Effect of Different Levels of Bael Fruit (*Aegle marmelos* L, Correa) Powder on Rats Inflicted with Hepatotoxicity

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Effect of Different Levels of Bael Fruit (*Aegle marmelos* L, Correa) Powder on Rats Inflicted with Hepatotoxicity

ABSTRACT

The study aimed to investigate the impact of four levels (2.5%, 5%, 10% and 15%) powder of bael fruit (Aegle marmelos L, Correa) on liver functions of rats suffering from hepatotoxicity. The experiment was performed on 42 male Albino rats allocated in 6 equal groups; first group fed on basal diet and kept as a control negative. The other rats (n=35) were intoxicated by subcutaneous injection with CCL4 in paraffin oil (2 ml/kg) twice a week for two consecutive weeks to induce hepatic toxicity, then those rats were subdivided into: control positive, (second group) fed on basal diet .Third, fourth, fifth and sixth groups fed on basal diet and bael fruit powder 2.5%, 5%,10% and 15%. The results showed that with increasing the amount of bael fruit powder, the lipid profile, AST, ALT, uric acid and urea nitrogen levels decreased significantly. The best result was belonged to rats that fed on diet containing bael fruit powder15%. the results showed decreasing in lipid profile such as total cholesterol, triglycerides, LDL-c and VLDL-c, decreasing in the liver enzymes AST and ALT, uric acid and urea nitrogen. It can be concluded that, supplementation of bakery products with high percentage of bael fruit powder (15%) exerts a positive impact on liver functions and other biochemical parameters.

Key words: Bael fruit, liver, histopathology.

INTRODUCTION

Liver is a vital organ that has a wide range of functions, including detoxification, plasma protein synthesis, and production of biochemicals necessary for digestion. Damage to the liver inflicted by hepatotoxic agents is of grave consequence. Today, liver damage is one of very common aliment in the world resulting in serious debilities ranging from severe metabolic disorders to even mortality **(Akilavalli *et al*., 2011).**

Reactive oxygen species (ROS) contribute to the pathogenesis of various acute and chronic liver diseases, such as acetaminophen overdose, haemochromatosis, alcoholic liver injury, toxin exposure and viral hepatitis (**Bruck *et al*., 2004 and Zorov *et al*., 2006).** ROS cause impairment of cellular membrane stability and cell death by lipid peroxidation **(Morcillo *et al*., 1999).** Thereby, it has become the key to prevent and cure hepatic damage by eliminating free radicals and preventing lipid peroxidation (**Han *et al*., 2004 and Gedik *et al*., 2005**).

Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world, including those in the developed countries, turning to complementary and alternative medicine (**Prakash *et al*., 2008).** Plant and natural products have been used in traditional remedies worldwide for the prevention and treatment of liver diseases (**Lahon and Das, 2011).**

Bael fruit (*Aegle marmelos)* belongs to family Rutaceae. Its golden colored fruit resembles golden apple hence the generic name-Aegle, and the species name is derived from marmelosin contained in the fruit. It is a divine tree having curative properties. Marmelosin derived from the pulp is laxative and diuretic **(Bag *et al*., 2009 and Brijesh *et al*., 2018).**

All parts of bael fruit tree have medicinal virtues and have a long tradition as herbal medicines **(Parmar and Kaushal, 1982).**

The roots and fruits of bael fruit possess antiamoebic activity **(Ponnachan *et al.*, 1993).**

Bael fruit exerted anticancer activity, hepatoprotective activity, radioprotective activity, antiulcer activity and antioxidant activity **(Sekar *et al*., 2011; Suvimol and Pranee., 2018 and Kahirvel *et al*. 2019).** Beal has different names: Bael (English), Bel (Urdu) and Aegle marmelos (Latin) (**Orwa *et al*., 2009; Sekar *et al*., 2011 and Tanmay *et al*., 2020)**. The tree has unusual branches with aromatic leaves, sweet scented and greenish-white flowers **(Patel *et al*., 2012).**

The shells have minute aromatic glands. The pulp is pale orange, sweet, resinous and highly aromatic **(Parmar and Kaushal, 1982; Sharma *et al*., 2007).** Bael fruit is rich in water, carbohydrates and fibers, protein, vitamins and minerals **(Parichha, 2004).** The pulp is very fragrant, pleasantly flavored and sweet to taste **(Roy and Khurdiya, 1995).** The ripe fruits are used to prevent sub-acute and chronic dysentery **(Das and Das, 1995)**.

