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Original Article

Effect of Stevia Rebaudiana on the Attenuating Effect of Rosuvastatin Against Dexamethasone Induced Fatty Liver Disease in Rats

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Abstract

Background: Current researches are greatly interested in non-alcoholic fatty liver disease (NAFLD) treatment using medicinal plants. *Stevia rebaudiana Bertoni* is a natural non-calorie bio-sweetener belongs to Asteraceae family. In light of the association between metabolic diseases and NAFLD, researchers are attempting to evaluate the effectiveness of anti-hyperlipidemic, anti-obesity, and anti-diabetic medications to treat NAFLD. The ideal impacts of stevia on these disorders have been supported. This research was designed to investigate the rosuvastatin efficacy alone and combined with stevia to develop a prophylactic regimen for dexamethasone-induced NAFLD.

Methods: Thirty male albino rats were used: Group I: control rats. Group II: rats received dexamethasone. Group III: rats received rosuvastatin and dexamethasone. Group IV: Rats received stevia and dexamethasone. Group V: Rats received rosuvastatin, stevia and dexamethasone. Liver enzymes, triglycerides, total cholesterol, and adiponectin, malondialdehyde, glutathione peroxidase and TNF-α were measured. Histopathological alterations and PPAR-α expressions in the liver were evaluated.

Results: Dexamethasone caused an elevation in total cholesterol, triglycerides, malondialdehyde, liver enzymes, and TNF-α with a reduction in adiponectin and glutathione peroxidase levels. There was a reduction in the expression of PPAR-α. Both rosuvastatin and stevia caused an improvement in lipid profile, liver enzymes, oxidative stress, TNF-α, and adiponectin levels. The expression of PPAR-α was increased in all treated groups compared with the dexamethasone-treated group. Furthermore, stevia significantly enhanced the action of rosuvastatin.

Conclusions: Rosuvastatin alone or combined with stevia offered some protective effect against dexamethasone-induced NAFLD. This may be due to their anti-inflammatory, synergistic antioxidative, and lipid lowering effects

Keywords: Dexamethasone, NAFLD, Oxidative stress, PPAR-α, Rosuvastatin, Stevia rebaudiana, TNF- α.

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Introduction

One of the most prevalent chronic liver illnesses in the Western world is non-alcoholic fatty liver disease. [1] It can progress from steatosis to necrosis and inflammation. Some patients who have this condition may progress to fibrosis, cirrhosis, and liver failure. [2] Furthermore, evidence multiple lines have revealed a strong link between NAFLD and insulin resistance, obesity, metabolic syndrome, and visceral adiposity, all contributing to the cardiovascular disease and type 2 diabetes development. [3] The American Association for the liver diseases study offers several recommendations to manage NAFLD, including weight loss, vitamin E, insulin-sensitizing medications, fibrates, and statins, despite the fact that there is no authorized medical treatment for NAFLD. However, these medications have some side effects and there is no agreement on the best drug therapy. [4, 5] Dexamethasone is one of the strongest synthetic glucocorticoids that can cause NAFLD. [6]

Statins and other lipid-lowering medications have been demonstrated to quickly reduce liver inflammation, steatosis, and fibrosis thus improving NAFLD. [7] Rosuvastatin is a statin-derived drug which competitively inhibits 3-hydroxy-3-methylglutaryl-coenzyme A reductase (3-HMG-CoA reductase) that catalyzes the HMG-CoA conversion to mevalonate, the first rate-limiting step in cholesterol production in the liver[8]

In current years, herbal medicines have. gained popularity due to their potential impact on NAFLD prevention and treatment, as well as their effectiveness and minimal risk of side effects. [9] Stevia rebaudiana Bertoni is one of these herbs. It is a perennial shrub from Asteraceae family. [10] Stevioside, steviolbioside, isosteviol, rebaudiosides (A, B, C, D, E, F), and dulcoside A are among the eight sweet diterpene glycosides that are naturally found in the Stevia leaves. [11]

It has a number of biological actions and is not harmful. [12] Stevia glycosides have several biological effects such as, anti-diabetic, antioxidant, antimicrobial, antihypertensive, and anticancer effects. [4] However, the direct effects of RSV alone or combined with stevia on DEX-induced NAFLD are unclear. We aimed to determine the potential protective effect of RSV alone or combined with stevia on the hallmark features of NAFLD induced by DEX in rats.

