Structural Changes of Diabetic,Hypercholesterolimic and Hepatoxified toxicated Rats as Influenced by Samani Date kernel Diets

BY

Mona E.M. Naga

Dept. of Home Economics ,Specific Education Faculty Menoufia University

Abstract

This study was carried out to determine the effect of natural date kernel powder on males of white rats weighing 150-200 g. Rats were divided into twelve group (each group consist of five rats) (three groups for control - negative group) and three groups control postive.The hypercholesterolemic rats were diseared by feeding them on the standard diet containing 1.5% cholesterol plus 10% fat from sheep tail for 15 days.

The indual diabetic rats were previously injected with alloxan at 150 mg / kg body weight into the peritoneal membran . Hepatointoxication was induced by CCl_4 injection (2ml/kg Bw)twice a week for 2weeks.

Other three group inflicted with hepatointoxication, hypercholesterolemia and diabetes mellitus.were fed on the basal diet with germinated powdered dates stones 5%. Other three group, inflicted with hepatointoxication, hypercholesterolemia and induced diabetes mellitus were fed on basal diet with extracts from dates seeds by methanol,after being dried and added to distilled water after methanol volatilization ,them administered orally at 1/2 ml level daily.

At the end of the experiment (45 days) rats wered slaughtered under anesthesia, after 12 hours of fasting . Liver ,hearts and pancreas removed,cleaned in saline solution then wiped samples were soaked in formalin then fixed in parafin and slaimed , and sections were prepared ,Investigated by light microscope and photographed .

It was found that the date palm extract and date kernel powder had a significant desirable effect on histological structure of the internal organs of rats suffering from hypercholesterolemia , diabetic mellitus & hepatotoxicity.

Keywords: Palm date kernel .Hepatointoxication, hypercholesterolemia, diabetes mellitus, rats.

INTRODUCTION

The date palm is largely grown in arid and semiarid regions of the world. World production of date seeds reached approximately 863,000 ton 2004. Date kernel are being wasted in large quantities or used mainly for animal (camels, cattle, sheep and poultry) feed. In addition, date seeds are used on a very limited scale in making a caffeine-free beverage with a coffee-like flavour***(Hussein et al., 1998; FAO, 2004; Rahman et al., 2007;and Habib and Ibrahim 2011).***

According to some authors *Phoneixdactylifera* extract has antibacterial effect against E. fecalis, indicating that this extract can be used for treating enteric diseases.Also,an important report of extracts of the flesh and pits of Phoenix dactylifera in gentamicin treated nephrotoxicity rat model showed significantly reduced increase in plasma creatinine and urea concentrations, and ameliorated the proximal tubular damage*)*.

Date seed extract (DSE), especially from germinated seeds,increases serum paraoxonase and arylesterase activities. These beneficial effects may be due to the presence of natural antioxidants such as phenolic compounds in the palm date seeds ***(Takaeidi et al., 2014)*** Germination of palm date seeds was said to improve the biological effect of seeds ***(Juan et al., 2016 &Burghardt et al., 2016).***

Hypercholesterolemia is typically due to a combination of environmental and genetic factors. Environmental factors,include obesity and dietary choices.Genetic contributions are usually due to the additive effects of multiple genes though occasionally may be due to a single gene defect such as in the case of familial hypercholesterolemia**.(Shields and shields., 2008)**

Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood, as identified as dyslipidemia, to describe the mainfestations of different disorders of lipoprotein metabolism. Although elevated low density lipoprotein cholesterol (LDL)is thought to be the best indicator of atherosclerosis risk ,dyslipidemia can also describe elevated total cholesterol (TC) or triglycerides (TG) or low levels of high density lipoprotein cholesterol (HDL) ***(Jacobson,1998).***

MATERIALS AND METHODS

-The used plants and their preparation: Palm date kernel (kernel) was washed well to get rid of any foreign material.The date pits were placed between two layers of cloth wetted by a spray of clean water twice a day for a month at room temperature 20-26^{o}C during the winter. Germinated kernel (1cm),were dried in direct sunlight, taking into account to be taken and kept during the night in order to prevent dirt & rain. After being germinated and dried, kernel were milled, in electrical mill, to get fine powder and kept in sealed plastic bag.The kernel powder was mixed with methanolic alcohol at 5% level, shaked at 60^{o}C for 12 hours., filtrated, centrifugated at 400 rpm for 10 min,and alcohol repelled in a rotary evaporator, at 60^{o}C. The powdered extract,which was diluted with distilled water(100 ml water & 50 gm powder) and used in by oral injection at a rate of 1/2 ml rat. Also, date kernel powder(unextracted) mixed with the basal diet(50gm powder/1kg of basal diet) to be used in feeding the rats.

-Experimental animals: sixty (60) adult male healthy albino rats, weighting 150-200 g each, were used in this study.

-Basal diet composition of tested rats: Basal diet composition of tested rats was according to **Reeves *et al.,* (1993).**

-Experimental design: The male albino rats were obtained from AgricultureResearchCenter,Giza, Egypt. Rats were housed in wire cages under the normal laboratory condition, and were fed on basal diet for a week as an adaptation period. Diet was offered to rats in special feed cups to avoid losing of feeds. Water was provided to the rats by glass tubes supported to one side of the cage, feed and water provided ad libium and checked daily.

Hepatointoxication was induced into rats by subcutaneous injection of carbon tetrachloride (CCl4)in parafin oil(50%u /l c2ml/kgb.B.W.) twice week (Hepatotoxified rats) for two weeks **(jayasker et al .,1997).**

Hypercholesterolemia was induced in normal healthy male albino rats by feeding rats on diet containing 1.5 % cholesterol plus 10 % sheep tail(Hypercholesterolemic rats) for 15 days according to the method described by **Ain (1993) .**

Diabetes was induced by intra peritoneal injection of alloxan 150 mg /kg body weight,according to the method described by **Desai and Bhide (1985).**

Each group consisted of five rats.

Grouping of feeding rats: Rats were distributed into 12 groups, 5rats each

Hepatoxified rats:

Group(1): Healthy rats were fed on basel diet only (control "-").

Group(2):CCl4 rats were fed on basal diet only (control "+").

