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The Protective Role of Rutin on some Biochemical and histological parameters induced-hyperlipidemia by Triton WR1339 in male rats

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ABSTRACT

Rutin (3,3, 4', 5, 7-pentahydrohyflavone-3-rhamnoglucoside) is a flavonoid of the flavonol type. Rutin is found in many plants and is also an important dietary constituent of food and plant-based beverages. Rutin has several pharmacological properties including antioxidant activities. Also, it was identified that rutin is the major low-density lipoprotein (LDL) antioxidant compound of mulberry in an study. The effects of rutin were tested by using it as induced-hyperlipidemia by Triton WR1339. Male rats were fed 4 weeks with rutin (30mg/kg) to study the hyperlipidemia effects of rutin on serum lipid levels, hepatic enzyme activity, and liver tissue. gavaged the animals with Triton WR1339 resulted in marked hyperlipidemia and increased the serum level of LDL cholesterol (LDL-C). Rutin (at 30 mg/kg) alone significantly reduced the levels of total cholesterol, and LDL-C and also markedly decreased liver enzymes and weight in animals with a high-cholesterol diet. Our findings show that 30 mg/kg of rutin alone or with other supplementation lowered liver weight and enzymes as well as serum total cholesterol and LDL. The hepatic histopathological results reflect the correlation of rutin with liver weight and the levels of serum total cholesterol and LDL-C. These results indicate that rutin has increased anti-hyperlipidemia effects in an animal model.

KEYWORDS: *Rutin, hyperlipidemia, Triton WR1339, male rats.*

I. INTRODUCTION

Rutin (3, 4,5,7-pentahydrohyflavone-3-rhamnoglucoside) is a flavonoid of the flavonol type. Rutin is found in many plants and is also an important dietary constituent of food and plant-based beverages. Rutin has several pharmacological properties including antioxidant activities. Lipid levels are a metabolic risk factor for cardiovascular disease and abnormalities in serum lipoprotein classes, and derangements in lipid metabolism rank among the most firmly-established and best-understood risks factors for atherosclerosis [1]. Serum cholesterol levels are regulated by the absorption of dietary cholesterol, excretion of cholesterol via faecal

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sterols or bile acids, cholesterol biosynthesis, and removal of cholesterol from circulation. Numerous previous studies have reported on the beneficial effects of hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and acyl-CoA: cholesterol acyltransferase inhibitors of hypercholesterolaemia and atherosclerosis [2,3]. Low density lipoprotein cholesterol (LDL-C) transports cholesterol from liver to tissues, whereas high-density lipoprotein cholesterol (HDL-C) facilitates the translocation of cholesterol from the peripheral tissues to the liver for catabolism. Therefore, HDL-C has a useful effect in reducing tissue cholesterol and an elevated ratio of HDL-C in serum is suggested together with a decreased level of LDL-C to reduce the risk of cardiovascular diseases [4].

The nonionic detergent, Triton WR1339 (Tyloxapol or an oxyethylated tertiary octyl phenol formaldehyde *poly*mer), is used by several studies to induce hypercholesterolemia in animals [5], [6]. Its function is to inhibit the activity of the enzyme lipoprotein lipase, resulting in the accumulation of triglycerides and VLDL in serum, beyond causes a significant increase in hepatic cholesterol biosynthesis by stimulating the activity of the enzyme HMG-CoA reductase [7].

II. MATERIALS & METHODS

Chemicals

Rutin and Triton WR-1339 (Tyloxapol) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Animals

Adult female rat (160–180 gm) were used. The animals were kept on a 12 h light/dark cycle, at room temperature (22± 2°C), with free access to food and water.

Liver enzymes and Lipid levels

Total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides were determined by enzymatic colorimetric methods using commercial kits (Labtest Diagnostica, MG, Brazil).

