



Journal of Home Economics
Volume 27, Number (3), 2017

<http://homeEcon.menofia.edu.eg>

**Journal of Home
Economics**

ISSN 1110-2578

**Biological Study on the Potential Effects of Bael Fruit
(*Aeglemarmelos L, Correa*) on Hypercholesterolemic Rats**

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Abstract:

The present study was conducted to investigate the potential effects of Bael fruit (*Aeglemarmelos L, Correa*) powder (BFP) on the biological activities of rats. The experiment was carried out on 30 male Albino rats divided into 5 equal groups (6 rats per each); the first group fed on basal diet and kept as a (control-). Rats in the other four groups fed on basal diet containing 1.5% cholesterol plus 0.25% bile salts for two weeks to induce hypercholesterolemia, then those rats were subdivided into the following groups: a group that remained as hypercholesterolemic (control+, second group), a group that fed on hypercholesterolemic diet plus 5% BFP (third group); a group that fed on hypercholesterolemic diet plus 7.5% BFP (fourth group); a group that fed on hypercholesterolemic diet plus 10% BFP (fifth group). The results showed that with increasing the amount of BFP or beverage, the total cholesterol, triglycerides, LDL-c, AST, ALT, urea nitrogen and glucose levels decreased significantly ($P \leq 0.05$) and the best result was belonged to the group of rats that fed on BFP by 10%. From the obtained results it can be concluded that, supplementation with high percentage of BFP, 10%, exerts a positive impact on the lipid profile and other biochemical parameters in hypercholesterolemic rats. As well as BFP is recommended to be ingested as fresh fruit to hypercholesterolemic patients.

Key words: Bael fruit, liver functions, kidney functions, serum lipid profile

Introduction

Bael (*Aeglemarmelos*) 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).

The bael tree is one of the most useful medicinal plants of India. All parts of this tree including stem, bark, root, leaves and fruits have medicinal virtues and a long tradition as herbal medicines (Parmar and Kaushal, 1982). Bael leaves and fruits are widely used in folk medicines for the treatment of diabetes mellitus (Gaur, 1969). The roots and fruits of *A. marmelos* possess antiamoebic and hypoglycemic activity (Ponnachan *et al.*, 1993).

In recent years *Aeglemarmelos* is reported for various medicinal properties viz, anticancer activity, antibacterial activity, hepatoprotective activity, radioprotective activity, antiulcer activity antidiabetic activity, antioxidant activity and anti-inflammatory activity (Sekar *et al.*, 2011). Due to its endless uses, *Aeglemarmelos* is also known as Mahaphala or Great fruit (Parichha, 2004).



Figure 1. Photos of *Aeglemarmelos* tree and its fruits

Nutritional value (% per 100 g wet pulp without seeds) of the fruit of *Aeglemarmelos*: water 64.2, protein 1.8, fat 0.2, mineral 1.5, fiber 2.2, carbohydrate 30.6, calcium 0.09, phosphorus 0.05, potassium 0.6, iron 0.3, vitamin A (IU) 186, Vitamin B1 (0.01), nicotinic acid (0.9), riboflavin (1.2), and vitamin C (0.01) (Parichha, 2004).

Since antiquity, the ripe fruits of bael have been used as a dietary source in the Indian subcontinent. The pulp which is yellow or orange in color is very fragrant (characteristic floral aroma), pleasantly flavored and sweet to taste (**Roy and Khurdiya, 1995**). In India, a popular drink colloquially called as “bael sherbet” is prepared from the ripe fruit. The soft pulp is scooped, deseeded and blended with milk, sugar and cardamom and is consumed as a cooling drink. The semi ripe fruits are used in making jam by adding sugar, citric acid and preservatives. The pulp is also converted into marmalade, murabba or syrups and is eaten with Indian bread (**Parmar and Kaushal, 1982 and Roy and Khurdiya, 1995**). In Indonesia, the pulp of the fully ripe fruit is scooped dressed with palm sugar and consumed for breakfast. In Thailand the fruits are cut into pieces, dried and packed in bags either whole or pulverized and packed as tea bags. They may also be preserved in syrup and is used as dessert or as an ingredient for preparing cakes (**Parmar and Kaushal, 1982 and Roy and Khurdiya, 1995**).