Group(3): CCl_4 rats were fed on palm date seeds powder 5%.
Group(4): CCl_4 rats were fed with 1/2ml palm date kernel extract**.**
Diabeticinduced rats :
Group(5): Healthy rats were fed on basel diet only (control "-").
Group(6):diabetic rats fed on basel diet only (control "+").
Group(7): diabetic rats were fed on palm date kernel powder 5%.
Group(8): diabetic rats fed with 1/2ml palm date kernel extract**.**
Hypercholesterolemic rats:
Group(9): Healthy rats were fed on basel diet only (control "-")
Group(10): rats were fed on hypercholesterolemic diet .
(control "+")
Group(11): rats were fed on hypercholesterolemic diet and palm date kernel powder 5%.
Group(12): rats were fed hypercholesterolemic diet with 1/2ml palm date kernel extract

Histopathological investigation:

At end of the experimental period (45 days), all rats were sacrificed, Autopsy samples were taken from the internal organs of rats and fixed in 10% buffered formlin for twenty-four hours. The obtained tissue sections were collected on glass slides, paraffinized and stained by hematoxylin and eosin (***Banchroft et al., 1996***) for histopathological examinations by the light microscope**.**

Results and Discussion

The Histopathological Investigation:-

1- Healthy rats after 45 days of feeding:

Liver of control negative rat:

Liver section of control negative l rats (group 1)

showed normal histological structure of hepatic lobule including central vein and concentrically arranged hepatocytes (photo 1).

Pancreas of control negative rats:

Microscopically, pancreas of the conttol negative rats from group 5 assigned for Alloxan . Showed normal histological structure of pancreatic parenchyma with no changes (photo 2).

Heart of control negative rats:

Microscopically heart of rats from(group 9) control negative assigned for the hypercholesterolemia showed the normal cardiac myocytes.(photo 5)

II- Diseased rats.

a-Liver sections (Hepatoxified rats) :

Microscopically, liver of hepatoxified rat from group (2) (control "+ " group) revealed congestion of control vein and cytoplasmic vacuolization of hepatocytes(Photo4).Some sections of group (2) showed hepatic necrosis associated with inflammatory cells infiltration (Photo 5). Liver of group (2)showed also edema in the portal triad (Photo 6),hyperplasia of epithelial lining bile duct and poral inflammation with inflammatory cells (Photo 7),congestion of central vein and hepatic sinusoids (Photo 8), congestion of hepatic sinusoids and cytoplasmic vacuolization of hepatocytes (Photo 7) and cytoplasmic vacuolization of hepatocytes (Photo 8).

Microscopically, liver of diabetic rats from group (2) (control "+") revealed also necrosis of sporadic hepatocytes and activation of kupffer cell (Photo9), binucleation of hepatocytes (Photo10) and congestion of hepatic sinusoids as well as cytoplasmic vacuolization of hepatocytes (Photo 11). Liver section group (2) (control "+") revealed also sinusoidal leukocytosis and activation of kupffer cells (Photo12), cytoplasmic vacuolization of hepatocytes and oedema (Photo13) and cytoplasmic vacuolization of hepatocytes plus congestion of hepatic sinusoids (Photo 14).

Also, liver of hepatic rats from group (2) (control "+") revealed kupffer cells activation and binucleation of hepatocytes(Photo 15), congestion of control vein and hepatic sinusoids (photo16),or only hepatic sinusoids (Photo 17),sinusoids leukocytosis (Photo 18).or cytoplasmic vacuolization of hepatocytes and edema (Photo 19).

Rats fed on 5% palm kernel diet powder revealed the normal unchanged histological structure of hepatic lobule (Photo20) and rats fed on palm kernel extract 1/2ml showed also the normal histological structure of hepatic lobule (Photo 21).

b-Pancreas sections (diabetic induced rats):

Microscopically diseased rats (hepatic ,diabetic or hypercholesterolemic) of group 6 (control "+") (basal diet) revealed many changes congestion of pancreatic blood vessel (Photos 22, 23& 24),thickening in the wall of pancreatic blood vessel (Photo 25),vacuolization of islets of langerhan,s (Photo 26&27), vacuolization of pancreatic acini (Photo 28), vacuolization of pancreatic acini and congestion of pancreatic blood vessels(Photo 29). On the other hand pancreas section of diabetic rats fed on palm kernel powder 5% (Photo 30).or palm kernel extract 1/2ml (Photo 31) revealed the normal histological structure of pancreatic parenchyma e.g. with no histological chores.

C- Heart sections:(Hypercholesterolemic rats):

These few showed congestive of myocardial blood vessels (Photo 32),few inflammatory cells infiltration (Photo 33),intramuscular oedema (Photo 34),necrosis of cardiac myocytes associated with inflammatory cells infiltration (Photo 35),focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (Photo 36), vacuolation of tunica media of blood vessels (photo 41),inter muscular oedema and congestion of myocardial blood vessels (photo 38&39)

On the other side sections of rats fed with palm kernel powder 5% revealed normal cardiac myocytes with no histological change (Photo 40,41& 42). Also heart sections of hyperchlorlestrolemic rats of palm kernel extract intake ½ ml revealed no biological changes (Photo 43,44,45,46&47).

AbdElaziz , and Ali(2014) found that the examination of liver histopathology revealed that *Phoenix dactylifera* seeds attenuate the incidence of liver lesions (including vacuolization and fibroblast proliferation) triggered by CCl4 intoxication.

Phenolic compounds of dates as either soluble or linked to fiber date seeds contain very high levels of phenolic antioxidants. Date seeds also contain an antibiotic ox tetracycline. (**Sami *et al.*,2017)**.

The final conclusion all the control postive groups for hepatic,diabetic &hypercholesterolemic rats revealed dramatic recovery of liver, pancreas and heart tissues respectively, on feeding palm kernel powder and palm kernel extract.

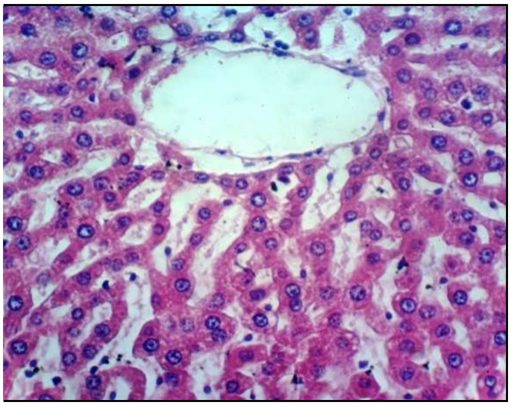
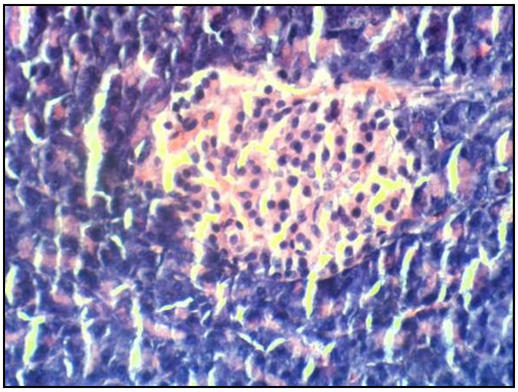
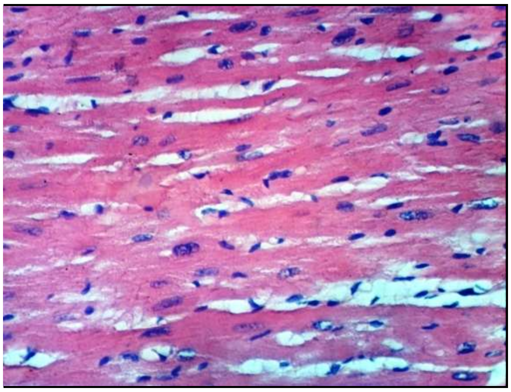


