Group –II (Positive control obese group) rats were fed on high fat diet, Group III: rats were fed on basal diet with administered orally date seed extract daily in a dose of 2 ml / kg b.wt. [(12)] for 10 weeks, Group IV rats were fed on high fat diet for 4 weeks followed by administration of date seed extract orally in a dose of 2 ml / kg b.wt. daily for 6 weeks beside continuation feeding on HFD.

E. Blood sampling:

At the end of the test period, blood sample collected from retro-orbital puncture after diethyl ether anesthesia**,** blood samples were drawn into dry tubes. Serum were separated after centrifuging the blood sample and stored at -20°C for subsequent analysis.

Tissue sample Preparation:

After soon evisceration, the liver of each rat was removed, washed in saline; Liver pieces were preserved in 10% formalin for histopathological studies. The pieces of liver were processed and embedded in paraffin wax. Section were taken and stained with hematoxylin and eosin according to **(14)**, other part of liver was homogenized and stored at - 20 ^{0}C for further determination of antioxidant enzymes activities and MDA level.

Tissue homogenate was prepared from the liver according to Combs et al. **(15)**.

F- Biochemical Investigations

Serum glucose level was determined colorimetrically according to the method of [(16)]. The serum cholesterol concentration was determined by quantitative enzymatic colorimetric method of **(16).** Determination of high-density lipoproteins cholesterol (HDL-cholesterol**)** level was carried out according to the method of **(17).** Serum triglycerides level was determined colorimetrically according to the method of **(16).** Low-density lipoproteins cholesterol was calculated as described by **(18)** as follows:

$$LDLcholestrol(mg/dl) = Totalcholesterol - (\frac{triglyceride}{5}$$

Very low-density lipoproteins cholesterol was calculated as described by **(18)** as follows:

VLDL- cholesterol (mg/dl) = Triglyceride /5

Atherogenic indices were calculated as described by **(19, 20).**

1- Cardiac risk ratio = Total cholesterol / HDL cholesterol

2- Atherogenic coefficient =
(Total cholesterol - HDL cholesterol) / HDL cholesterol

3- Atherogenic index of plasma =
log (Triglyceride / HDL cholesterol)

Lipid Peroxidation and Antioxidant Enzyme

Measurement of Malondialdehyde (MDA Concentration):

Liver lipid peroxidation product such as malondialdehyde (MDA) was determined by the method of **(21)**. MDA reacts with thiobarbituric acid (TBA) in an acid medium giving a colored TBA-complex measured colorimetrically at 520-535 nm against blank and MDA values were expressed as n moles MDA/mg protein

Measurement of Superoxide Dismutase Activity:

Superoxide dismutase (SOD) activity was estimated according to **(22)**. The optical absorbance was measured at wave length 560 nm against blank reagent. SOD=

Reading (absorbance) of (SOD) / mg protein.

Glutathione reduced (GSH) activity was determined in liver homogenate according to the procedure described by [23] by using Kits obtained from Gama Group Company in Egypt.

Protein Determination: The total protein concentration of supernatant was determined by the method of [24].

Statistics: The values were expressed as means ± standard error (SE). One way ANOVA was used to compare between the values of treated groups and that of the control (G1) All statistical analyses were performed using SPSS (Statistical package for Social Sciences 10.0 for windows) [25].

RESULTS

The obtained data in figure 1, 2 showed that a significant increase in serum total cholesterol and triglyceride concentration in obese group (G2) all over experimental period compared to control group (G1) while date seed extract group (G3) showed a significant decrease in serum total cholesterol and triglyceride concentration all over experimental period compared to control group (G1). Treated obese date seed extract group (G4) showed significant decreased in serum total cholesterol and triglyceride concentration compared to obese group (G2).

The obtained data in figure 3 showed that a significant decrease in serum HDL-cholesterol concentration in obese group (G2) after 8 weeks compared to control group (G1) while date seed extract group (G3) showed a significant increase in HDL-cholesterol all over experimental period compared to control group (G1) as well as treated obese date seed extract group (G4) showed significant increased in HDL-cholesterol compared to obese group (G2).

The obtained data in figure 4, 5 showed that a significant increase in LDL-cholesterol and VLDL-cholesterol in obese group (G2) all over experimental period compared to control group (G1) while date seed extract group (G3) showed a significant decrease in LDL-cholesterol and VLDL-cholesterol concentration all over experimental period compared to control group (G1). Treated obese date seed extract group (G4) showed significant decreased in LDL-cholesterol and VLDL-cholesterol concentration compared to obese group (G2).