From the previous concept the present work was a trial to spot the light on the beneficial effects of bael fruit powder as hepatoprotective agents in rats.

MATERIALS AND METHODS

Material

Fruits of Bael fruit were obtained from El-Zohrya Botanical Garden, Giza, Egypt. Kits were purchased from Biodiagnostic Co., Egypt. Forty two male Albino rats of Sprague Dawley strain weighing about (200±10g) obtained from animal house of National Research Center, Egypt.

Methods:

Preparation of bael fruit Powder:

Fruits were washed with running tap water. The pulp was removed from the peel, cut into slices (thickness 1.5-2 mm) and dried by solar energy. The whole quantity was distributed on solar cabinet dryer chamber of solar energy laboratory in the National Research Center was used for solar dehydration at Dokki. Power was used and the temperature was recorder continuously (relative humidity from 11 to 12%, air velocity 500m^2 / hr. The dried fruit pulp was ground into fine powder.

Chemical composition of bael fruit powder:

Bael fruit was chemically analyzed of protein, fat, fiber, carbohydrates, ash and moisture according to **A.O.A.C. (2005).**

Biological assay:

Forty two male albino fed on basal diet was formulated according to **(Reeves *et al*., 1993)** for 1 week for adaptation. The basal diet consists of casein 12.5 %, Corn oil 10%, choline chloride 0.25 %, vitamin mixture 1 % **(Campbell., 1963),** salt mixture 4% **(Hegseted., 1941),** cellulose 5 %, and the remainder is corn starch

After the period of adaptation on basal diet, the rats were divided into 6 equal groups, the first group fed on basal diet as a negative control, the second main category (n=35) rats were intoxicated by subcutaneous injection of CCl_4 in paraffin oil (1:1 v/v; 2 ml/kg) twice a week for two consecutive weeks to induce hepatic toxicity **(Jayasekhar *et al*., 1997).**

The second group fed on basal diet (positive control), the third group fed as the positive control group diet with addition of bael fruit powder 2.5% (BFP2.5%), fourth, fifth and sixth group fed as the positive control group diet with addition of bael fruit powder 5%, 10% & 15% respectively.

At the end of the experimental period (6 weeks), blood samples were collected and centrifuged to obtain serum for

estimating some biochemical parameters, i.e. serum cholesterol **(Allain *et al*., 1974),** Triglycerides (TG) **(Fossati, and Prencipl., 1982),** High density lipoprotein cholesterol (HDL-c) **(Lopes-Virella *et al*., 1977),** Low density lipoprotein cholesterol (LDL-c) and Very low density lipoprotein cholesterol (VLDL-c) **(Friedewald *et al*., 1972),** Aspartate amino transferase (AST) and Alanine amino trasferase (ALT) **(Reitman and Frankl.,1957)**

Statistical analysis

Data are presented as means ± SD and the analysis was conducted using SPSS program, Version 16.0 **(2007).**

RESULTS AND DISCUSSION

Chemical composition of bael fruit /100 g:

Table (1) showed the chemical composition of bael fruit, (% per 100g wet pulp without seeds) the % of protein, fat, fiber, carbohydrates, ash and moisture were (1.8, 0.5, 2.6, 32.5, 1.6 and 61%) respectively. Our result in the same line with **(Parichha, 2004)** who showed that, the bael fruit is rich in water, carbohydrates and fibers, it is also a good source of protein, vitamins and minerals

Effect of bael fruit powdered on BWG, FER and Relative Organs weight of hepatotoxic rats:

As shown in Table 2, final body weight increased in control negative rats as compared to the positive control group. On the other hand, giving beal fruit powder significantly increased body weight gain, as compared to the positive control group.