In the Ayurvedic system of medicine, bael fruits are considered as an excellent remedy for diarrhea (**Das and Das, 1995**). The unripe fruits are bitter, acrid, sour, and astringent, and aids digestion and stomach irritation. The half-ripe fruit is astringent, digestive and anti-diarrheal. The ripe fruits are supposed to be more useful than the raw and are used to prevent sub-acute and chronic dysentery. The fruit pulp acts as a mild stimulant to the intestinal mucus membrane and stops diarrhea. The ripe fruits are aromatic, cooling and acts as a laxative (**Das and Das, 1995**).

Hypercholesterolemia is directly associated with an increased risk for coronary heart disease and other sequelae of atherosclerosis (**Pitter et al., 2002**). Many herbal medicinal preparations have potential hypocholesterolemic activity and encouraging safety profiles (**Thompson-Coon and Ernst., 2003**). Preparations that derived from natural sources often contain compounds that contribute to antioxidant defense system and apparently play a role in the protection against heart and degenerative diseases (**Brijesh et al., 2009**). From the previous concept, the present work was conducted to spot the light on the beneficial effects of BFP as hypocholesterolemic agents in rats.

Materials and Methods

Material

Fresh mature fruits of *Aegle marmelos* were collected during the month of August-September 2017 from El-Zohrya Botanical Garden, Giza, Egypt.

Chemicals

All chemicals used in this experiment were of analytical grade. Kits used for the quantitative determination of the different parameters were purchased from Biodiagnostic Co., Dokki, Giza, Egypt. Casein (85% protein), vitamins and minerals constituents were obtained from El-Gomhoriya Company for trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. Cellulose and D-L methionine were purchased from Morgan Company for Chemicals, Cairo, Egypt. Corn oil and corn starch were obtained from the local market.

Thirty male Albino rats of Sprague Dawley strain, weighing about 180-195g were obtained from an animal house of NRC, Cairo, Egypt.

Methods:

Preparation of bael fruit powder (BFP)

Fruits of bael were washed with running tap water. The pulp was removed from the peel, cut into slices and dried by hybrid solar convective drying system, belonging to the solar energy Department, National Research Center (NRC), Dokki, Egypt. The dried fruit pulp was ground into fine powder and kept in tightly closed containers at room temperature for further use.

Chemical composition of BFP

Bael Fruit was chemically analyzed to determine its protein, fat, carbohydrate, fiber, ash and moisture content according to AOAC, (2005).

Experimental Design:

Rats were housed in well aerated cages under hygienic condition and fed on basal diet for one week for adaptation *ad-libitum* in animal house NRC. The basal diet consists of casein 12.5 %, corn oil 10%, choline chloride 0.25 %, vitamin mixture 1 % (Campbell, 1963), salt mixture 4% (Hegsted, 1941), cellulose (5%) and the remainder (71.07 %) is corn starch (Reeves *et al.*, 1993), and this diet was modified in its content of (casein and starch) before giving to the groups fed on BFP.

After the period of adaptation on basal diet, the rats were divided into 5 equal groups (6 rats per each), the first group fed on basal diet as

a negative control, the other groups fed on basal diet containing cholesterol (1.5%) and bile salt (0.25%) for two weeks to induce hypercholesterolemia, then divided into the following groups: The second group fed on basal diet plus 1.5% cholesterol plus 0.25% bile salts (positive control), groups 3, 4 and 5 were fed as the positive control group plus BFP by ratio of 5 , 7.5 and 10%, respectively.

