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Effect of Nutmeg *(Myristica fragrans)* on Hyperlipidaemia in Rats

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Abstract: The present work seeks to investigate the effect of nutmeg *(Myristica fragrans)*on hyperlipidaemia for rats fed on high fat diet. Twenty five male albino rats Sprague Dawley were divided into 5 groups fed on (1) basal diet (control -), (2) basal diet and high fat (control $^{+)}$The rats of third, fourth and fifth groups were fed on hypercholesterolemia diet supplemented with nutmeg (5, 10 and 15%) respectively. At the end of experimental Period (35 day).The parameters of serum total lipid, total cholesterol, triglycerides, high density lipoprotein cholesterol HDL, low density lipoprotein cholesterol LDL, very low density lipoprotein VLDL and Atherogenic Index (AI) in hyperlipidaemia rats' were estimated and histopathological changes also were examined. The obtained results revealed significant increase in body weight gain ratio for rats fed on hypercholesterolemia diet comparing with rats fed on basal diet. While it could be observed that all groups fed on hypercholesterolemic diets supplemented with nutmeg at different levels (5, 10 and 15%) had marked decrease in body weight gain ratio and food efficiency ratio comparing with control positive group. Moreover, nonsignificant difference recorded in food intake for all groups fed on basal diet or groups fed on hypercholesterolemic diets with or without nutmeg at the same above concentrations. On the other hand, there were significant increase of serum total lipids, total cholesterol and triglycerides ($p < 0.01$) in comparison with control negative group. While, significant decrease of the above same parameters for rats fed on hypercholesterolemic diet supplemented with nutmeg 5 and 10% comparing look place with control positive group. The obtained results indicated the significant decrease of serum low density lipoprotein cholesterol LDL-c, very low density lipoprotein cholesterol VLDL-c and atherogenic index (AI) for rats fed on hypercholesterolemic diets supplemented with nutmeg (5 and10%) comparing with control positive group. Moreover significant decrease in high density lipoprotein cholesterol HDL-c for all hypercholesterolemic groups which fed on hypercholesterolemic diets at different levels of nutmeg (5, 10 and 15%) comparing with control negative and control positive groups However Anyhow, the histopathological picture of liver, kidney and heart indicated that the addition of nutmeg diets at different levels for rats had slight effects on the microscopic structure.

Key words: Hypercholesterolemic · Total cholesterol · LDL-c · VLDL-c · HDL-c · Atherogenic Index (AI)

INTRODUCTION

Spices have been used since ancient times not only for enhancing the flavor of foods but also for their preservative and medicinal properties. *Myristica fragrans* (family: Myristicaceae) is a tropical, evergreen tree native to the Moluccas or Spice Island of Indonesia. Nutmeg is the dried seed kernel of *Myristica fragrans* and is widely used as spice and also to flavor many kinds of baked goods and vegetables. Nutmeg seeds contain 20% to 40% of a fixed oil, commonly called nutmeg butter. This oil contains myristic acid, trymiristin and glycerides of lauric, tridecanoic, stearic and palmitic acids [1, 2]. Nutmeg also yields 8% to 15% of an essential oil that is believed to be partially responsible for the effects associated with nutmeg intoxication. The essential oil contains myristicin, elemicin, eugenol and safrole [1-3].

The essential oils of nutmeg and mace are very similar in chemical composition and aroma, with wide color differences (brilliant orange to pale yellow). Mace oil appears to have higher myristicin content than nutmeg oil [4]. Also present in the oil are sabinene, cymene alpha-thujene, gamma-terpinene and monoterpene alcohols in smaller amounts. Phenolic compounds found in nutmeg are reported to have antioxidant properties [1, 2, 5, 6]. Other isolated compounds include the resorcinols malabaricone B and malabaricone C, [7] as well as lignans and neolignans [8, 9].

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Nutmeg possesses antifungal, hepatoprotective [10] and antioxidant properties [11]. Recent studies indicate that it is useful against damage caused by gamma radiation [12] and also in the improvement of mouse memory [13]. Anti-inflammatory [14], antidysentric [15], analgesic and hypotensive [16], activities of nutmeg have also been reported, in addition to its insulin-like biological activity [17]. Compounds isolated from the seeds of this plant have been reported to possess strong platelet antiaggregatory activity [18].