III. RESULTS

Body Weight:

The results of the study after statistical analysis of the experimental groups as in Table (1) showed a significant increase ($P < 0.05$) in the average body weight in the positive control group T1 injected with triton 100) mg / kg compared with the negative control group (C) That drenched distilled water for the duration of the experiment. Also, there was a significant increase ($P < 0.05$) in the T2 treatment that dose the routine (30) mg / kg compared to the negative control group (C), while it showed a significant decrease ($P < 0.05$) compared to the positive control group T1. The T3 treatment, which dose the routine and injected with triton concurrently, showed a significant decrease ($P < 0.05$) compared to the negative control group (C) as well as the positive control group T1 and treatment T2 and showed a significant increase ($P < 0.05$) compared to the treatment T4. As for the T4 treatment that was injected with triton and then injected with the routine, it showed a significant decrease ($P < 0.05$) in the weight ratio compared to the negative control group (C) and positive control T1 and the

rest of the treatments. 0.05) compared with the negative control group (C) and coefficients T3 and T4 showed a significant decrease (P <0.05) compared to coefficients T1 and T2.

Liver weight

The results of the statistical analysis of the experimental groups as in Table (1) showed a significant increase (P <0.05) in the weight of the liver in the T1 group that was injected with triton compared to the control group C. Also, a significant increase (P <0.05) was observed in T2 treatment A comparison with the control group C with a significant decrease (P <0.05) when compared with treatment T1. The T3 treatment showed a significant increase (P <0.05) compared to the control group C and treatment T4 and showed a significant decrease (P <0.05) when compared to the positive control group T1 and treatment T2. The T4 treatment showed a significant increase (P <0.05) when compared to the control group C and showed a significant decrease (P <0.05) compared to the rest of the factors. A significant increase (P <0.05) was observed in the T5 treatment compared to the negative control group (C) and the T3 and T4 coefficients and showed a significant decrease (P <0.05) compared to the positive control T1 and treatment T2.

Table (1) shows the effect of routine on body and liver weight in rats induced hyperlipidemia

Liver weights (Gm)	(Gm) Ratio	Final weights (Gm)	First weights (Gm)	Groups
6.5 ± 0.02 F	25 ± 0.025 D	215 ± 1.25 E	190 ± 0.521	C
9.6 ± 0.03 A	54 ± 0.52 A	248 ± 1.15 A	194 ± 0.241	T1
8.9 ± 0.05 B	37 ± 0.35 B	232 ± 1.02 B	195 ± 0.325	T2
7.7 ± 0.02 D	21 ± 0.052 E	217 ± 1.45 D	196 ± 0.592	T3
7.6 ± 0.04 E	15 ± 0.015 F	211 ± 1.30 F	196 ± 0.625	T4
8.7 ± 0.01 C	26 ± 0.022 C	221 ± 1.05 C	195 ± 0.751	T5
0.021	0.742	3.025	0.00	LSD0.05

Total cholesterol (TC)

The results of the statistical analysis shown in Table (2) showed a significant increase ($p < 0.05$) in the total cholesterol concentration in the serum of male white rats in the positive control group (T1) compared to the negative control group (C). The results also showed a significant decrease ($p < 0.05$) in the total cholesterol concentration in treatment T2 compared with the negative control group (C) and positive control T1. The results showed a significant increase ($p < 0.05$) in the total cholesterol concentration in treatment T3 compared with the negative control group (C) and treatment T2 and a significant decrease ($p < 0.05$) compared with positive control T1. While the results of the T4 treatment showed a significant decrease ($p < 0.05$) compared to the negative control group (C) and the T1 and T3 treatments showed a significant increase compared to the T2 treatment. The results of treatment T5 showed a significant increase ($p < 0.05$) compared to the negative control group (C), T2 and T4 treatments, and a significant decrease ($p < 0.05$) compared to the positive control T1 and treatment T3.

Triglycerides

The results of the statistical analysis shown in Table (2) showed a significant increase ($p < 0.05$) in the serum triglycerides concentration in the blood serum of the male white rats in the positive control group T1 compared with the negative control group (C). The results of treatment T2 showed a significant decrease ($p < 0.05$) in the concentration of triglycerides compared with the negative control group (C) and positive control T1. The results also showed a significant increase ($p < 0.05$) in the concentration of triglycerides in treatment T3 compared with the negative control group (C) and treatment T2 and a significant decrease ($p < 0.05$) compared with positive control T1. The results showed a significant increase ($p < 0.05$) in treatment T4 compared to negative control (C) and treatment T2 and a significant decrease ($p < 0.05$) compared to positive control T1 and treatment T3. The results indicated a significant increase ($p < 0.05$) in T5 treatment compared with negative control (C) and T2, T3 and T4 factors and a significant decrease ($p < 0.05$) compared to positive control T1.