At the end of the experimental period (8 weeks), rats were fasted overnight, then anaesthetized & incised longitudinally and blood samples were collected from the aorta. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. serum cholesterol (**Allain et al., 1974**), triglycerides (**Fossati and Prencipl., 1982**), HDL-c (**Lopes-Virella et al., 1977**), LDL-c and VLDL-c (**Friedewald et al., 1972**), aspartate amino transferase (AST) and Alanine amino transferase (ALT) (**Ritman and Frankl.,1957**) and glucose (**Srikanth et al., 2004**).

Statistical analysis

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

Results and Discussion

Chemical analysis of BFP

Data in Table (1) showed the chemical analysis of BFP for protein, fat, fiber, carbohydrates, ash and moisture were recorded 1.9, 0.4, 2.7, 31.5, 1.5 and 62%, respectively.

Table (1): Proximate chemical composition of the fruit of BFP (g/100g wet basis)

Component	g/100g	Component	g/100g
Protein	1.9	Moisture	63.1
Carbohydrate	31.5	Ash	1.5
Fat	0.4	Fiber	2.7

Effect of BFD on serum lipid profile of induced hypercholesterolemic rats

Table (2) illustrated the effect of BFP on lipid fractions. The values of serum cholesterol triglycerides, LDL-c and VLDL-c (mg/dl) showed significant increase ($P \leq 0.05$) for control positive group in

compared with control negative group while HDL-c value (mg/dl) for control positive group was significantly ($p \leq 0.05$) lower than that of control (-) group. Data in this table showed that, total cholesterol (mg/dl) were increased significantly ($P \leq 0.05$) for rats fed on hypercholesterolemic diet (control +). The statistical analysis showed a significant decrease in total cholesterol of all treated groups with BFP when compared with (control +). The lowest decrease in all treated group in cholesterol was recorded in BFP10% (87.34 ± 0.46). Also, the best result of serum triglycerides level was observed in the group fed on basal diet containing BFP10% (59.15 ± 1.03). The antioxidative effect of Bael fruits extract was explained by **Manjula and Kumar (2016)** who found that its fruit extract has a potent *in vitro* antioxidant activity which was correlated with its content of bioactive compounds. The ameliorated effect of Pael 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 hypercholesterolemic diet containing BFP 5%, BFP 7.5% and BFP10% showed significant ($P \leq 0.05$) increase compared with (control +) and the best results for HDL-c was from the group fed on BFP10% (49.73 ± 0.44) followed by that fed on BFP 7.5% (55.19 ± 0.59). Low density lipoprotein cholesterol (LDL-c) of all treated rats with basal diet containing bael fruit powdered decreased significantly ($P \leq 0.05$) compared with control (+). Meanwhile these treatments for rats led to increase LDL-c significantly, compared to (control -). Very low density lipoprotein cholesterol (VLDL-c) of rats fed on basal diet containing cholesterol (hypercholesterolemic diet) increased significantly ($P \leq 0.05$) compared to control (-) group. Treating hypercholesterolemic rats with bael fruit powdered (5, 7.5 and 10%) led to a significant ($P \leq 0.05$) reduction in serum VLDL-c compared with (control +). The lipid lowering effect of the BFP extract might be due to the action of flavonoids and other phenolic compounds, triterpenoids, alkaloids, steroids and glycosides. Normalized rate of lipogenesis is due to the insulin-like activity of triterpenoids (**Sakurai et al., 2002**) or

activating normoglycemia by the insulinotropic effect of flavonoids (Pinent *et al.*, 2008).