MATERIALS AND METHODS

Materials: Nutmeg seeds powder was obtained from local market of Cairo- Egypt (Harraz market). Cholesterol (white crystalline powder), bile salt, casein, vitamins, minerals and cellulose were obtained from El-Gomhariya Pharm. and Chem. Ind. Comp., Cairo, Egypt. While, starch and corn oil were obtained from local market.

Methods

Standard Diet: The standard diet consisted of casein (15%), corn oil (5%), vitamin mixtures (1%), salt mixture (4%), fiber (cellulose) (5%), Starch (70%) and choline chloride (0.2%) [19].

Hypercholesterolemic Diet: The hypercholesterolemic diet composed of basal diet + cholesterol (l%) [20] + saturated fat (lamb fat 10%) [21].

A-Experimental design

Animals: Twenty five male albino rats, Sprague Dawley, each weighting 100±10 g were obtained from the Agricultural Research Center, Dokki Cairo (ARC). These rats were allowed to be acclimatized to laboratory condition for one week prior to the experiment and fed on basal diet. Animals were randomly divided into five groups, each group contained five rats. The first group was fed on basal diet (control -). Rats of second group were fed on hypercholesterolemic diet (control +). The rats of third, fourth and fifth groups were fed on hypercholesterolemic diets supplemented with nutmeg powders at different levels (5%, 10% and 15%), respectively. At the end of experiment (35days), animals were fasted over night before sacrificed. Blood samples were collected in test tubes from each rat and centrifuged at3000rpm for 15min. Then serum was kept frozen (-20°C) until analyzed. Organs (liver, heart and kidney) were separated and weighted, then kept in formalin solution (10%) until histopathological examination.

Biological Determination: Food efficiency ratio was calculated using following equation:

$$FER\% = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Food intake (g)}} \times 100$$

Body weight gain:

$$BWG\% = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Biochemical Analysis: Determination of lipids parameters carried out by enzymatic methods: Total lipid,[22] Total cholesterol (TC) [23], high-density lipoprotein cholesterol (HDL-c) [24] and triglycerides (TG)) [25], while (LDL-c and VLDL-c) [26]. Atherogenic Index (Total cholesterol / HDL-cholesterol) and Total protein [27].

Statistical Analysis: Results were expressed as the mean±SD. Data were [28] statistically analyzed using one-way analysis of variance (ANOVA).

5-Histopathological examination: Rat's internal organs were subjected to histological examination [29].

RESULTS AND DISCUSSION

Effect of experimental diets on food intake, body weight gain (BWG) and feed efficiency ratio (FER) in hyperlipidaemic rats: Effect of nutmeg *(Myristica fragrans)* on body weight gain ratio, food intake and food efficiency ratio was presented in Table 1. Data in Table 1 and Figures 1 and 2 showed significant increases in body weight gain ratio (BWG) for rats fed on hypercholesterolemic diet (control positive) comparing with rats fed on basal diet (control negative). While, it could be observed that all groups fed on hypercholesterolemic diet supplemented with nutmeg by different levels (5, 10&15%) had marked decrease in body weight gain ratio (BWG) and food efficiency ratio (FER) comparing with control positive group. The highest value of FER was (0.083±0.022) in the positive control group, while the lowest value was (0.036±0.011) in group fed on hypercholesterolemic diet supplemented with (15%) nutmeg. Moreover, nonsignificant difference could be recorded in food intake for all groups fed on basal diet (control negative) or on hypercholesterolemic diet with or without nutmeg.

Table 1: Effect of nutmeg at different levels on body weight gain (B.W.G.), food intake (F.I.) and food efficiency ratio (F.E.R)

Treatments	Initial weight(g)	Final weight (g)	BWG(g)	BWG (%)	Food Intake (g/day)	F.E.R
Control (-)	161.50±7.89 a	190.00±3.30 b	28.50±6.50 ab	17.422±4.41 ab	12.00±0.40 a	0.068 ±0.022 a
Control (+)	165.50±5.25 a	202.15±4.16 b	36.65±3.67 a	22.145 ±3.27 a	12.63 ±1.25 a	0.083±0.019 a
Nut 5%	154.75±5.56 a	178.10±10.56 ab	23.35±5.98 b	15.088±3.59 b	13.50 ±2.08 a	0.049 ±0.008 b
Nut 10%	156.25±8.30 a	176.27± 5.24 a	20.02±3.24 b	12.812± 2.82 b	13.625 ±1.49 a	0.042 ±0.004 b
Nut 15%	155.00±9.35 a	171.60±14.90 a	16.60±7.07 b	10.709±3.94 b	13.25 ±2.50 a	0.036 ±0.011 b

**Values with the same letters indicate insignificant difference (p=0.01) and vice versa.

Table 2: Effect of Nutmeg at different levels on Organ /Body weight ratio.