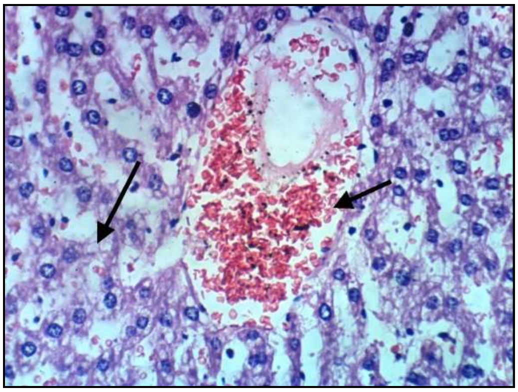
Photo (1): Liver of control,healthy untreated rat control (-) (basal daiet) showing the normal histological structure of hepatic lobule iincluding central vein and concentrically arranged hepatocytes (H & E X 400).



Photo(2): Pancreas of healthyrat from group 5conterol (-) (basal diet) showing the normal histological structure of pancreatic parenchyma (H & E X 400).



Photo(3): Heart of healthy rat from group 9 control (-)(basal diet) showing normal cardiac myocytes. (H & E X 400).



Photo(4): Liver of rat from(group 2)control (+) basal diet) showing congestion of central vein and cytoplasmic vacuolization of hepatocytes (H & E X 400**).**

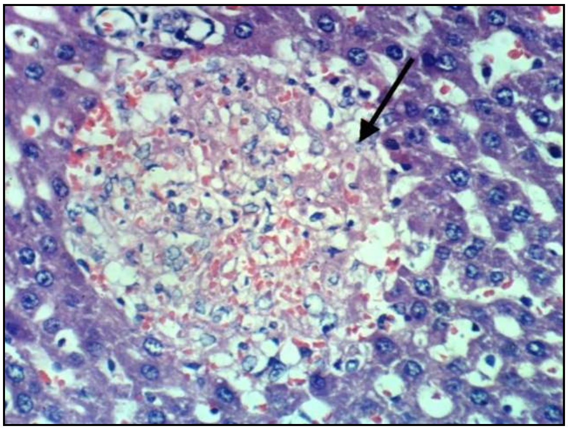


Photo (5): Liver of rat from(group 2)conterol (+)(basil diet) showing focal hepatic necrosis associated with inflammatory cells infiltration (H & E X 400).

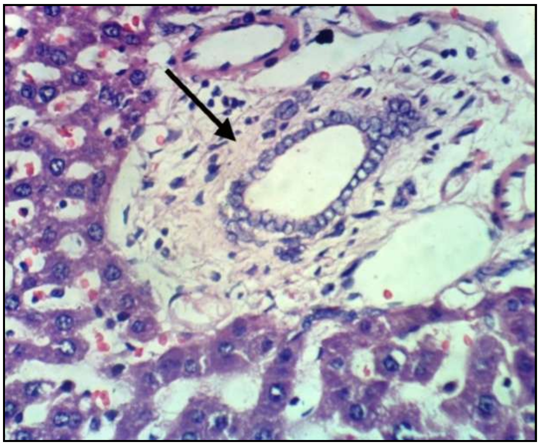


Photo (6): Liver of rat from(group2) control (+) (basil diet) showingedema in the portal triad (H & E X 400).

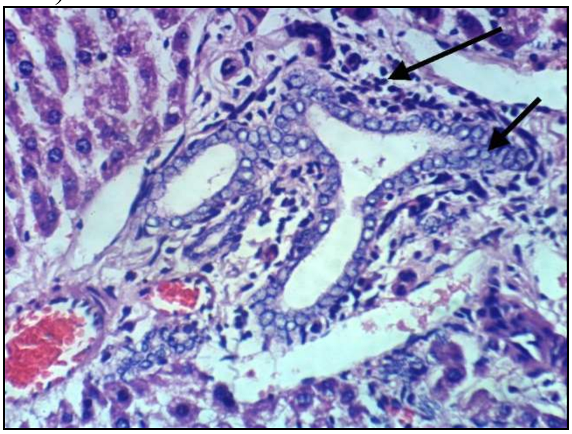


Photo (7): Liver of rat from(group 2)control (+)showing Hyperplasia of epitheliallining bile duct and portal infiltration with inflammatory cells (H & E X 400).

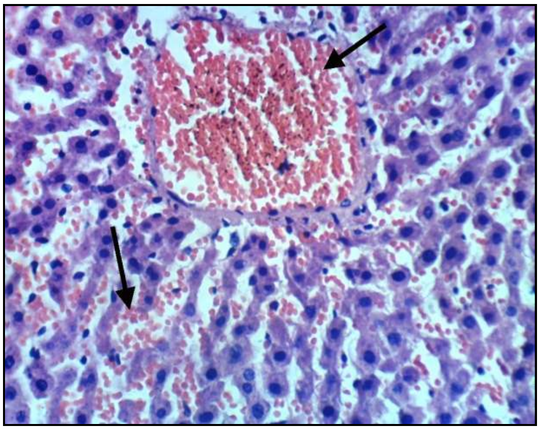


Photo (9): Liver of rat from(group 1) control (+)showing congestion of central vein and hepatic sinusoids (H & E X 400).

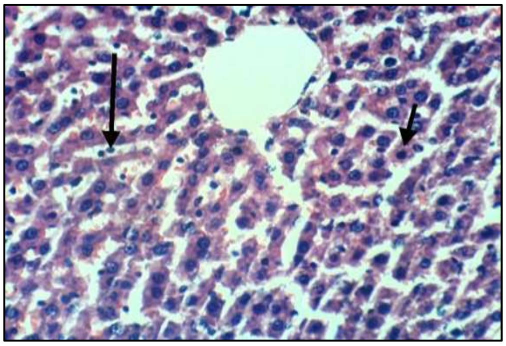


Photo (9): Liver of rat from group(2) control (+)showing necrosis of sporadic hepatocytes and activation of Kupffer cells (H & E X 400).

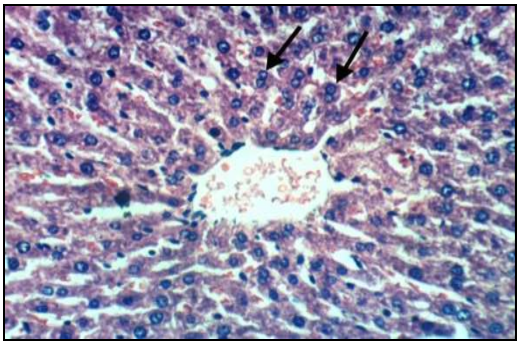


Photo (10): Liver of rat from(group 2) (control +) showing binucleation of hepatocytes (H & E X 400).