LDL-C

The results of the current study shown in Table (2) indicated a significant increase ($p < 0.05$) in the serum LDL-C concentration in T1 positive control group compared with negative control (C). The T2 treatment showed a significant decrease ($p < 0.05$) in the LDL-C concentration compared to the negative control (C) and positive control T1. The results of treatment T3 showed a significant increase ($p < 0.05$) in the LDL-C concentration compared with negative control (C) and treatment T2 and a significant decrease ($p < 0.05$) compared to positive control T1. The results of treatment T4 showed a significant increase ($p < 0.05$) in the LDL-C concentration compared with negative control (C) and treatment T2 and a significant decrease ($p < 0.05$) compared to positive control T1 and treatment T3. Regarding the T5 treatment, the results showed a significant decrease ($p < 0.05$) in the LDL-C concentration compared with the negative control (C) and the T1, T3 and T4 coefficients and a significant increase ($p < 0.05$) compared with the T2 treatment.

HDL-C

The results of the statistical analysis shown in Table (2) showed a significant decrease ($p < 0.05$) in the serum HDL-C concentration in the T1 positive control group compared to the negative control (C). A significant decrease ($p < 0.05$) was observed in HDL-C concentration in treatment T2 compared to negative control (C) and significant increase ($p < 0.05$) compared to positive control T1. A significant decrease ($p < 0.05$) was observed in HDL-C concentration in treatment T3 compared to negative control (C) and positive control T1 and treatment T2

and a significant increase ($p < 0.05$) compared to treatment T4. The results of treatment T4 showed a significant decrease compared to negative control (C), as well as other treatments. As for the results of treatment T5, a significant decrease ($p < 0.05$) was observed compared to group (C) and T2 treatment and a significant increase ($p < 0.05$) compared to positive control T1 and T3 and T4 coefficients.

Table (2) shows the effect of routine on cholesterol, triglycerides and lipoproteins in rats in which hyperlipidemia was introduced.

HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)	TC (mg/dL)	Groups
48.152 ±2.951 A	31.486 ±1.325 D	41.829 ±2.015 E	72.197 ±3.25 D	C
37.954 ±2.31 D	41.659±0.956 A	72.003 ±2.105 A	100.603 ±5.21 A	T1
46.816 ±1.252 B	21.31 ±1.874 F	38.637 ±2.551 F	68.566 ±2.52 F	T2
34.291 ±1.324 F	36.511 ±1.654 B	52.071 ±1.851 C	88.593 ±3.65 B	T3
32.861 ±1.521 E	36.045 ±1.251 C	45.023 ±1.697 D	71.751 ±2.95 E	T4
41.329 ±1.520 C	29.327 ±1.215 E	55.028 ±0.954 B	82.209 ±3.521 C	T5
1.15	0.52	1.25	4.05	LSD0.05

Liver enzymes (AST, ALT, ALP)

The results of the statistical analysis of the current study, as shown in Table (3), showed a significant increase ($p < 0.05$) in the level of ALP, ALT, and AST in the serum of male white rats in the positive control group T1 compared to negative control (C). The T2 treatment showed a significant decrease ($p < 0.05$) in the ALP and AST levels compared to the negative control group (C) and no significant differences ($P > 0.05$) in the ALT level. It also showed a significant decrease ($p < 0.05$) in the level of ALP, ALT and AST compared to positive control T1. While treatment T3 indicated a significant increase ($p < 0.05$) in the level of ALP, ALT and AST compared to negative control (C) and treatment T2 and showed a significant decrease ($p < 0.05$) compared to positive control T1. As for the results of treatment T4, there was a significant decrease ($p < 0.05$) in the ALP level compared to the negative control group (C) and the rest of the treatments while it showed a significant increase ($p < 0.05$) in the level of ALT and AST compared to the negative control (C) and the treatment T2 and indicated to There was a significant decrease ($p < 0.05$) in the level of ALT and AST compared to positive

control T1 and coefficients T3 and T5. The results of treatment T5 showed a significant increase ($p < 0.05$) in the level of ALP, ALT and AST compared to the negative control group (C) and the coefficients T2 and T4 and showed a significant decrease ($p < 0.05$) compared to positive control T1.