The obtained data in figure 6, 7, 8 showed that a significant increase in cardiac risk, atherogenic coefficient and atherogenic index in obese group (G2) all over experimental period compared to control group (G1) while date seed extract group (G3) showed a significant decrease in cardiac risk, atherogenic coefficient and atherogenic index all over experimental period compared to control group (G1). Treated obese date seed extract group (G4) showed significant decreased in cardiac risk, atherogenic coefficient and atherogenic index compared to obese group (G2).

The obtained data in figure 9 showed that a significant increase in serum glucose in obese group (G2) all over experimental period compared to control group (G1) while date seed extract group (G3) showed a significant decrease in serum glucose all over experimental period compared to control group (G1). Treated obese date seed extract group (G4) showed significant decreased in serum glucose compared to obese group (G2).

The obtained data in figure 10 showed that a significant increase in concentration of malondialdehyde (MDA) in obese group (G2) all over experimental period compared to control group (G1) while date seed extract group (G3) showed a significant decrease in MDA level all over experimental period compared to control group (G1). Treated obese date seed extract group (G4) showed a significant decreased in MDA level compared to obese group (G2).

The obtained data in figure 11, 12 showed that a significant decrease in activity of

superoxide dismutase and reduced glutathione concentration in obese group (G2) all over experimental period compared to control group (G1) while date seed extract group (G3) showed a significant decrease in superoxide dismutase and reduced glutathione all over experimental period compared to control group (G1). Treated obese date seed extract group (G4) showed a significant decreased in superoxide dismutase and reduced glutathione compared to obese group (G2).

Effect of date seed extract on liver histopathology in different groups of rats:

Parallel to the biochemical changes, the histopathological examination of the liver revealed some morphological changes. The histopathological examination of liver of rats of control group (G1) showed normal histological structure and appearance of hepatocytes all over experimental period in figure (13). The histopathological examination of liver of rats of obese group (G2) that fed on high fat diet all experimental period showed that signs of fatty change in which the hepatocyte cells appeared swollen with presence of circumscribed vacuoles may present around the nucleus or pushing the nucleus to one side giving signet ring appearance in figure (14). The distribution varied according to the time of the experiment. It appeared focal after 4 weeks and increase in severity with the time of experiment. At the end of experiment the liver showed focal areas of coagulative necrosis in figure (15). The histopathological examination of rat liver of date seed extract group (G3) showed that normal histological structure and appearance of hepatocytes all over experimental period in figure (16). The histopathological examination of liver of rats of treated obese date seed extract group (G4) showed that fatty changes varied in distribution and severity, it severe after 4 and 6 weeks of experiment in figure (17), but less in severity after 8 weeks of experiment in which it appeared around the central vein only in figure (18), and most of hepatocytes showed hydropic degeneration. The liver appeared normal after 10 weeks of experiment in figure (19).

DISCUSSION

From the obtained results, it was clear that high fat diet increased the level of cholesterol, triglyceride, LDL-cholesterol, VLDL-cholesterol, Cardiac index atherogenic coefficient and atherogenic index. High fat diet intake generally increases energy storage mainly as a triglyceride and raises circulating free fatty acid level [26], also increased Serum atherogenic coefficient because of high fat diet increased the level of cholesterol and decrease the level of HDL cholesterol lead to increased Serum atherogenic coefficient [19, 20]. This finding was in agreement with that obtained by [27] who reported that high fat diet increase level of cholesterol, triglycerides, LDL and VLDL and hyperlipidemia. Feeding a high fat diet resulted in elevating the plasma glucose level. This finding was confirmed by that of [28] who reported that feeding a high fat diet for 10 weeks resulted in elevating the plasma glucose level.

The obtained results revealed that date seed extract caused decreased the level of cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, Cardiac index, atherogenic coefficient and atherogenic index in treated obese date seed extract group, this effect is due to date seed fibers content which increases the activity of plasma lecithin cholesterol acyl transeferase (LCAT) that enhances hepatic bile acid synthesis and increases

degradation of cholesterol to fecal bile acid [29, 30].

The obtained data revealed that date seed extract have stronger effect of increased level of HDL cholesterol, and reach level of HDL cholesterol in treated obese date seed extract group because of its high content of both oleic and linoleic acid which increased the level of high density lipoprotein (HDL), that have essential role in transport total cholesterol from peripheral tissues to liver for synthesis of bile acid, vitamin D and steroid hormones [8] as well as lowering the level of low density lipoprotein (LDL) content in blood [9].