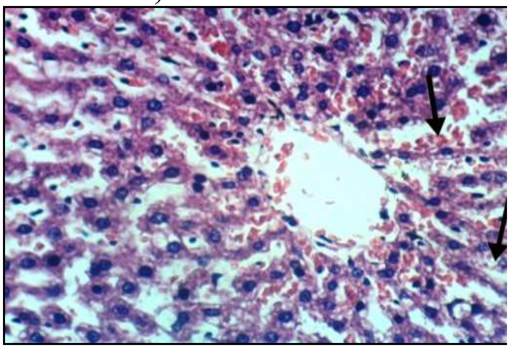


Photo (11): Liver of rat from(group 2) (control +) showing congestion of hepatic sinusoids and cytoplasmic vacuolization of hepatocytes (H & E X 400).

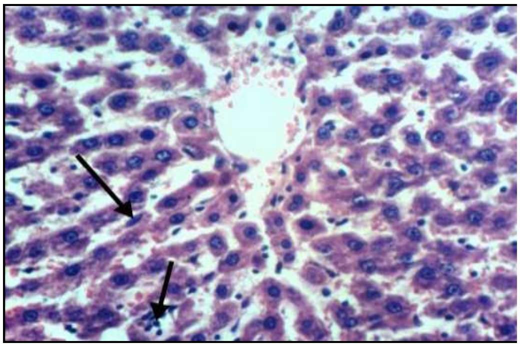


Photo (12): Liver of rat from(group 2) (control +) showing sinusoidal leukocytosis and activation of Kupffer cells (H & E X 400).

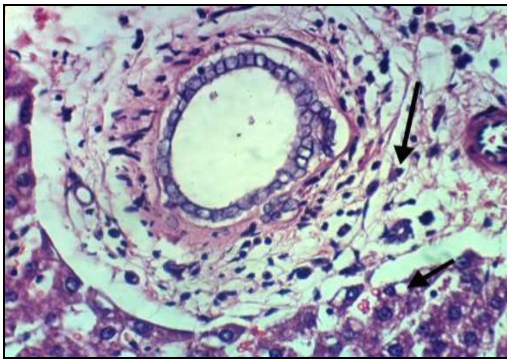


Photo (13): Liver of rat from(group 2) (control +) showing cytoplasmic vacuolization of hepatocytes and portal oedema (H & E X 400).

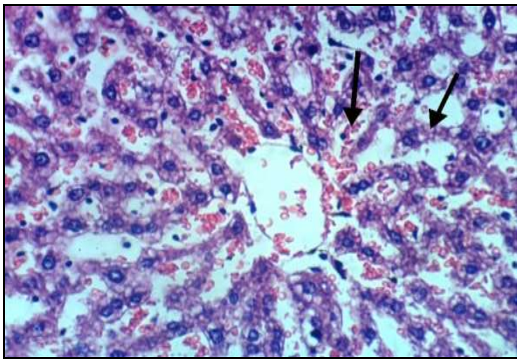


Photo (14): Liver of rat from (group 2) (control +) showing cytoplasmic vacuolization of hepatocytes and congestion of hepatic sinusoids (H & E X 400).

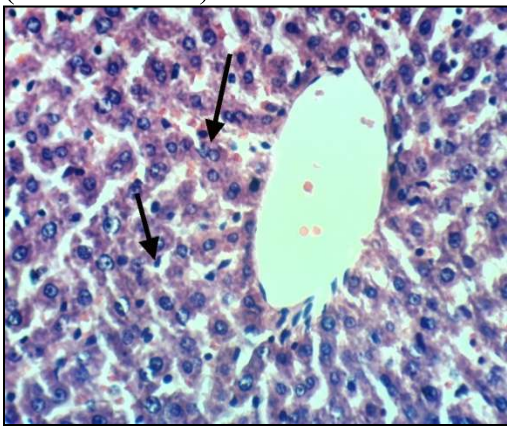


Photo (15): Liver of rat from(group 2) (control +) showing Kupffer cells activation and binucleation of hepatocytes (H & E X 400).

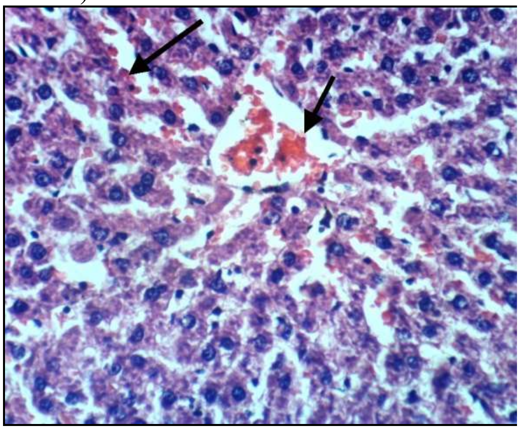


Photo (16): Liver of rat from(group 2) (control +) showing congestion of central vein and hepatic sinusoids (H & E X 400).

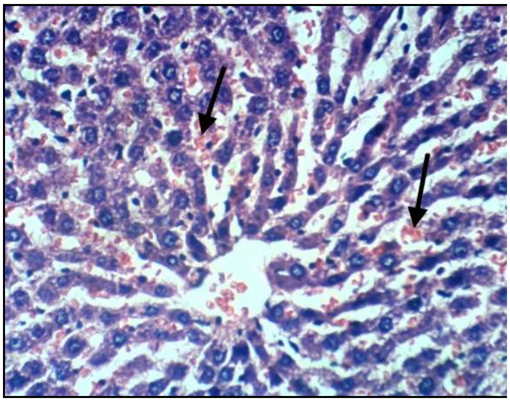


Photo (17): Liver of rat from (group 2) (control +) showing congestion of hepatic sinusoids (H & E X 400).

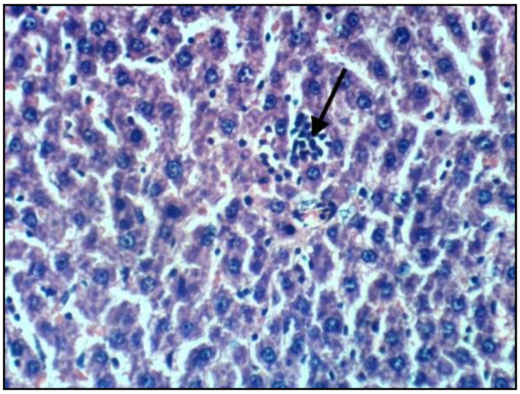


Photo (18): Liver of rat from (group 2) (control +) showing sinusoidal leukocytosis (H & E X 400).