Treatments	Liver /B.W (%)	Kidney /B.W (%)	Heart /B.W (%)
Control (-)	1.655 ±0.20 a	0.297 ±0.05 b	0.17 ±0.03 a
Control (+)	1.809 ±0.41 a	0.40 ±0.06 a	0.167 ±0.03 a
Nut 5%	2.06 ±0.59 a	0.486 ±0.06 a	0.208 ±0.01 a
Nut 10%	1.62 ±0.13 a	0.445 ±0.04 a	0.211 ±0.01 a
Nut 15%	2.146 ±0.51 a	0.438 ±0.03 a	0.214 ±0.02 a

**Values with the same letters indicate insignificant difference (p=0.01) and vice versa

Table 3: Effect of nutmeg at different levels on total lipids, triglycerides and total protein.

Treatments	Total Lipids (mg/dl)	Triglycerides (mg/dl)	Total protein (g/dl)
Control (-)	288.25 ±8.22 d	82.4 ±2.24 d	3.69±0.440 a
Control (+)	483.75 ±7.76 b	179.5 ±4.12 a	3.78±0.470 a
Nut 5%	439.50 ±5.80 c	146.25 ±12.03c	3.586±0.284 a
Nut 10%	444.50 ±8.34 c	162.75 ±4.78 b	3.644±0.124 a
Nut 15%	571.50 ±11.67 a	171.75 ±5.90 ab	3.648±0.561 a

**Values with the same letters indicate nonsignificant difference (p=0.01) and vice versa

Table 4: Effect of nutmeg at different levels on total Cholesterol, HDL-C, LDL-C, VLDL-C and AI.

Treatments	Total Cholesterol (mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL (mg/dl)	AI
Control (-)	76.50 ±1.29 d	43.00 ±1.82 a	17.02 ±1.95 d	16.48 ±0.44 d	1.78 ±0.06 d
Control (+)	161.50 ±8.69 a	35.50 ±1.29 b	90.10 ±7.41 b	35.90 ±0.82 a	4.54 ±0.15 a
Nut 5%	103.25 ±4.71 c	29.50 ±1.29 c	44.50 ±4.44 c	29.25 ±2.40 c	3.50 ±0.21 c
Nut 10%	143.75 ±12.50 b	32.75 ±3.86 c	76.65 ±11.41 b	34.35 ±1.18 b	4.42 ±0.51 b
Nut 15%	173.50 ±9.14 a	33.50 ±2.38 c	107.45±11.08 a	32.55 ±0.95 b	5.21 ±0.57 a

Values with the same letters indicate insignificant difference (p=0.01) and vice versa

HDL-C: High Density Lipoprotein Cholesterol.

LDL-C: Low Density Lipoprotein Cholesterol.

VLDL-C: Very Low Density Lipoprotein Cholesterol.

AI: Atherogenic Index (Total cholesterol / HDL).

Effect of experimental diets on organs / body weight (BW) ratio: Data in Table 2 showed insignificant differences in liver and heart to body weight ratio for all groups fed on basal diet (control positive) or groups fed on hypercholesterolemic diet with or without being supplementation by nutmeg. In addition, significant increase in kidney to body weight ratios were observed for all groups fed on hypercholesterolemic diet with or without supplemented at different levels of nutmeg (5, 10 andl 5%) comparing with negative control group.