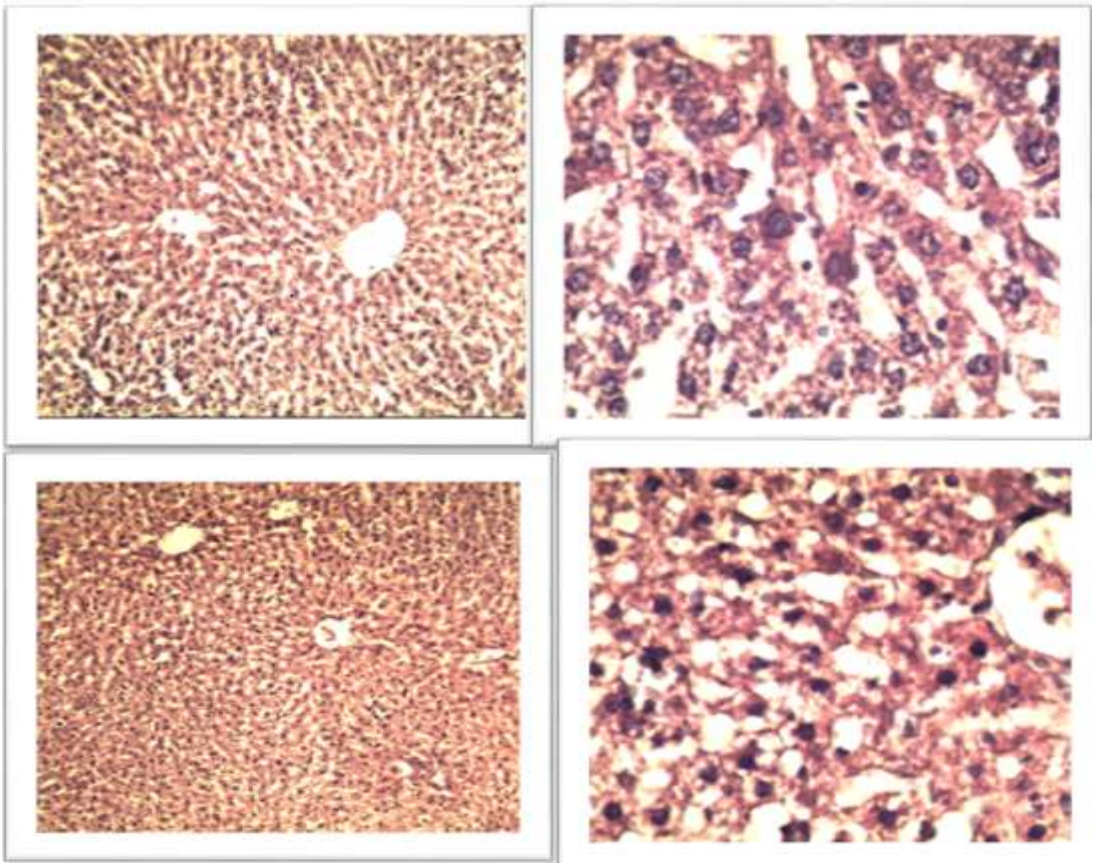
Table (3) shows the effect of the routine on liver enzymes (ALP, ALT, AST) in newly developed rats with hyperlipidemia.