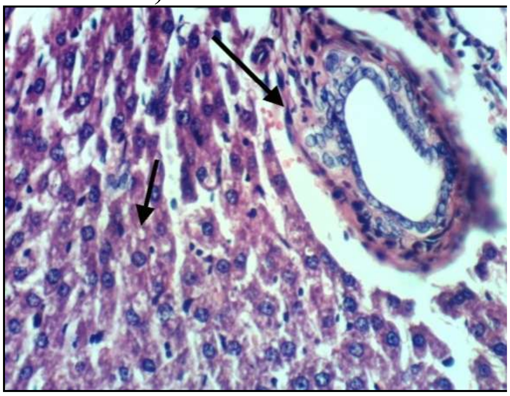


Photo (19): Liver of rat from (group 2) (control +) showing cytoplasmic vacuolization of hepatocytes and fibroblast proliferation around bile duct (H & E X 400).

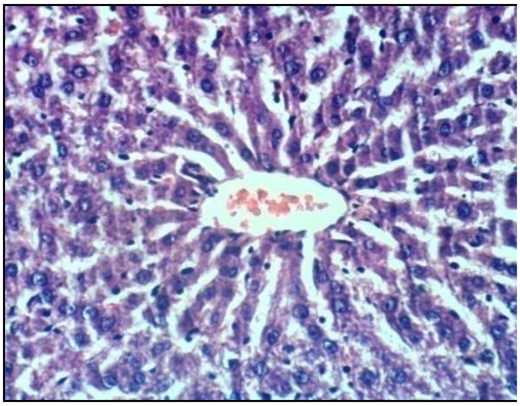


Photo (20): Liver of rat from (group 3) palm date kernel powder 5% showing the normal histological structure of hepatic lobule (H & E X 400).

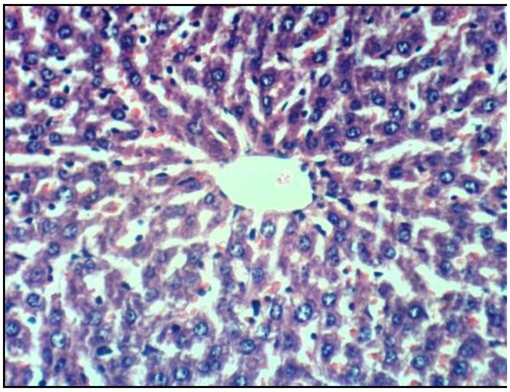


Photo (21): Liver of rat from group 4 showing the normal histological structure of hepatic lobule (H & E X 400).

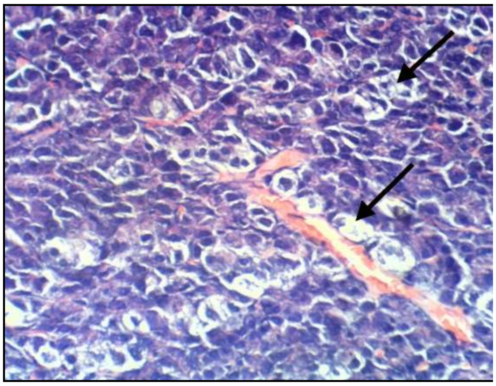


Photo (22): Liver of rat from group 4showing the normal histological structure of hepatic lobule (H & E X 400).

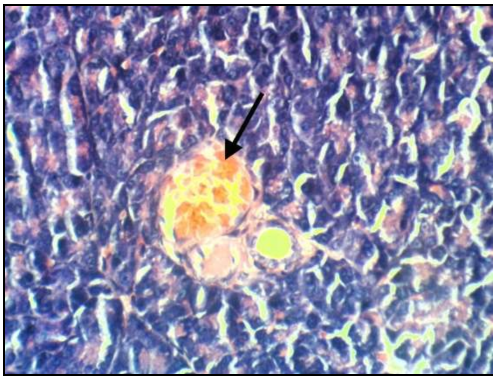


Photo (22): Pancreas of rat from group 6showing congestion of pancreatic blood vessel (H & E X 400).

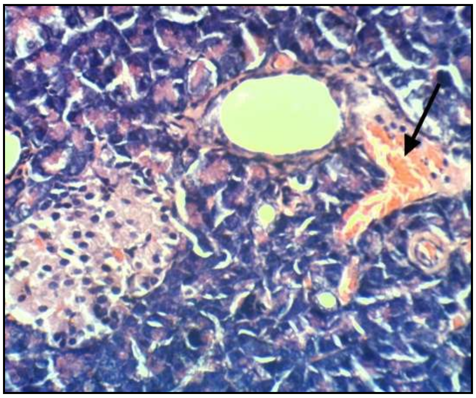


Photo (23): Pancreas of rat from group 6 showing congestion of pancreatic blood vessel (H & E X 400).

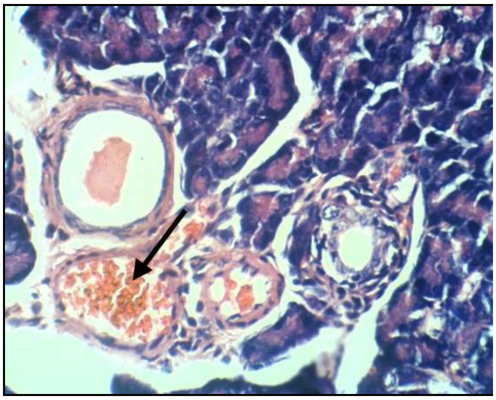


Photo (24): Pancreas of rat from group 6 showing congestion of pancreatic blood vessel (H & E X 400).

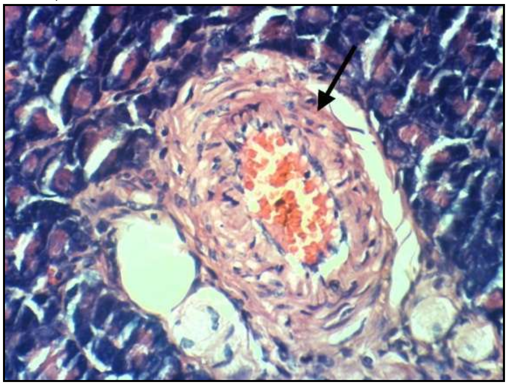


Photo (25): Pancreas of rat from group 6showing congestion and thickening in the wall of pancreatic blood vessel (H & E X 400).

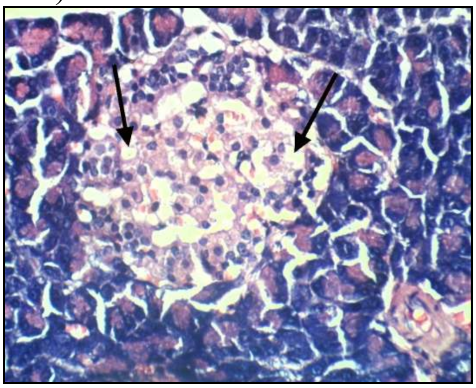


Photo (26): Pancreas of rat from group 6 photoshowing vacuolations of cells of islets of Langerhan's (H & E X 400).