Table (2): Effect of BFP on serum lipid fractions of induced hypercholesterolemic rats

Parameters Groups	Lipid fractions (Mean ± SD, mg/dl)				
	Cholesterol	Triglycerides	HDL	LDL	VLDL
Control(-)	77.82± 1.57D	42.16±0.99E	71.24±0.87A	16.78±0.59B	8.08±0.68D
Control(+)	171.30±2.76A	118.19±0.91A	28.45±0.67E	41.15±0.71AB	22.60±0.37A
BFP (5%)	133.81±0.69B	69.84±0.43B	58.87±0.53B	43.79±0.69A	13.82±0.82B
BFP(7.5%)	111.69±11.43C	65.38±1.09C	55.19±0.59C	37.77±1.01AB	13.74±0.42C
BFP (10%)	87.34±0.46D	59.15±1.03D	49.73±0.44D	37.49±1.35AB	11.79±0.26C
L.S.D	9.674	1.681	1.159	2.677	1.003

All results are expressed as mean ±SD, values in the same column which have different superscript letters are significantly different at P≤0.05.

Effect of BFP on serum uric acid, urea nitrogen and creatinine levels of induced hypercholesterolemic rats

Results in Table (3) indicated that uric acid value, urea nitrogen and creatinine (mg/dl) of the control positive group showed highly significant (P≤0.05) increase as compared to (control -) group the mean values were 2.146 ± 0.126 and 28.736 ± 0.447 vs. 1.376 ± 0.058, 15.873 ± 0.220 respectively. the mean values of uric acid decreased in the groups fed on BFP by 5, 7.5 and 10% compared with controls. Also, the highest decrease in serum urea nitrogen in all treated groups was found in the group fed on hypercholesterolemic diet containing 5% BFP. While the highest increase in serum urea nitrogen was observed in 10% BFP group.

Table (3): Effect of BFP on serum uric acid and urea nitrogen levels (mg/dl) of induced hypercholesterolemic rats

Parameters Groups	Uric acid Mean ± SD	Urea nitrogen Mean ± SD	Creatinine Mean ± SD
Control (-)	1.376±0.058D	15.873±0.220D	0.523±0.069B
Control (+)	2.146±0.126A	28.736±0.447A	0.700±0.106A
BFP (5%)	2.013±0.025A	20.693±0.523B	0.527±0.063B
BFP (7.5%)	1.820±0.036B	18.450±1.187C	0.484±0.073B
BFP (10%)	1.563±0.102C	17.463±1.364C	0.391±0.020B
L.S.D	0.154	1.585	0.132

All results are expressed as mean ±SD, values in the same column which have different superscript letters are significantly different at P≤0.05.

Effect of BFP on liver functions of induced hypercholesterolemic rats

Results of AST and ALT are presented in Table (4), AST in all treated groups recorded significant decrease ($P \leq 0.05$) except BFB by 10% when compared with (control +). On the other hand, the lowest levels of AST enzymes were found in group of rats fed on hypercholesterolemic diet containing 10% BFP (21.026 ± 0.846). Also, results obtained from this table showed a significant increase ($P \leq 0.05$) in the mean values of ALT enzyme in the group fed on control + when compared with all treated groups. The best results were observed in the groups that fed on BFP by 10 and 7.5%, respectively. In similar study, **Manjula and Kumar (2016)** reported that phytochemicals screening of Pael fruits ethanolic extract revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, coumarins and triterpenoids.

Table (4): Effect of BFP on liver functions (IU/L) of induced hypercholesterolemic rats

Groups	AST (Mean \pm SD)	ALT (Mean \pm SD)
Control(-)	14.750 \pm 0.170E	6.877 \pm 0.776B
Control(+)	33.863 \pm 0.506A	14.187 \pm 0.774A
BFP (5%)	27.553 \pm 0.247B	10.427 \pm 1.913AB
BFP (7.5%)	24.406 \pm 0.610C	12.943 \pm 1.580AB
BFP (10%)	21.026 \pm 0.846D	11.873 \pm 1.371AB
L.S.D	0.974	6.719

All results are expressed as mean \pm SD. Values in a given column which have different letters are significantly different at $P \leq 0.05$

Effect of BFP on serum glucose levels of induced hypercholesterolemic rats

Data in Table (5) showed that, serum glucose levels in positive control group increased significantly ($P \leq 0.05$), as compared to negative control group 167.997 ± 2.559 mg/dl vs 88.273 ± 1.141 mg/dl respectively. Feeding rats on BFP 10% showed significant ($P \leq 0.05$) decrease in serum glucose levels as compared to positive control group. This agrees with the finding of **Kamalakkannan and Prince (2005)** who observed that Pael fruits extract administration improved the functional state of the pancreatic cells and partially reversed the damage caused by streptozotocin to pancreatic islets. The effect of *A.marmelos* fruit extract can be attributed to its antioxidant activity by scavenging free radicals and improving the antioxidant status.