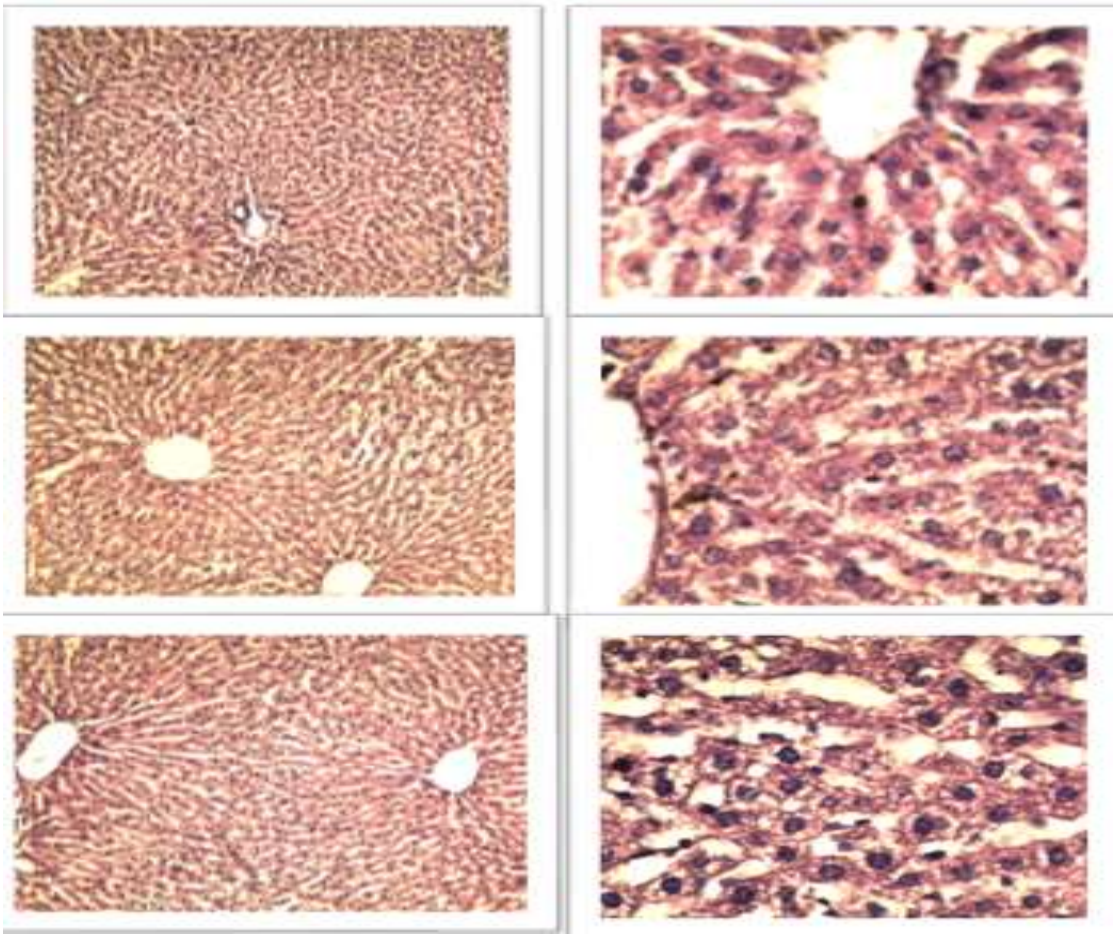
AST (U/L)	ALT (U/L)	ALP (U/L)	Groups
15.442 ±0.05 E	10.536 ±0.75 E	80.64111 ±1.25 D	C
26.186 ±0.052 A	22.022 ±0.06 A	121.539 ±3.25 A	T1
14.921 ±0.5 F	10.69 ±0.04 E	76.103 ±1.52 E	T2
22.217 ±0.41 C	15.038 ±0.03 C	91.48 ±1.62 B	T3
16.733 ±0.32 D	11.487 ±0.025 D	69.859 ±0.98 F	T4
23.532 ±0.25 B	17.41 ±0.012 B	88.39 ±1.32 C	T5
1.01	1.56	4.01	LSD0.05

Histological changes in the liver

The histological sections of the liver in the control group (C) showed the natural form consisting of hepatic cells Hepatocytes with clear nuclei arranged in the form of monolithic lines with some and among them there are sinusoids and the clarity of Kupffer cells within the sinuses as in pictures (1,2). As for the group treated with triton (100 mg / kg) (positive control T1), histological examination showed that it has necrosis in the hepatocytes with the irregularity of the shape of the cells with the presence of a clear fatty degeneration resulting from the accumulation of fat inside the hepatocytes of the hepatocytes, which led the nucleus to Aspect and infiltration of inflammatory cells (ring shape) with severe bleeding, as in the pictures (3,4). Whereas, the histological sections of the liver in group T2 treated with routine (30 mg / kg) showed the normal form consisting of hepatic cells Hepatocytes with clear nuclei arranged in the form of monolithic lines with some and among them there are sinusoids and the clarity of Kupffer cells within the sinuses as in images (4,5). As for the T3 group that was treated with routine (30 mg / kg) and injected with triton (100 mg / kg) simultaneously, histological examination of it showed a little clear necrosis of hepatic cells with a little fatty degeneration