Effect of nutmeg on serum total lipids, triglycerides and total protein in hyperlipidaemic rats: Effects of nutmeg *(Myristica fragrans)* on serum lipids profile parameters of hypercholesterolemic rats were illustrated in Table 3, Fig. 3 and 4. Data indicated that all hypercholesterolemic groups which fed on hypercholesterolemic diet with or without supplementation at different levels of nutmeg (5, 10 and 15%), there were significant increase in serum total lipids and triglycerides TG ($p < 0.01$) comparing with control negative group. Moreover, the same data showed

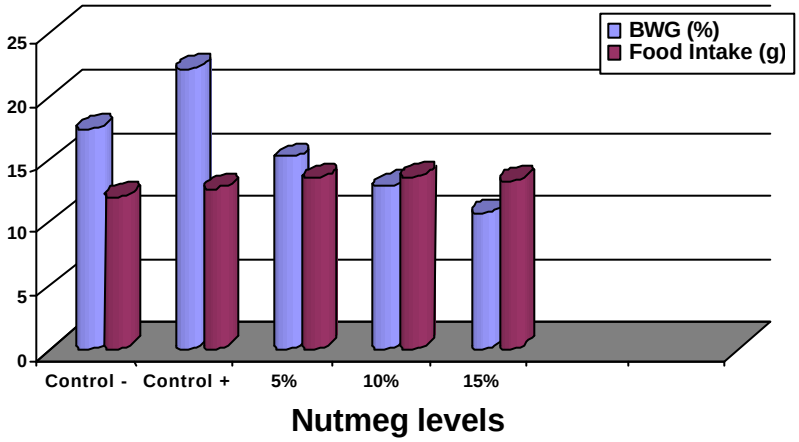

Fig. 1: Effect of nutmeg at different levels on BWG and FI

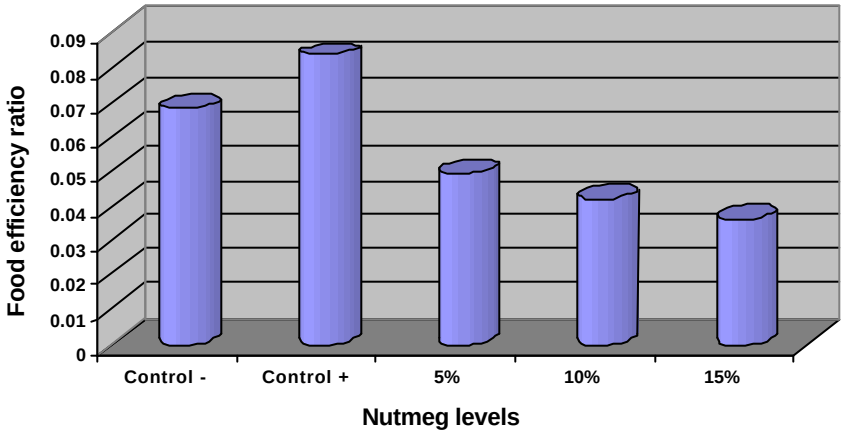

Fig. 2: Effect of nutmeg at different levels on food efficiency ratio (FER)