From the obtained results it can be concluded that, supplementation with high percentage of BFP(10%) exerts a positive impact on the lipid profile and other biochemical parameters in hypercholesterolemic rats. As well as BFP fruit is recommended to be ingested as fresh fruit to hypercholesterolemic patients.

Table (5): Effect of BFP and beverage on serum glucose levels (mg/dl) of induced hypercholesterolemic rats

Groups	Glucose (Mean \pm SD)
Control(-)	88.273 \pm 1.141D
Control(+)	167.997 \pm 2.559A
BFP (5%)	116.960 \pm 0.765B
BFP (7.5%)	115.847 \pm 4.523B
BFP (10%)	104.340 \pm 1.431C
L.S.D	6.686

All results are expressed as mean \pm SD. Values in a given column which have different letters are significantly different at $P \leq 0.05$

Histopathological examination of heart

Microscopically, heart of rats from group control negative are showing normal myometrial muscle striations and nucleation, (Figure 1). Meanwhile, heart of rats from group 2 control positive group: heart showing area of hemorrhage and congested blood vessel with thickened wall (Figure 2). Heart treated group BFP (5%) are showing moderately dilated and congested intermuscular blood vessel (Figure 3). Heart Treated group BFP (7.5%) showing slightly dilated and congested intermuscular blood vessel (Figure 4). Heart treated group BFP 10% are showing normal myometrial muscle striations and nucleation. Treated group BFP (10%) are showing normal myometrial muscle striations and nucleation (Figures 5 and 6).

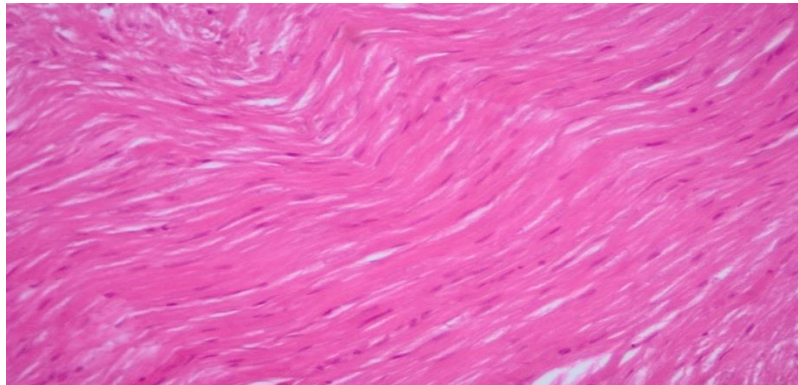


Fig. (1): Control Negative group: Heart showing normal myometrial muscle striations and nucleation, (H&E X 400).

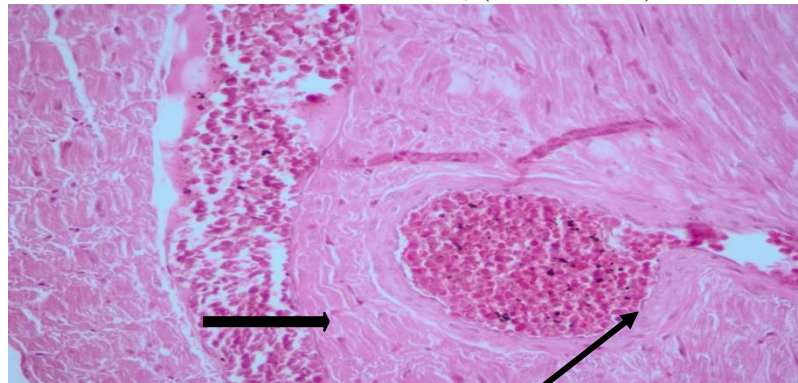


Fig. (2): Control Positive group: Heart showing area of hemorrhage (arrow head), and congested blood vessel with thickened wall (arrow), (H&E X 400).