Date seed extract have hypoglycemic effect, stimulating certain cells to differentiate in pancreatic cells [10].

Our result revealed that the high fat diet intake lead to increased the concentration of MDA and decreased the activity of superoxide dismutase (SOD) and concentration of reduced glutathione. These investigations were in agreement with many authors [31, 32] they are demonstrated that high fat diet increased MDA concentration, as well as suppresses the antioxidant reserve and reduce the concentration of superoxide dismutase (SOD) [33].

On the other hand date seed extract decreased the concentration of MDA these finding was in agreement with (34). Also date seed extract increased the concentration of antioxidant enzymes as superoxide dismutase (SOD), due to the date seed extract contain several antioxidant components including phenolic compounds, selenium, vitamin C, oleic acid and carotenoids, which are essential for superoxide [6].

These finding was in agreement with some authors [35] reported that the high fat diet leads to signs of fatty change in hepatocyte cells. The date seed extract group showed a gradual improvement in liver morphology and reaches the liver cells in treated obese date seed extract in normal histopathological pattern.

CONCLUSION

The administration of date seed extract caused decrease in total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides level, atherogenic indices, glucose, MDA status and increase in HDL-cholesterol and antioxidant enzymes as GSH and SOD activities in treated obese rats, also decrease fat droplet and retain liver to normal condition.

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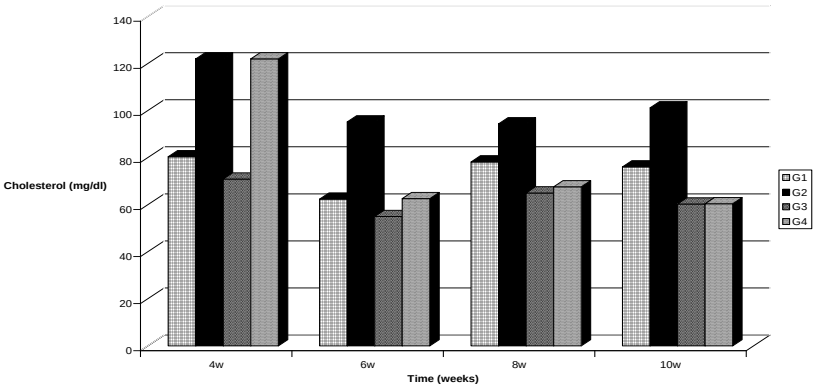

Fig. 1: **Effect of date seed extract on serum total cholesterol concentration (mg/dl) in different rat groups.**

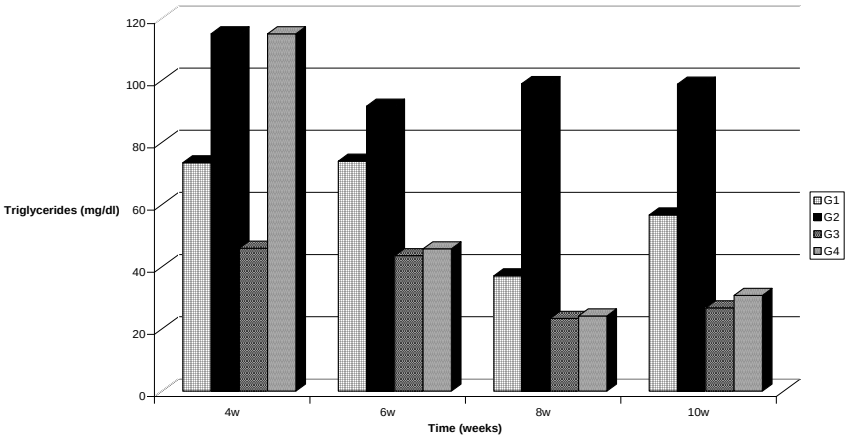

Fig. 2: Effect of date seed extract on serum triglycerides concentration (mg/dl) in different rat groups.

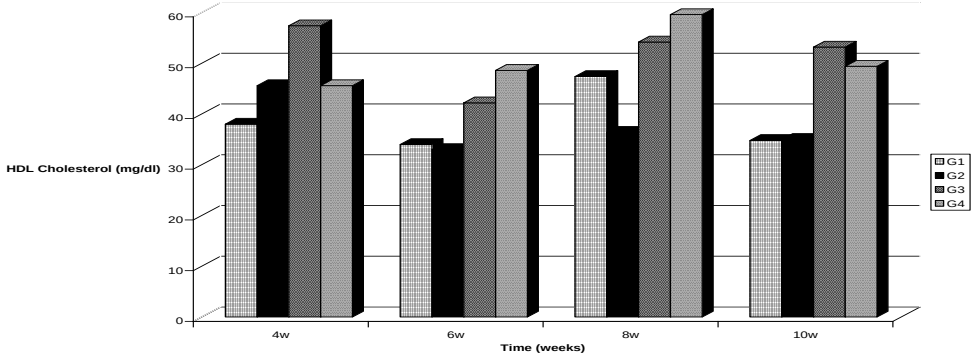

Fig. 3: Effect of date seed extract on serum HDL cholesterol concentration (mg/dl) in different rat groups.