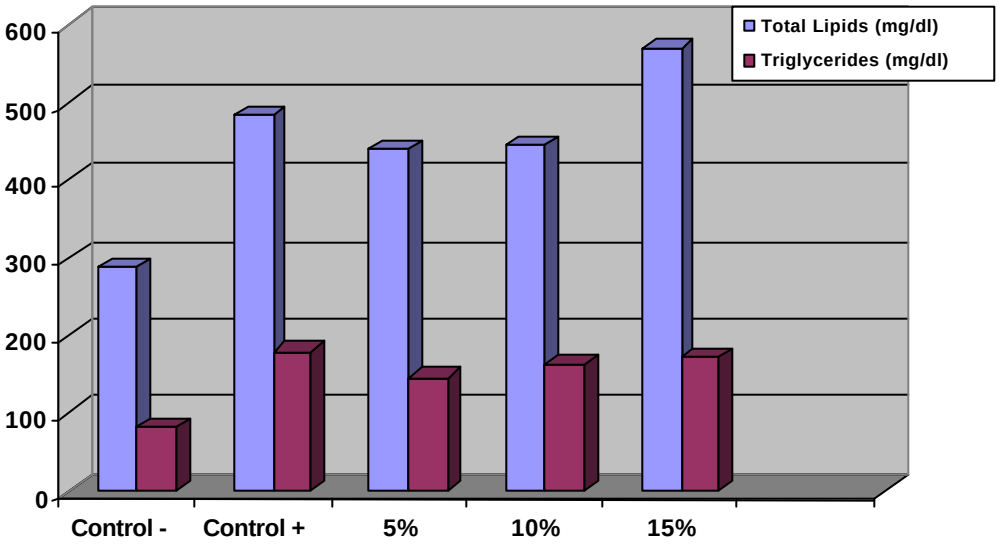

Fig. 3: Effect of nutmeg at different levels on total lipids and triglycerides

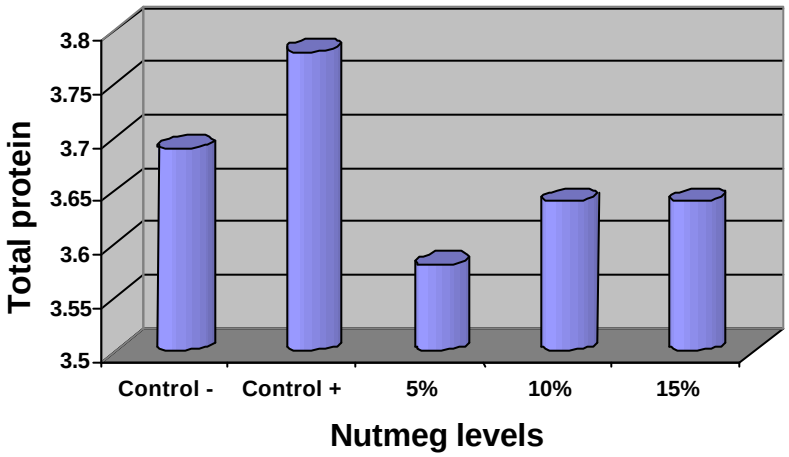

Fig. 4: Effect of nutmeg at different levels on total protein

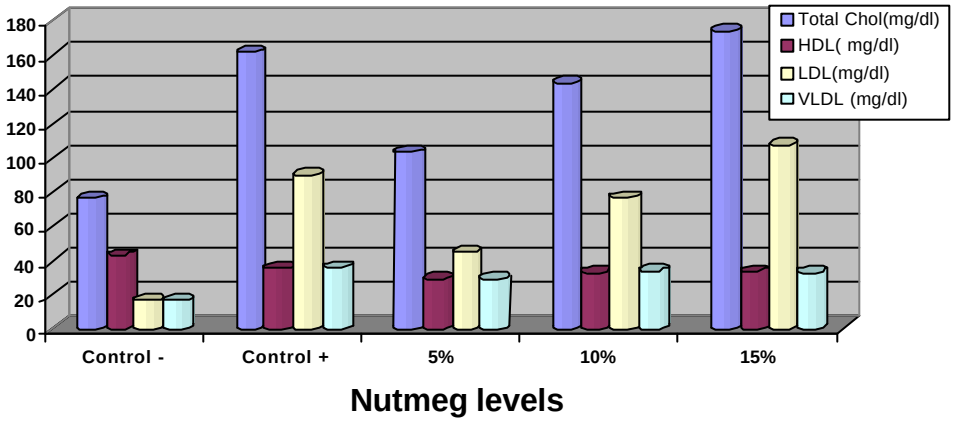

Fig. 5: Effect of nutmeg at different levels on total cholesteol, HDL, LDL and VLDL

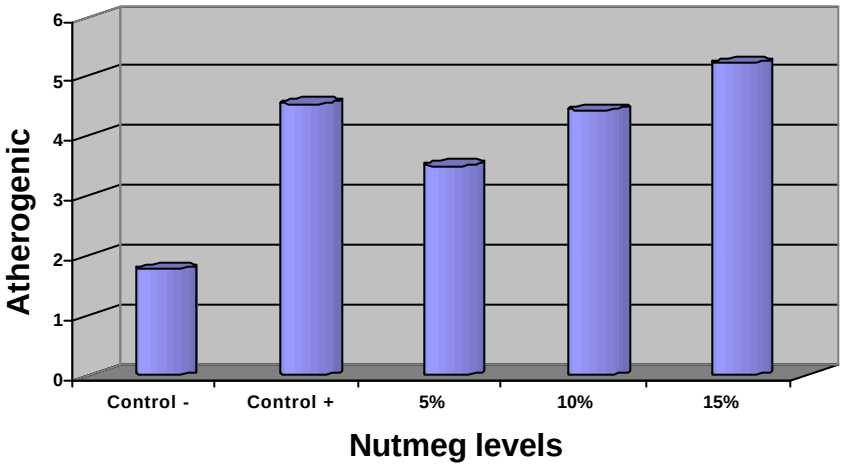

Fig. 6: Effect of nutmeg at different levels on atherogenic index (Al)