Feed efficiency ratio which reflects the feed intake/gain ratio was reduced by CCL_4 while beal fruit powder consumption induced significant increase in FER, as compared to the positive control group. Rats given CCL_4 exhibited bigger heart, liver, kidneys and brain than negative control animals. However, these

increases in vital organs were improved by BFP supplementation. On the other hand rats given BFP show variations in organs weight than control. Indeed, the body weight is a reflection of the health state and the body metabolism (**Bhatia and Khera, 2013**).

Effect of bael fruit powdered on serum lipids fractions of hepatotoxic rats:

Table (3) illustrated the effect of bael fruit powdered on lipid fractions. The values of serum cholesterol, TG, LDL-c, VLDL-c mg/dl) and LDL/HDL showed significant increase ($P<0.05$) for control positive group in compared with control negative group while HDL-c value (mg/dl) for control positive group was significantly lower than that of control (-) group. Data in this table showed that, total cholesterol (mg/dl) increased significantly for rats (control +). The statistical analysis showed a significant decrease in total cholesterol of all treated groups with bael fruit powder when compared with (control +). The lowest decrease in all treated group in cholesterol was recorded in BFP15% (205.60 ± 0.45). Also, the best result of serum triglycerides level was noticed in rats fed on basal diet with BFP15% (120.19±0.01) followed by 10% BFP (121.79±1.48) The antioxaditive effect of bael fruit extract was explained by **Manjula and Kumar (2016)** who found that bael fruit extract has a potent in vitro antioxidant activity which was correlated with its content of bioactive compounds. The ameliorated effect of bael fruit fruit extract on lipid peroxidation may be attributed to the antioxidative phytochemicals present in it especially flavonoids. Flavonoids are the most promising agents for treatment of oxidative stress-related disease (**Babu *et al.*, 2013**).

HDL-c is an effective scavenger of cholesterol molecules from several locations, possibly even from some early plaque formation. Therefore, HDL-c has been considered to be a good lipoprotein and the cholesterol associated with HDL has been

referred to be good cholesterol. HDL-c among all groups fed on diet containing BFP 2.5%, BFP 5%, BFP10% and BFP15% showed non-significant changes in serum HDL-c, except group of rats treated with 15% BFP compared with (control +) therefore the best results for HDL-c was from the group fed on BFP15% (28.93± 0.81) followed by to other treated groups.

All animals treated with basal diet containing bael fruit powdered showed significant decrease in LDL-c versus control (+).While these treatments for rats led to increase LDL-c significantly, compared to (control -).

VLDL-c of rats fed on basal diet showed decrease versus control (+) group. Treating rats with bael fruit powdered (2.5%, 5%, 10% and 15%) led to a significant reduction in serum VLDL-c compared with (control +). The improvement in the lipid profile of the extract may be attributed to its content of flavonoids and other phenolics, triterpenoids, alkaloids, steroids and glycosides and the insulin-like activity of triterpenoids seems to be responsible for the normalized lipogenesis (**Sakurai *et al*., 2002**) or due to activating normal glycemic level by the insulin tropic effect of flavonoids (**Pinent *et al*., 2008)**.

Effect of bael fruit powdered on some kidney functions of hepatotoxic rats:

As shown in table (4) illustrated the effect of bael fruit powdered on some kidney functions of hepatotoxic rats. Uric acid value and urea nitrogen (mg/dl) of the control positive group showed highly significant increase as compared to (control -) group the mean values were (4.27± 0.10 and 31.59 ± 0.54 vs. 1.15± 0.23, 14.29 ± 0.49) respectively. While values of uric acid decreased in the groups fed on (BFP2.5%, BFP5%, BFP10% and BEP15%) compared with (Control+). Also, the highest decrease in serum urea nitrogen was found in the group fed on diet with

BFP15%. While the highest increase in serum urea nitrogen was noticed in BFP2.5% group.

Antioxidative effect of bael fruit was explained by **Manjula and Kumar (2016)** who found that bael fruit has a potent in vitro antioxidant activity which was correlated with its content of bioactive compounds. The ameliorated effect of bael fruit on lipid peroxidation may be attributed to the antioxidative phytochemicals present in it especially flavonoids. Flavonoids are the most promising agents for treatment of oxidative stress-related disease (**Babu *et al*., 2013**).