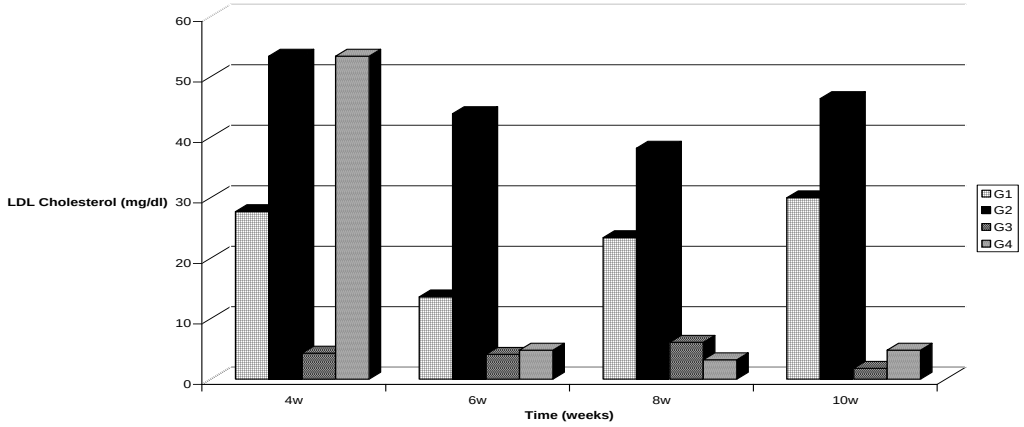

Fig. 4*:* Effect of date seed extract on serum LDL cholesterol concentration (mg/dl) in different rat groups.

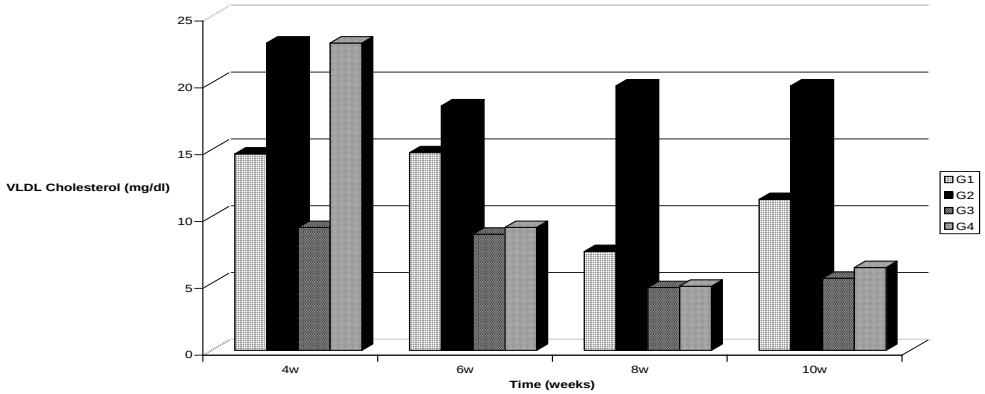

Fig. 5: Effect of date seed extract on serum VLDL cholesterol concentration (mg/dl) in different rat groups.