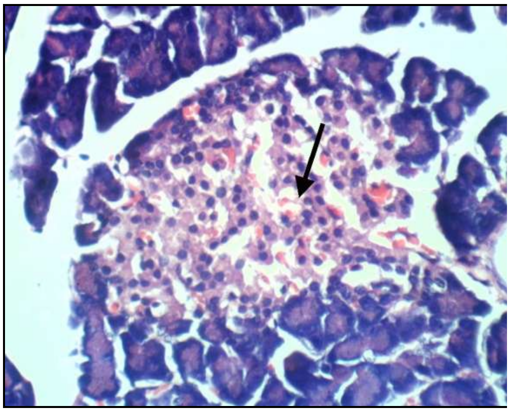


Photo (27): Pancreas of rat from group 2showing vacuolations of cells of islets of Langerhan's (H & E X 400).

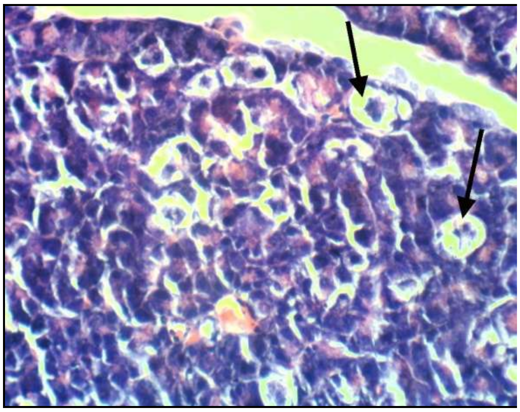


photo. (28): Pancreas of rat from group 8showing vacuolations of cells of pancreatic acini (H & E X 400).

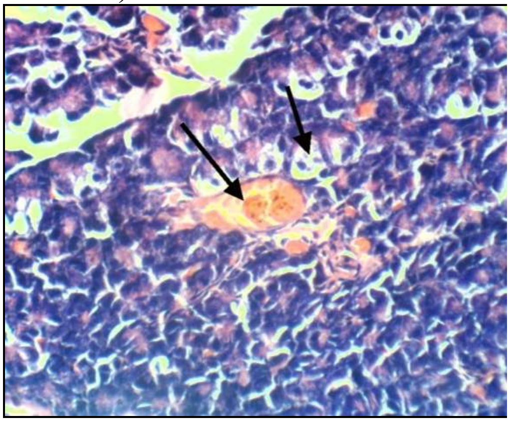


Photo (29): Pancreas of rat from group 8photoshowing vacuolations of cells of pancreatic acini and congestion of pancreatic blood vessels (H & E X 400).

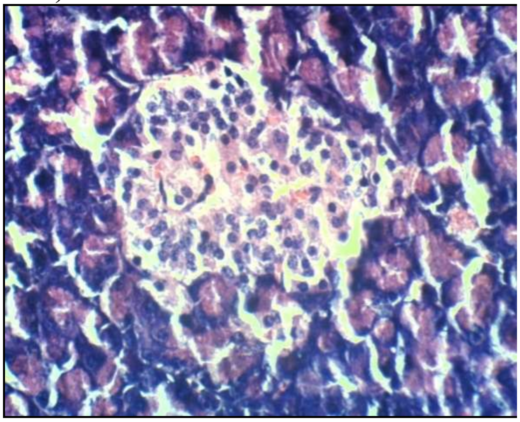


Photo (30): Pancreas of rat from group 8 showing no histopathological changes(H & E X 400).

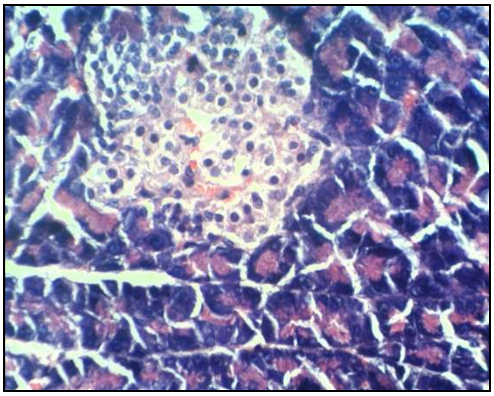


Photo (31): Pancreas of rat from group 8photoshowing the normal histological structure of pancreatic parenchyma (H & E X 400).

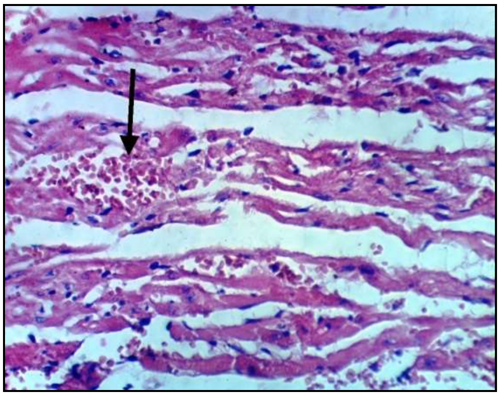


Photo (32): Heart of rat from(group 10) hypercholoestrima showing congestion of myocardial blood vessels (H & E X 400).

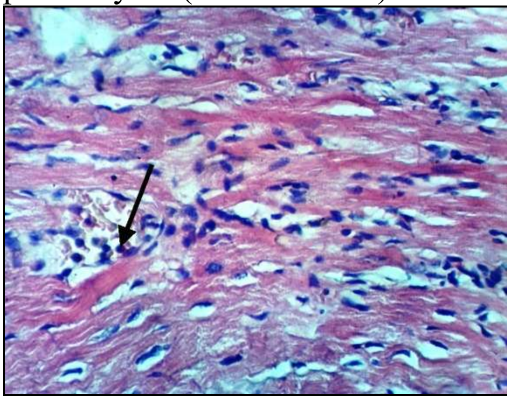


Photo (33): Heart of rat from(group 10) hypercholoestrima showing few intermuscular inflammatory cells infiltration (H & E X 400).

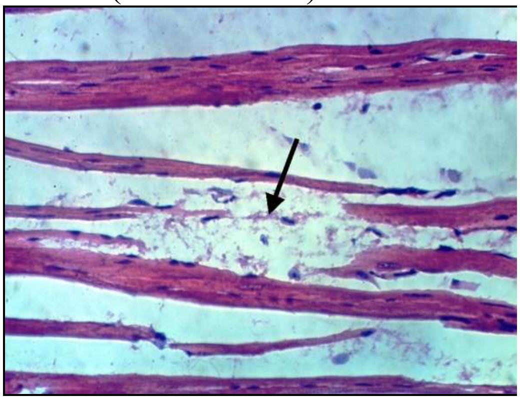


Photo (34): Heart of rat from (group10) hypercholoestrima showing intermuscularoedema (H & E X 400).