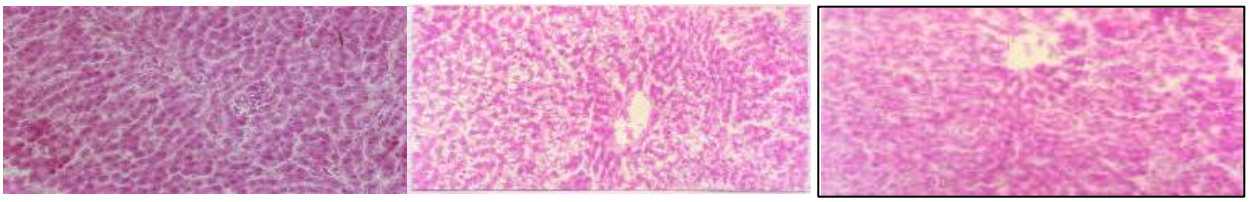

Photo. (1) Liver control (-) Photo. (2)Liver Control(+) Photo. (3) Liver (5%)

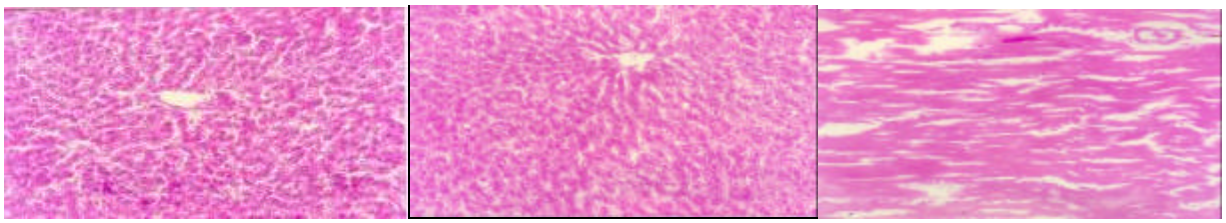

Photo. (4) Liver (10%) Photo (5). Liver (15%) Photo. (6) Heart control(-)

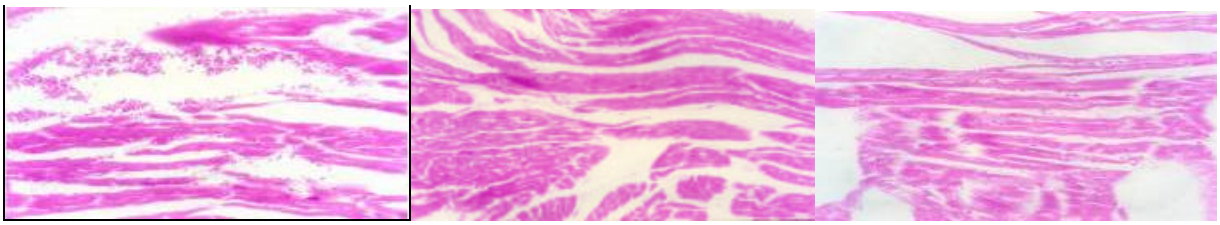

Photo. (7) Heart control(+) Photo. (8) Heart (5%) Photo. (9) Heart (10%)

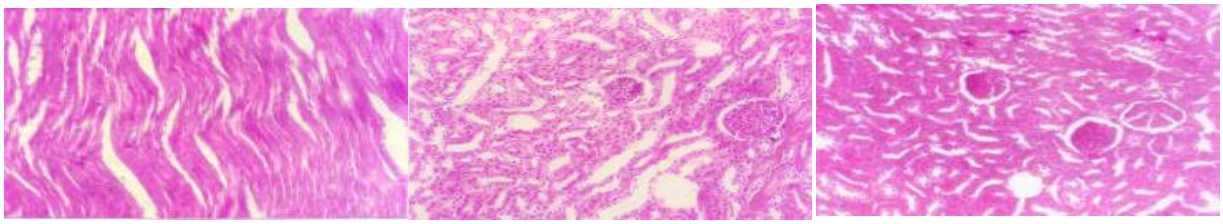

Photo. (10) Heart (15%) Photo. (11) Kidney (5%) Photo. (12)Kidney (10%)