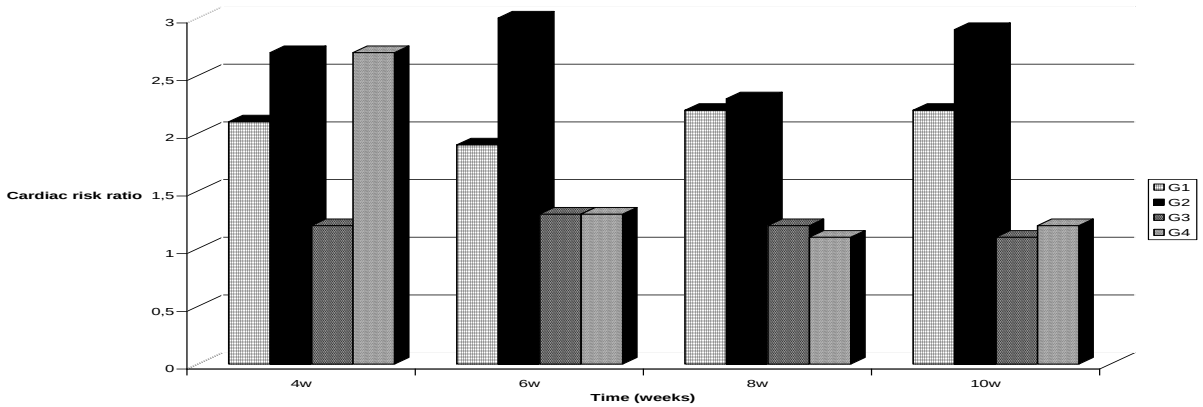

Fig. 6: Effect of date seed extract on serum cardiac risk ratio in different rat groups.

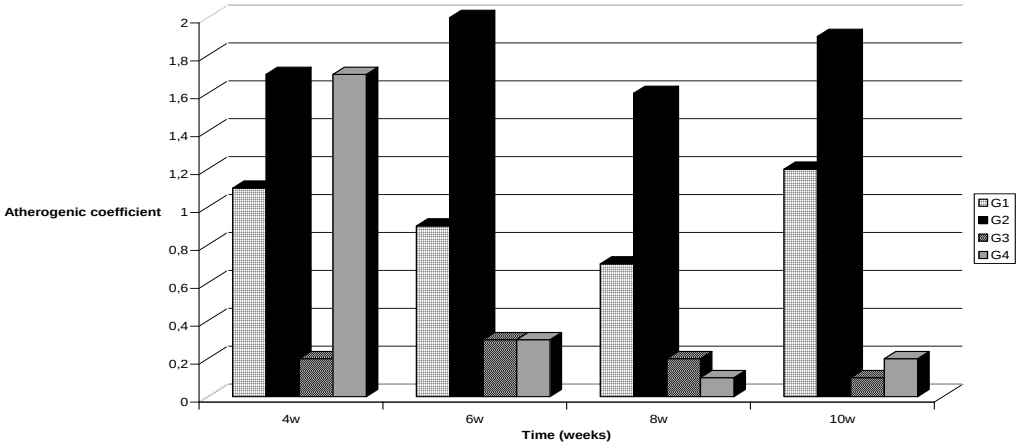

Fig. 7: Effect of date seed extract on serum atherogenic coefficient in different rat groups.

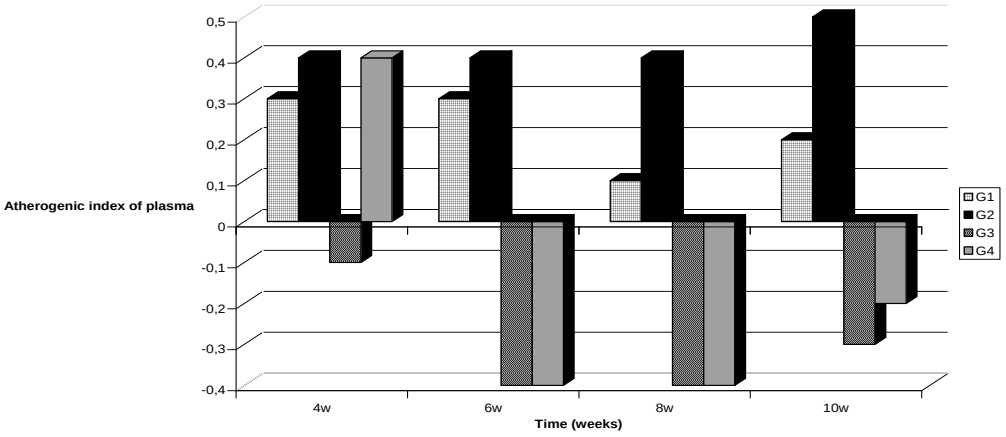

Fig. 8: Effect of date seed extract on serum atherogenic index of plasma in different rat groups.