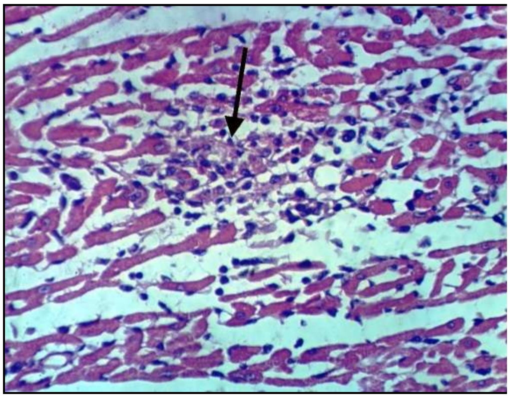


Photo (35): Heart of rat from(group 10) hypercholoestrima showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).

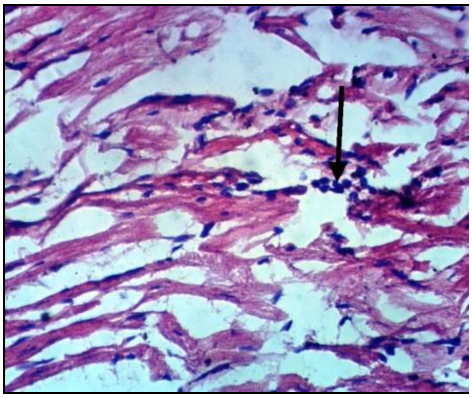


Photo (36): Heart of rat from(group 10) hypercholoestrima showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).

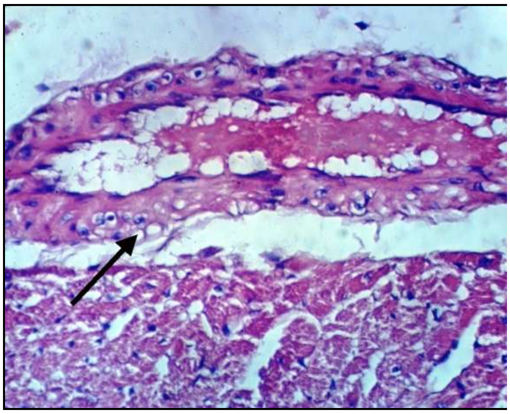


Photo (37): Heart of rat from(group 10) hypercholoestrima showing vacuolation of tunica media of blood vessel (H & E X 400).

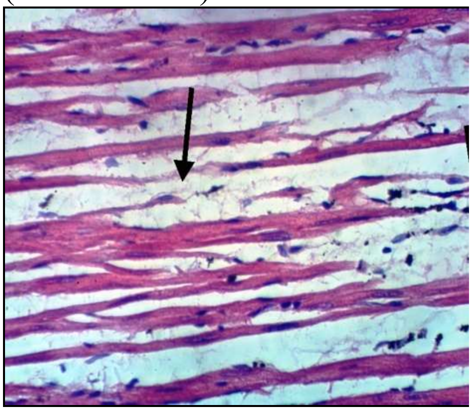


Photo (38): Heart of rat from(group 10) hypercholoestrima showing intermuscularoedema(H & E X 400).

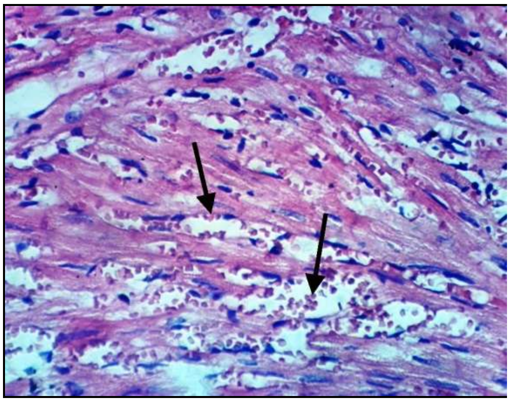


Photo (39): Heart of rat from (group 10) hypercholoestrima showing congestion of myocardial blood vessels (H & E X 400).

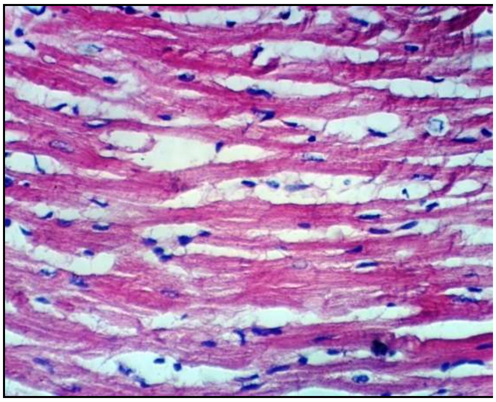


Photo (40): Heart of rat from (group 11)palm date kernel powder 5%hypercholoestrima showing normal cardiac myocytes(H & E X 400).

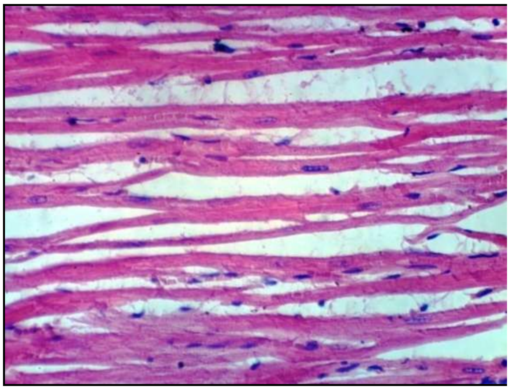


Photo (41): Heart of rat from (group 6)palm date kernel powder 5% showing normal cardiac myocytes (H & E X 400).

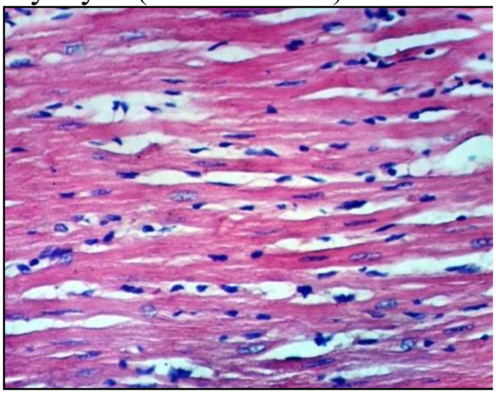


Photo (42): Heart of rat from (group 11)palm date kernel powder 5%showing no histopathological changes (H & E X 400).