compared to the other treatment resulting from the accumulation of fat within the hepatocytes of the hepatocytes This led to the nucleus being pushed to the side and infiltration of the inflammatory cells with moderate hemorrhage, as in the pictures (6,7). As for the T4 group treated with triton (100 mg / kg) and then routine (30 mg / kg), histological examination showed that there was no strong necrosis in the liver cells with a slight fatty degeneration compared to the other treatment, resulting from the accumulation of fat inside the liver cells cytoplasm This led to the nucleus being pushed to the side and infiltration of the inflammatory cells as in the pictures (8,9). As for the T5 group that was treated with routine (30 mg / kg) and then triton (100 mg / kg), histological examination showed that it has necrosis in the hepatocytes with the irregularity of the shape of the cells with a fatty degeneration slightly resulting from the accumulation of fat inside the hepatocytes, which led to Push the nucleus to the side and infiltrate the inflammatory cells (ring shape) with moderate hemorrhage, as in the pictures (10,11).





IV. DISCUSSION

Rats are generally considered to be resistant to naturally occurring and experimentally-induced atherosclerosis [23]. High doses of dietary cholesterol combined with bile acids and experimentally induced hypolipidemia have been demonstrated to lead to the development of atherosclerotic lesions in rats [24]. Therefore, in the current study hyperlipidemia was induced in rats, evident from the total cholesterol level in serum after feeding the animals for 4 weeks. In this context, the serum lipid profile, liver enzymes, and tissue were investigated. According to the results, the effects of different doses of rutin on reducing serum total cholesterol .The effects of rutin groups .Diet for 4 weeks caused an increase in oxidative stress in the liver, resulted in an increase in AST and ALT levels, and induced fatty liver [25,20]. Therefore, in the present study, ALT and AST, apart from cholesterol, were also evaluated to reveal the protective effects of rutin on hepatic enzymes. As a result, it seems that supplementing a high-cholesterol diet with 30 mg/kg rutin could lower AST and ALT activities. As the liver weight : body weight ratio indicates, it is possible that animals that received rutin 30 mg/kg alone with their hyperlipidemia diets have less fat accumulation in the liver. In other studies, the influence of the flavonoids on the endogenous regulation of cholesterol biosynthesis has been discussed and antiatherosclerotic effects of flavonoids have been explained in several studies [6,27,28]. On the other hand, quercetin, one of the metabolites of rutin, lowered the serum lipid and hepatic cholesterol levels in high-cholesterol-fed rats. Quercetin also decreased HMGCoA reductase activity. It was suggested that supplementation of quercetin dihydrate and gallate promotes an increase in faecal sterols, which in turn leads to

a decreased absorption of dietary cholesterol as well as lowered serum and hepatic cholesterol [29,30]. Thus, it is possible that rutin, by converting to its active metabolite, decreased HMG-CoA reductase activity and decreased absorption of dietary cholesterol. Moreover, both rutin and quercetin have an antioxidant effect, and rutin showed clear synergism with quercetin in an study [31]. It has also been reported that quercetin and rutin could suppress lipid peroxidation in biological membrane systems such as mitochondria, erythrocytes, and others [32,33]. Thus, the other possibility for the hepatoprotective effects of rutin may be related to antioxidant activity to prevent liver injury. However, further studies are needed to clarify the exact mechanism. *in vitro* Another possible explanation for the decreased liver damage and lower liver enzymes for the 30 mg/kg rutin alone group with a high-cholesterol diet in animals depends on the anti-inflammatory effects of both drugs. Anti-inflammatory effects of rutin were found in several studies [34,35]. Furthermore, it is suggested that rutin has effective anti-inflammatory effects in the chronic model of inflammation [36]. Statins also have an anti-inflammatory effect that is independent of changes in the cholesterol level [37,38]. Furthermore, the neuroprotective effects of statins against a variety of CNS diseases have been studied [39,40].

In conclusion, our findings indicate that 30 mg/kg rutin alone or with other supplementation lowered liver weight as well as serum total cholesterol and LDL. The hepatic histopathological results reflect a correlation of rutin with both liver weight and the levels of serum total cholesterol and LDL-C. However, histopathological results show that liver injury in rats given 30 mg/kg rutin . Our data clearly indicate that the antihypercholesterolaemic effects of rutin in an animal model increased in combination therapy. It seems that several mechanisms may contribute to the hyperlipidemia effects of rutin.

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