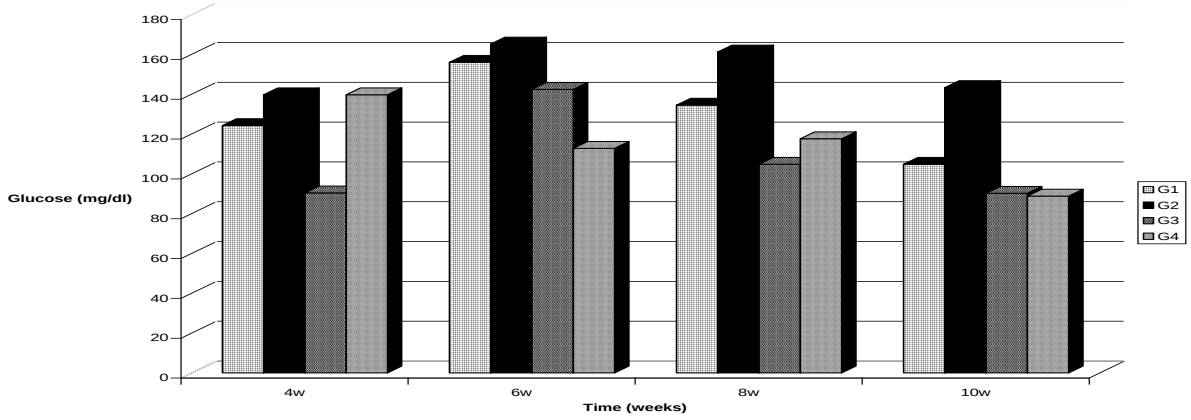

Fig. 9: Effect of date seed extract on serum glucose concentration (mg/dl) in different rat groups.

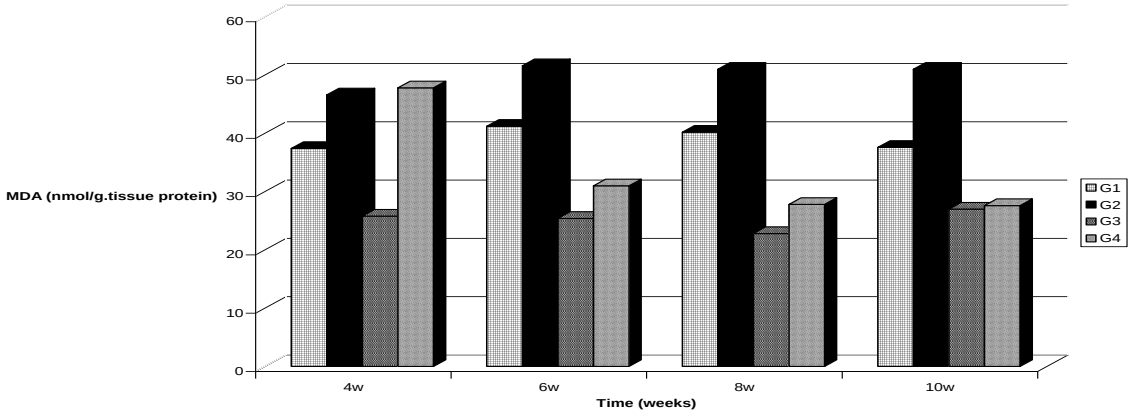

Fig. 10: Effect of date seed extract on malondialdehyde (MDA) concentration (nmol /g tissue protein) in liver tissues of different rat groups.

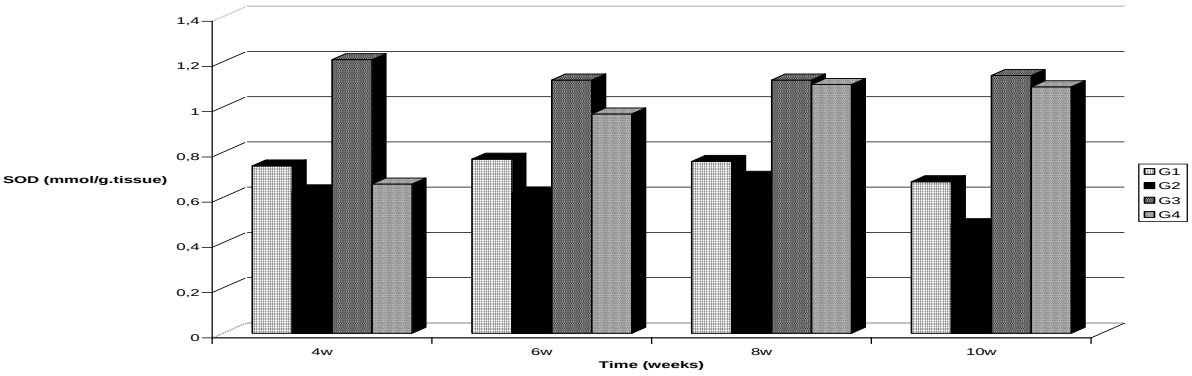

Fig. 11: Effect of date seed extract on superoxide dismutase (SOD) concentration (µmol /mg tissue protein) in liver tissues of different rat groups.