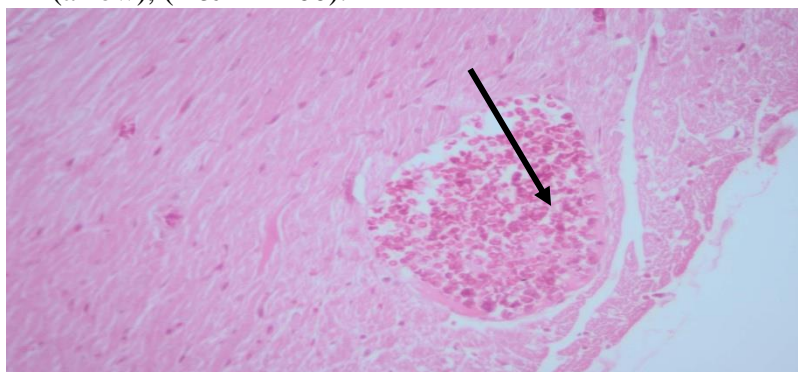


Fig. (3): Treated group BFP5%: Heart showing moderately dilated and congested intermuscular blood vessel (arrow), (H&E X 400).

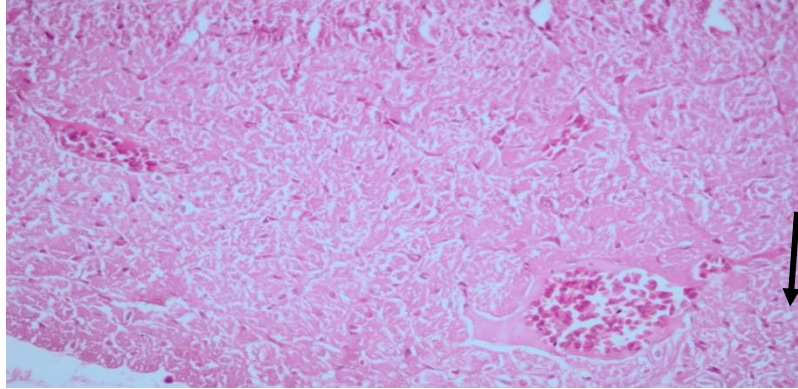


Fig. (4): Treated group BFP7.5%: Heart showing slightly dilated and congested intermuscular blood vessel (arrow), (H&E X 400).

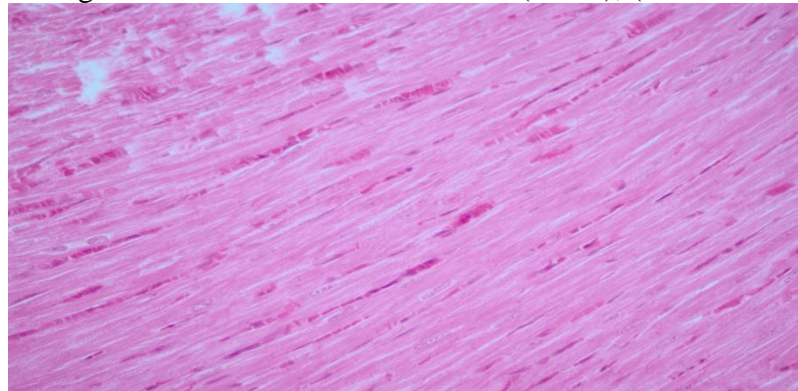


Fig. (5): Treated group BFP10%: Heart showing normal myometrial muscle striations and nucleation, (H&E X 400).