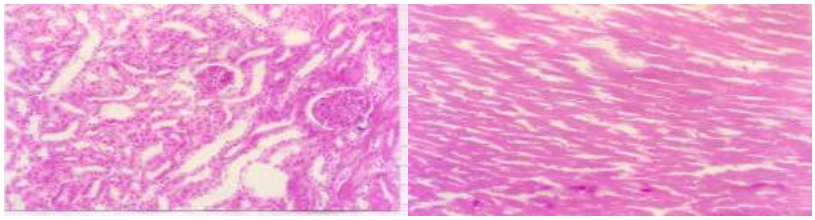

Photo. (13) Kidney (15%) Photo. (14)Kidney control(+)

that significant decrease in total lipids and triglycerides occurred for rats fed on hypercholesterolemic diet supplemented with nutmeg (5 and 10%) comparing with control positive group. On the other hand, rats fed on hypercholesterolemic diet supplemented with nutmeg by (15%) had marked increase in total lipids comparing with control positive group. These results are in agreement with those reported by Bainton *et al.* [30] and Panel [31]. In addition, the data shows nonsignificant changes in total protein for all groups fed on nutmeg by different levels (5, 10 and15%)) when comparing with control negative.

Effect of nutmeg on serum total cholesterol, HDL, LDL, VLDL and AI in hyperlipidaemia rats: Table 4, Fig. 5 and 6 show the effect of nutmeg at different levels on total cholesterol, HDL, LDL, VLDL and AI. The obtained results indicated that a significant increase in serum total cholesterol (TC), low density lipoprotein cholesterol LDL-c, very low density lipoprotein cholesterol VLDL-c and atherogenic index AI ($p < 0.01$) comparing with control negative group. In addition, from the same data illustrated in same table significant decrease in serum low density lipoprotein cholesterol LDL-c, very low density lipoprotein cholesterol VLDL-c and atherogenic index AI ($p < 0.01$) for rats fed on hypercholesterolemic diet supplemented with nutmeg by (5 and 10%) comparing with control positive group. On the other hand, rats fed on nutmeg by (15%) had significant increase in serum low density lipoprotein cholesterol LDL-c and marked increase in atherogenic index AI comparing with control positive group. Also all hypercholesterolemic groups which fed on different levels of nutmeg (5, 10 and 15%) showed significant decrease in high density lipoprotein cholesterol HDL-c when comparing with control negative and control positive groups. These results are in agreement with those reported by Hyun Sook Kwon [11], Panel [31] and Wilson [32].

Histopathological examination for

Liver: Microscopically, examination of liver from rat in control (-) group revealed no histopathological change (Photo.1).While; liver of rat from control (+) group fed on hypercholesterolemic revealed vacuolar degeneration of hepatocytes (Photo.2). However, histopathological finding of rat liver fed of third and fourth groups fed at different levels of nutmeg powder (5and10%) showed small vacuoltion in the cytoplasm of some hepatocytes (Photo.3&4). Moreover liver of rat from group (5) nutmeg (15%) revealed small vacuoles in sporadic hepatocytes (Photo.5). Meanwhile histopathological examination of liver for rats fed on nutmeg (15%revealed slight improvement in the histopathological picture when compared with control (+) group.

Heart: Microscopically, examination of sections heart for rat in control (-) group revealed no histopathological change (Photo 6).While, heart of control (+) group rats, fed on hypercholesterolemic diet, showed intermuscular hemorrhage (Photo.7). Meanwhile, heart of rats from groups (3 and 4) (nutmeg 5 and 10%) revealed no histopathological changes (Photos 8 and 9). However, heart of rat from group (5) fed on nutmeg (15%), revealed no histopathological changes (Photo 10). From the obtained examination of heart section of rat from group (5) fed with (15%nutmeg) revealed slight improvement of the histopathological view when compared with Control (+).

Kidney: Histopathological examination of kidney sections of rats in groups (3, 4 and 5) fed on different levels of nutmeg (5, 10 and 15%) revealed no histopathological changes (Photo 11, 12 and 13).Also kidney of rats fed on hypercholesterolemic diet showed any histopathological changes (Photo 14).

CONCLUSION

From the above- mentioned results, it could be concluded that the diet containing nutmeg at different levels reduced serum levels of total lipids, triglycerides, total cholesterol and LDL-cholesterol in the hyperlipidemia rats. This is may be that the nutmeg has some anti-hyperlipidaemic effect. Also, the obtained results indicate that there was improvement in the histopathological picture in liver and heart as a result of these additives.

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