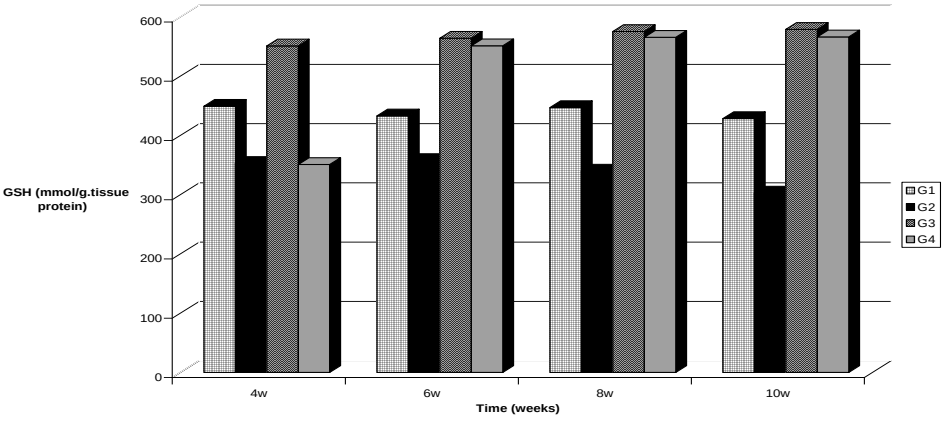

Fig. 12: Effect of date seed extract on reduced glutathione concentration (mmol /g tissue protein) in liver tissues of different rat groups.

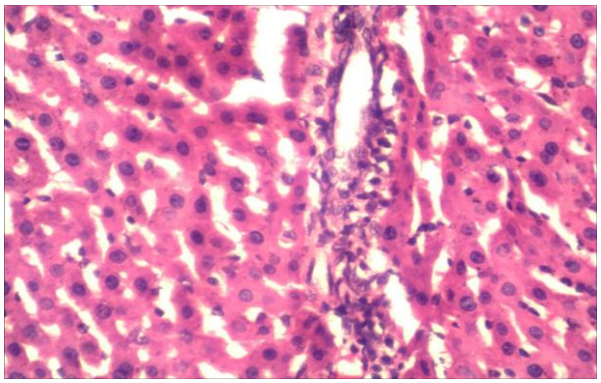

Fig. 13: Liver of rats fed on basal diet (G1) after 4 weeks showing normal structure of liver cells. H&E

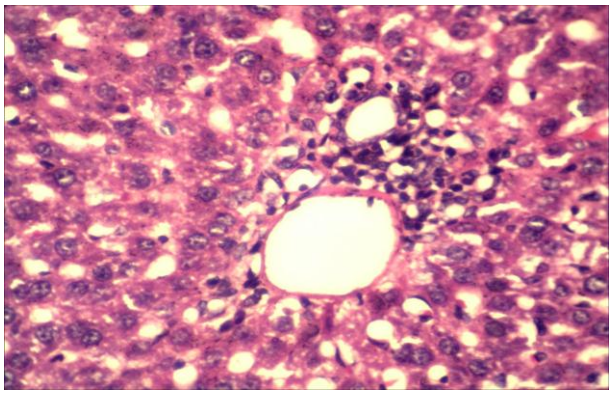

Fig. 14: Liver of rats fed on high fat diet (G2) at 4 weeks showing fatty changes around the central vein. H&EX 400.

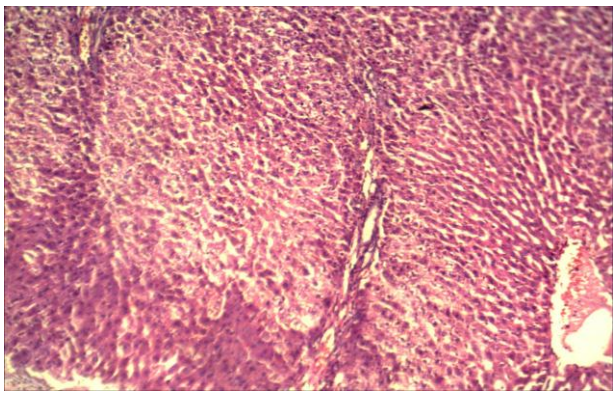

Fig. 15: Liver of rats fed on high fat diet (G2) at 10 weeks showing focal areas of coagulative necrosis. H&E X 100