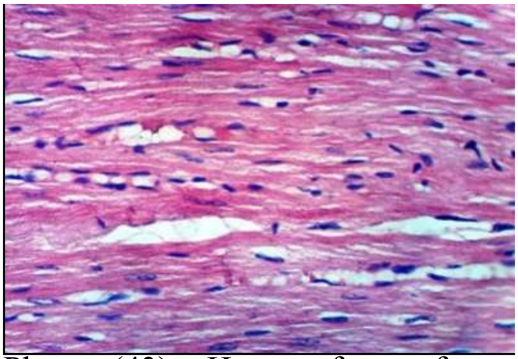


Photo (43): Heart of rat from (group 12)palm date kernel extract1/2ml showing no histopathological changes (H & E X 400).

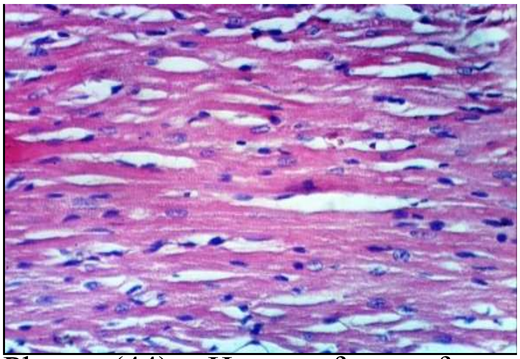


Photo (44): Heart of rat from (group 12)palm date kernel extract1/2ml showing normal cardiac myocytes (H & E X 400).

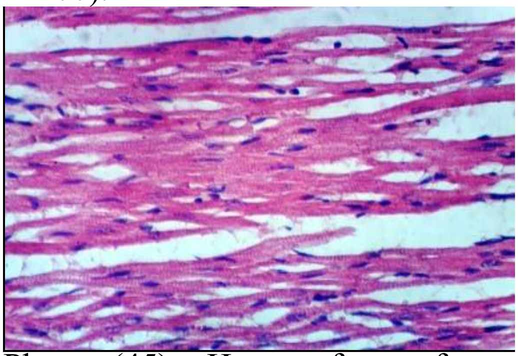


Photo (45): Heart of rat from (group 12)palm date kernel extract1/2ml showing normal cardiac myocytes(H & E X 400).

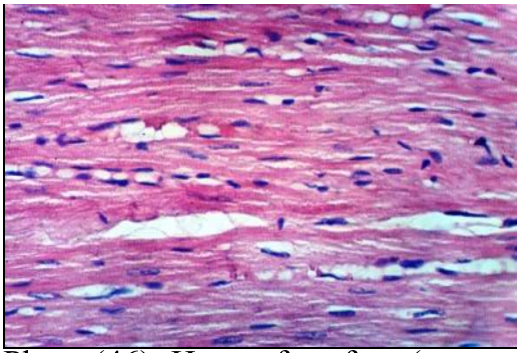


Photo (46): Heart of rat from(group 12)palm date kernel extract1/2ml showing no histopathological changes (H & E X 400).

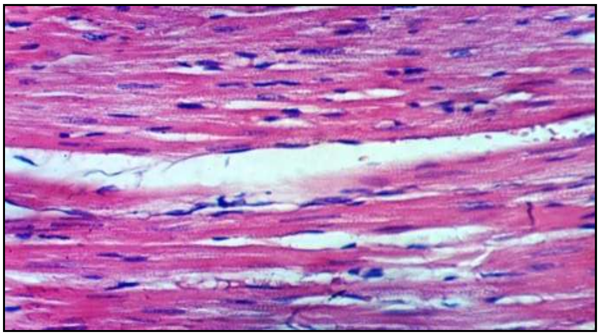


Photo (47): Heart of rat from (group 12)palm date kernel extract1/2ml showing no histopathological changes (H & E X 400).

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التغيرات البنائية نتيجة للسكرى وإرتفاع الكوليسترول والتسمم الكبدى فى الجرذان بتأثير الأغذية المحتوية علي أنوية البلح السماني

د/منى ابراهيم محمد نجا
قسم الاقتصاد المنزلي (تغذية وعلوم الأطعمة) - كلية التربية النوعية
جامعة المنوفية

المستخلص العربى

أجريت هذه الدراسة لتحديد تأثير مسحوق نواة التمر الناضجة على ذكور الجرذان البيضاء التي تزن (١٥٠-٢٠٠ جم) ، وقد تم تقسيم مجموعات الجرذان المستخدمة الى إثنى عشر مجموعة ، ثلاثة مجموعات للمقارنة السالبة (-) ، وثلاثة مجموعات للمقارنةالموجبة (+) وهى مجموعات الفئران المصابة بالتسمم الكبدى و ارتفاع نسبة الكوليسترول ومجموعه البول السكرى ، وقد تمت التغذية على الغذاء الاساسى المضاف إليه مسحوق نوى التمر بنسبة (٥٪).

وكذلك ثلاث مجموعات أيضا مصابة بالتسمم الكبدى وإرتفاع الكوليسترول والسكرى . حيث تمت تغذية الجرذان بالغذاء الاساسى مع مستخلص نواه التمر بالفم (٢/١ مل يوميا) وقد تم تجهيز مستخلص نواة التمر بالميثانول ، وبعد طرد المذيب تمت إذابة الناتج فى ماء مقطر قبل إعطائة بالفم للجرذان .

كما تمت تغذية ذكور الجرذان البيضاء السليمة بالكوليسترول الخام عن طريق إضافتها للغذاءالاساسى (١.٥٪ من الكوليسترول) بالإضافة إلى(١٠٪ من دهون ذيل الخراف) لمدة (١٥ يوم) مما أدى لارتفاع الكوليسترول فى السيرم.

وقد تم اصابه ذكور الجرذان البيضاء السليمة بالسكرى بالحقن فى الغشاء البريتوني بالالوكسان (١٥٠ ملغم / كغم من وزن الجسم).

كما أدى الحقن برابع كلوريد الكربون (CCl_4 بنسبة ٢ ملليلتر /١كجم وزن الجسم) مرتين فى الأسبوع لمدة أسبوعين متتاليين الى الإصابة بالتسمم الكبدى .

في نهاية التجربة(٤٥ يوم) تم ذبح الجرذان والحصول على الكبد والبنكرياس والقلب وتم حفظها فى الفورمالين ثم تم الفحص للقطاعات المأخوذه بالميكروسكوب وتصويرها .

كانت أفضل النتائج الهستوباثولوجية المتحصل عليها:التغذية على بذور نخيل التمر المنبتة التى تم استخلاصها بمحلول بمعدل (٢/١ مللى يوميا) خلال فتره التجربة . علما بان مسحوق بذور البلح كان له أيضا تأثير ملحوظ مرغوب على الجرذان المصابة بارتفاع الكوليسترول والسكرى والتسمم الكبدى والتى أظهرت تحسناً واضحاً في أنسجة وخلايا الأعضاء لكل من القلب والكبد والبنكرياس .

الكلمات المفتاحيه: ارتفاع الكوليسترول فى الدم – البول السكرى –إلتهاب الكبد – مسحوق نوى البلح والمستخلص منه.