Effect of bael fruit powdered on liver functions (IU/L) of hepatotoxic rats:

Results of AST and ALT are presented in table (5), AST recorded significant decrease except BFP 2.5% when compared with (control +). While, the lowest levels of AST enzymes were found in group of rats fed on diet containing BFP15% (44.53±1.22). Also, results obtained from this table showed a significant increase in the values of ALT enzyme in the positive control group versus all treated groups and the best results were observed in the groups that fed on the BFP (15% & 10%) respectively .In fact, bael fruit is an important medicinal plant and its fruits have been used in the treatment of various diseases. **Manjula and Kumar (2016)** reported that, phytochemical screening of bael fruit ethanolic fruit extract revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, coumarins and triterpenoids by high contents to which its ameliorative effect on the diseased liver may be attributed.

Pieced together, our data proclaim in nouncertain voice that, supplementation with high level of bael fruit powdered (15%) exerts a positive impact on the liver enzymes and other biochemical parameters in hepatic diseased rats. As well as bael

fruit is recommended to be ingested as fresh fruit to hepatic diseased patients.

Table (1): Chemical composition of bael fruit (% per 100g wet pulp without seeds)

Component	%
Protein	1.8
Fat	0.5
Fiber	2.6
Carbohydrate	32.5
Ash	1.6
Moisture	61

Table (2): Effect of bael fruit powdered on BWG, FER and Relative organs weight of hepatotoxic rats

Treatment	BWG %	FER	Organ weight/body weight (%)				
			Heart	Liver	Spleen	Kidney	Brain
Control (-)	36.16±1.11^{A}	0.15±0.01^{A}	0.33±0.45^{B}	3.75±0.11^{E}	0.25±0.04^{C}	0.71±0.01^{C}	0.77±0.014^{D}
Control (+)	9.26±0.64^{E}	0.01±0.09^{E}	0.44±0.15^{A}	6.34±0.71^{A}	0.45±0.06^{A}	0.95±0.05^{A}	2.42±0.23^{A}
BFP 2.5%	9.59±1.02^{E}	0.02±0.01^{E}	0.43±0.14^{A}	6.18±0.05^{A}	0.43±0.05^{A}	0.93±0.06^{A}	2.36±0.40^{A}
BFP 5%	11.16±0.90^{D}	0.05±0.01^{D}	0.41±0.43^{AB}	5.99±0.24^{B}	0.35±0.06^{B}	0.88±0.05^{AB}	1.98±0.10^{AB}
BFP10 %	24.45±1.80^{C}	0.08±0.01^{C}	0.40±0.01^{B}	4.88±0.38^{C}	0.28±0.07^{CB}	0.78±0.05^{BC}	1.52±0.031^{BC}
BFP15 %	28.41±0.82^{B}	0.09±0.01^{B}	0.36±0.01^{C}	4.35±0.12^{D}	0.24±0.08^{C}	0.75±0.14^{BC}	1.20±0.10^{CD}

Control (-): Control Negative. **Control (+):** Control Positive.
BFP2.5%: Bael Fruit powder2.5% BFP5%: Bael Fruit powder 5%
BFP10%: Bael Fruit powder10% BFP15%: Bael Fruit powder15%
Values are expressed as mean ± SD. ***Significance at p<0.05.***
Values which don't share the same letter in each column are significantly different.

Table (3): Effect of bael fruit powdered on serum lipids fractions of hepatotoxic rats