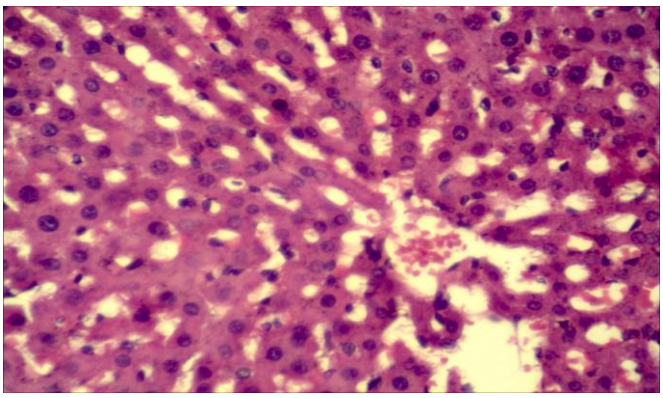

Fig. 16: Liver of rats of date seed extract group (G3) at 10 weeks showing normal appearance of liver cells. H&EX 400.

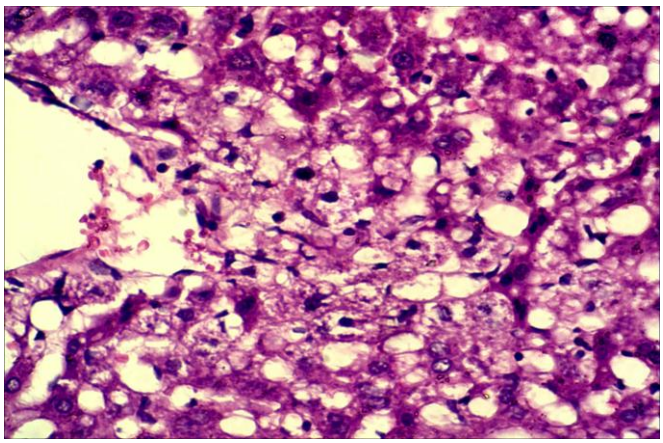

Fig. 17: Liver of rats of treated obese date seed extract group (G4) at 6 weeks showing severe fatty changes in hepatocytes. H&EX 400

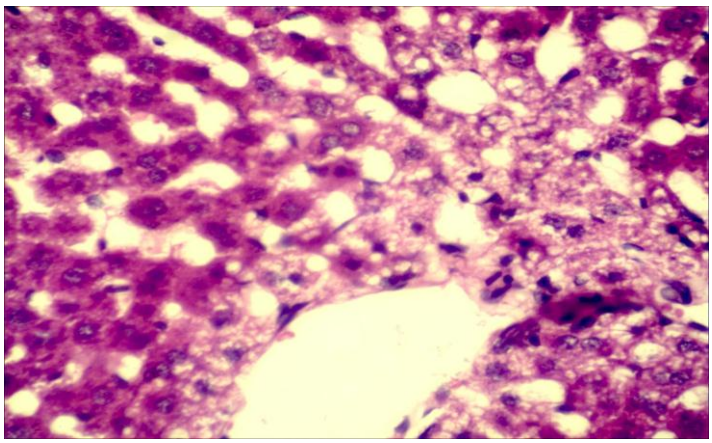

Fig. 18: Liver of rats of treated obese date seed extract group (G4) at 8 weeks showing fatty changes around the central veins only. H&EX 400.

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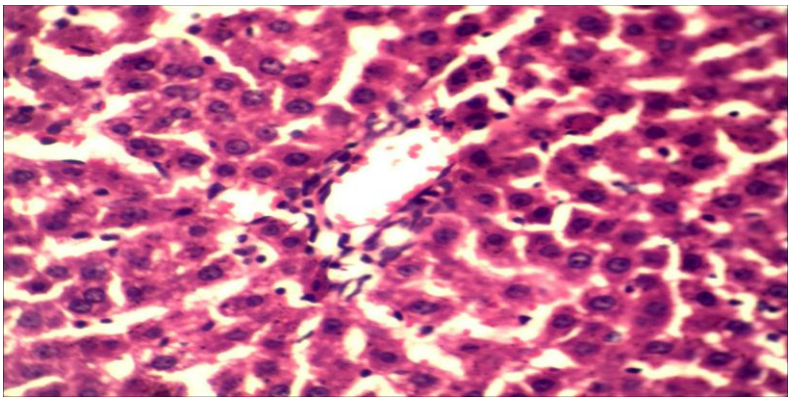

Fig. 19: Liver of rats of treated obese date seed extract group (G4) at 10 weeks showing normal appearance of liver cells. H&EX 400.