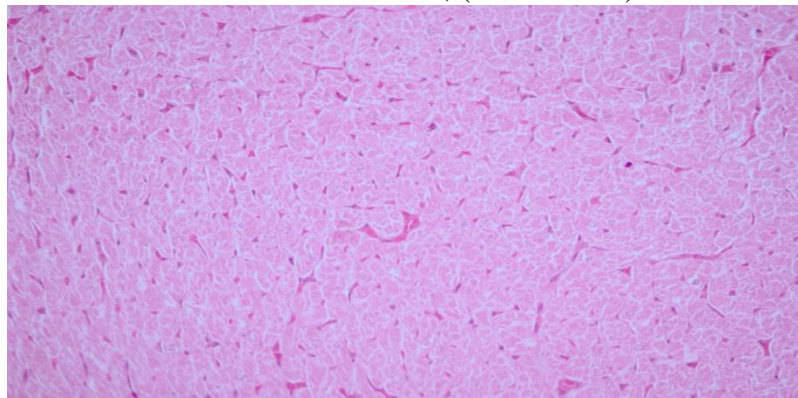


Fig. (6): Treated group BFP10%: Heart showing normal myometrial muscle striations and nucleation, (H&E X 400).

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دراسة بيولوجية على التأثيرات المحتملة لجرعات مختلفة من مسحوق فاكهة بيل (*Aeglemarmelos L., Correa*) على الفئران التي تعاني من ارتفاع مستوى الكوليستيرول

رشا حاجي غلوم حسن أشكناني

كلية التربية الأساسية ، قسم الإقتصاد المنزلي، الهيئة العامة للتعليم التطبيقي والتدريب، الكويت

الملخص :

يهدف هذا البحث إلى دراسة تأثير مسحوق فاكهة بيل في علاج الفئران التي تعاني من ارتفاع مستوى كوليستيرول الدم. تم استخدام ثلاثون فأراً من سلالة الألبينو وقد قسمت إلى خمس مجموعات: المجموعة الأولى تغذت على الغذاء الأساسي (المجموعة الضابطة السالبة)، أما الأربعة مجموعات الأخرى فقد تم رفع مستوى الكوليستيرول لديها ثم قسمت هذه الفئران إلى تحت المجموعات التالية: المجموعة الثانية تغذت على الغذاء العالي في الكوليستيرول (المجموعة الضابطة الموجبة)، والمجموعة الثالثة تغذت على الغذاء العالي في الكوليستيرول المضاف إليه 5 % من مسحوق فاكهة بيل، والمجموعة الرابعة تغذت على الغذاء العالي في الكوليستيرول المضاف إليه 7.5 % من مسحوق فاكهة بيل، والمجموعة الخامسة تغذت على الغذاء العالي في الكوليستيرول المضاف إليه 10 % من مسحوق فاكهة بيل. ولقد أسفرت النتائج عن حدوث نقص معنوي ($p \leq 0.05$) في مستوي الكوليستيرول الكلي والجليسيريدات الثلاثية وكوليستيرول الليبوبروتينات الدهنية المنخفضة الكثافة ودرجة نشاط إنزيمات الكبد ومستوى اليوريا ومستوى الجلوكوز في الفئران التي تغذت على غذاء عالي في الكوليستيرول والمضاف إليه نسبة عالية من مسحوق ثمار فاكهة بيل. وكانت أفضل النتائج للمجموعة التي تغذت على مسحوق ثمار فاكهة بيل بنسبة 10%. وتوصى الدراسة بتدعيم الوجبات والمنتجات الغذائية المقدمة لمرضى القلب الذين يعانون من ارتفاع مستوى كوليستيرول الدم بمسحوق ثمار فاكهة بيل 10% وذلك لما أظهره من نتائج ايجابية في تحسين صورة دهون الدم ووظائف كل من الكبد والكلى.

الكلمات المفتاحية: مسحوق ثمار فاكهة بيل، وظائف الكبد، وظائف الكلى، صورة دهون الدم.