Treatment Groups	Lipid Fraction (mg/dl)					
	Cholesterol	Triglycerides	HDL	LDL	VLDL	LDL/HDL
Control (-)	89.24±0.21^{F}	39.16±0.12^{D}	60.37±0.05^{A}	20.06±0.33^{F}	7.83±0.03^{D}	0.30±0.04^{C}
Control (+)	227.36±2.16^{A}	133.21±3.77^{A}	24.32±0.92^{C}	161.54±0.67^{A}	26.60±0.65^{A}	5.99±0.22^{AB}
BFP 2.5%	220.93±0.60^{B}	130.55±1.39^{A}	24.78±3.35^{CB}	154.44±0.12^{A}	26.11±0.29^{A}	5.85±0.11^{B}
BFP 5%	217.02±1.22^{C}	126.39±1.61^{B}	25.25±0.93^{CB}	150.88±0.89^{C}	25.16±0.16^{B}	5.78±0.68^{AB}
BFP10%	211.83±1.34^{D}	121.79±1.48^{C}	27.76±0.93^{C}	141.77±0.93^{D}	24.35±0.29^{CB}	5.44±0.16^{AB}
BFP15%	205.60±0.45^{E}	120.19±0.01^{C}	28.93±0.81^{B}	131.73±0.85^{E}	24.03±0.08^{C}	5.30±0.10^{B}

Control (-): Control Negative. **Control (+):** Control Positive.
BFP2.5%: Bael Fruit powder2.5% BFP5%: Bael Fruit powder 5%
BFP10%: Bael Fruit powder10% BFP15%: Bael Fruit powder15%
Values are expressed as mean ± SD. ***Significance at p<0.05.***
Values which don't share the same letter in each column are significantly different

Table (4): Effect of bael fruit powdered on some kidney functions of hepatotoxic rats

Treatment	Uric Acid	Urea Nitrogen
Control (-)	1.15±0.23^{E}	14.29±0.49^{F}
Control (+)	4.27±0.10^{A}	31.59±0.54^{A}
BFP 2.5%	3.90±0.12^{B}	28.79±0.57^{B}
BFP 5%	3.88±0.01^{C}	26.49±0.86^{C}
BFP10%	2.98±0.01^{CD}	24.08±0.20^{D}
BFP15%	2.41±0.48^{D}	22.79±0.39^{E}

Control (-): Control Negative. **Control (+):** Control Positive.
BFP2.5%: Bael Fruit powder2.5% BFP5%: Bael Fruit powder 5%
BFP10%: Bael Fruit powder10% BFP15%: Bael Fruit powder15%
Values are expressed as mean ± SD. ***Significance at p<0.05.***
Values which don't share the same letter in each column are significantly different

Table (5) :Effect of bael fruit powdered on liver functions (IU/L) of hepatotoxic rats.

Treatment	AST	ALT
Control (-)	43.67 ± 0.71^{E}	37.61 ± 0.81^{D}
Control (+)	60.53 ± 0.27^{A}	53.72 ± 0.87^{A}
BFP 2.5%	58.63 ± 0.78^{B}	53.29 ± 1.65^{A}
BFP 5%	55.17 ± 0.10^{C}	47.47 ± 1.05^{B}
BFP10%	52.50 ± 0.86^{D}	44.42 ± 2.27^{C}
BFP15%	44.53 ± 1.22^{E}	39.30 ± 0.87^{D}

Control (-): Control Negative. **Control (+):** Control Positive.
BFP2.5%: Bael Fruit powder2.5% BFP5%: Bael Fruit powder 5%
BFP10%: Bael Fruit powder10% BFP15%: Bael Fruit powder15%
Values are expressed as mean ± SD. ***Significance at p<0.05.***
Values which don't share the same letter in each column are significantly different

Histopathological examination of liver:

Liver of rat (control-) showing the normal histological structure of hepatic lobule (Photo 1). Meanwhile, liver of injected rat with ccl_4 and fed on basal diet (control+) showing vacuolations of hepatocytes and focal hepatic necrosis associated with leucocytic cells infiltration (Photo 2). Moreover, liver of rat suffering from chronic liver diseases and treated daily with (Bael Fruit powder 2.5%) showing necrosis of sporadic hepatocytes (Photo 3). Liver of rat suffering from chronic liver diseases and fed daily on diet containing (Bael Fruit powder 5%) showing necrosis of sporadic hepatocytes (Photo 4). Liver of rat suffering from chronic liver diseases and treated daily with (Bael Fruit powder 10%) showing vacuolation of Centro lobular hepatocytes (Photo 5). Liver of rat suffering from chronic liver diseases and fed daily on diet containing (Bael Fruit powder 15%) showing no histological changes (Photo 6).

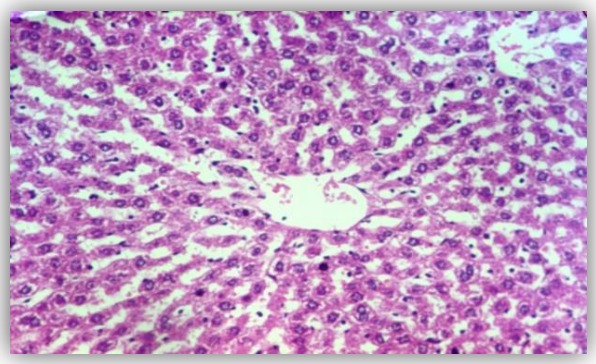

Fig. (1): Liver of rat (control -) showing the normal histological structure of hepatic lobule.

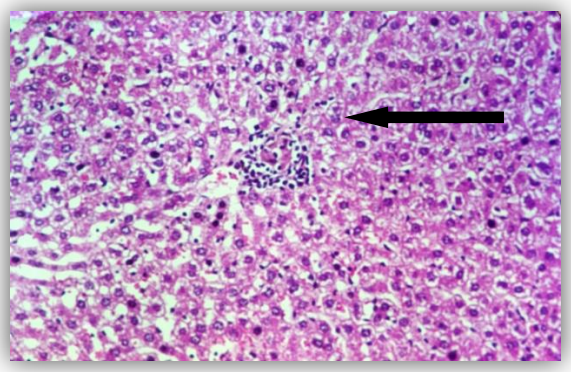

Fig. (2): Liver of rat (control +) showing vacuolations of hepatocytes and focal hepatic necrosis associated with leucocytic cells infiltration.

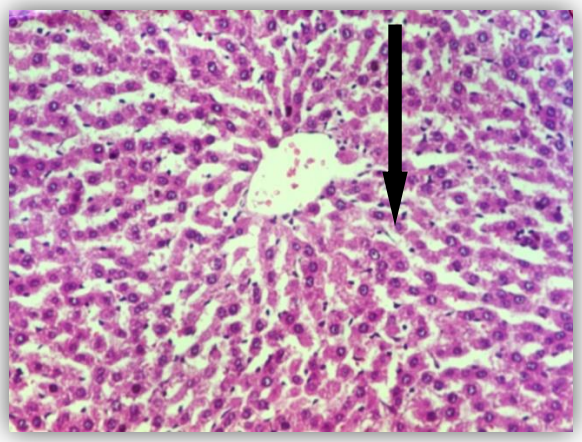

Fig. (3): Liver of rat suffering from chronic liver diseases and treated daily with (BFP2.5%) showing necrosis of sporadic hepatocytes.

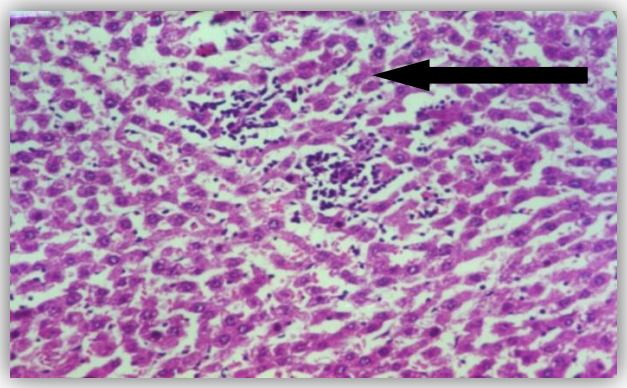

Fig. (4): Liver of rat suffering from chronic liver diseases and fed diet containing (BFP5%) showing necrosis of sporadic hepatocytes.

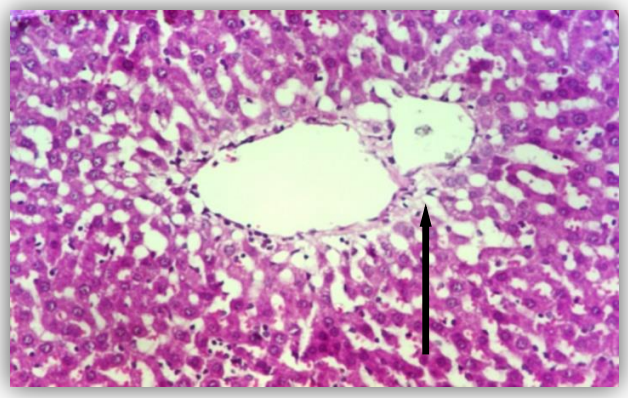

Fig. (5): Liver of rat suffering from chronic liver diseases and treated daily with (BFP 10%) showing vacuolation of Centro lobular hepatocytes.

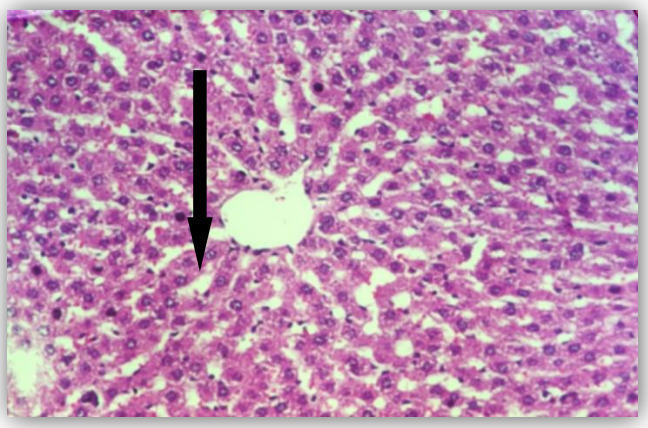

Fig. (6): Liver of rat suffering from chronic liver diseases and fed diet containing (BFP15%) showing no histological changes.

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تأثير مستويات مختلفة من مسحوق فاكهة بيل على الفئران المصابة بالتسمم الكبدي

المستلخص العربى

تهدف الدراسة إلى تتبع تأثير إضافة 2.5% ،5%، 10% ، 15% من مسحوق فاكهة بيل في وجبة الفئران المصابة بالتسمم الكبدى على الصحة و وظائف الكبد. تم توزيع 42 ذكر فئران بالغ لسلالة ألبينو على 6 مجموعات. المجموعة الأولى (كنترول سالب) حيث تناولت الغذاء الاساسى الخاص بالفئران، أما المجموعات من 2-6 فقد حُقنت تحت الجلد برابع كلوريد الكربون مع زيت البرافين (1:1 حجم/حجم 2 مللجم/ كجم) مرتين إسبوعياً لمدة اسبوعين متتاليين لإحداث خلل كبدي واعتبرت المجموعة الثانية (كنترول موجب) حيث تناولت الغذاء الاساسى فقط، أما المجموعة الثالثة والرابعة والخامسة والسادسة فقد تناولت الفئران الغذاء الأساسي مضافا إلية 2.5 % ، 5%، 10% ، 15% مسحوق فاكهة بيل على التوالى. و أسفرت النتائج عن حدوث نقص معنوي في صورة دهون الدم وكذلك إنزيمات الكبد واليوريا نيتروجين وحامض البوليك في الفئران التى تغذت على الغذاء الاساسى المضاف إلية مسحوق فاكهة الايجل مارميلوس. وكانت أفضل النتائج للمجموعة التى تغذت على مسحوق ايجل مارميلوس 15% حيث أظهرت النتائج أنخفاض مستويات الكوليستيرول الكلى والجليسريدات الثلاثية وكوليستيرول الليبوبروتينات المنخفضة الكثافة و المنخفضة الكثافة جداً، كما انخفضت مستويات انزيمات الكبد وأيضاً تحسنت وظائف الكلى حيث انخفض حامض اليوريك واليوريا نتيتروجين. وتوصى الدراسة بتدعيم المخبوزات بمسحوق فاكهة الإيجل مارميلوس وذلك لما أظهرته من نتائج إيجابية في تحسين وظائف الكبد وبعض القياسات البيوكيميائية الأخرى.

الكلمات المفتاحية: فاكهة بيل – الكبد – الهيستوباثولوجى