Materials and Procedures

1. Chemicals and kits

DEX and RSV were graciously provided by Egyptian International Pharmaceutical Industries Company (EIPICO) in the form of powder. Carboxy methyl cellulose (CMC) was utilized as a solvent for RSV and stevia. CMC was supplied from Sigma Aldrich company for chemicals in Egypt. Tumor necrosis factor alpha (TNF-α) (CAT. NO. CSB-E11987r) enzyme linked immunosorbent assay kit was obtained from CUSABIO, China. Adiponectin (CAT. NO. 201-11-0759) ELISA kit was purchased from Sunred biotechnology, China. Malondialdehyde (MDA) (CAT. No. MD 25 29) and glutathione peroxidase (GPx) (CAT. No. GP 2524) kits were supplied from Biodiagnostic, Egypt. Total cholesterol (TC) (CAT. No. CH 12 20) and triglycerides (TG) (CAT. No. TR 20 30) kits were purchased from Biodiagnostic, Egypt. Alanine aminotransferase (CAT. No. 264 001) and aspartate aminotransferase (AST) (CAT. No. 260 001) kits were obtained from Spectrum Diagnostics, Egypt.

2. Extraction procedure of Stevia

During the flowering stage, Stevia rebaudiana leaves, family Asteraceae, were collected from a farm in Assiut, Egypt. To remove dust particles, the stevia leaves were cleaned with water then they were dried. 5 kg of air-dried, powdered stevia leaves were macerated in 100% methanol (10L) for 7 days with occasional stirring and shaking before being filtered. The filtrate containing solvent was dried using a rotary evaporator with a 40°–45°C temperature setting. The solvent-free residue weighted 835 g. The extraction was conducted in accordance with the method described by previous studies. [13-15]

3. Animals

Thirty adult male albino Wistar rats weighing 170 to 210 g were housed in the laboratory for one week. a controlled environment was provided with constant humidity (50%), room temperature (23 ± 4 °C) and 12-hour cycles of light and dark.

4. Experimental design

This study was conducted in 5 groups, every group included six rats. Group I: control rats that were administered 2 ml of 0.5% CMC solution orally. Group II: rats that were injected by DEX 8 mg/kg. Group III: Rats were administered the combination RSV 20 mg/kg + DEX. Group IV: Rats were administered with the combination stevia 400 mg/kg + DEX. Group V: Rats were administered with the combination RSV 20 mg/kg + stevia extract 400 mg/kg + DEX. RSV and stevia extract were given for 12 successive days but DEX was given in the last 6 days. According to previous studies, the doses of the agents used were calculated as follows: the dose of stevia extract was used according to Abdel-Aal et al. [16], RSV dose was used according to Fraulob et al. [17] and DEX dose was used according to Mathai et al. [18] DEX was injected intraperitoneally. Stevia extract and RSV were administered by stomach tube after being dissolved in freshly prepared 0.5% CMC.

5. Blood collection and preparation of serum

On the 13th day, all rats were fasted overnight for 12 hours. Diethyl ether 1.9% (0.08 ml / Liter of container volume) was used to anaesthetize the rats, then they were sacrificed. Cardiac puncture was performed to collect blood samples and then the blood was placed in pre-labeled centrifuge tubes. Fasting blood glucose (FBG) level was determined using the glucometer (Accu-Chek Performa, Germany). Blood was centrifuged at a speed of 3000 rpm for 10 minutes. Serum was rapidly preserved at -20°C till analysis and utilized for measurement of adiponectin, TC, TG, AST and ALT levels.

6. Tissue sampling

The liver of each rat rinsed with ice-cold saline after being dissected and then it was weighed and liver index was calculated. Specimens from ever liver were preserved in 10% formalin until immunohistochemical and histopathological analysis. Another sample from each liver was weighed separately and homogenized in an ice-cold potassium phosphate buffer (pH 7.4). After centrifugation of tissue homogenates (at 10000 rpm for 10 minutes), the supernatant that collected was kept at -80°C to be used for measurement of GPx, MDA and TNF-α levels.

7. Measurements of body weight (BW), liver weight and calculation of liver index

On the 13th day before scarification, all rats were individually weighed. ice-cold saline was used for rinsing the liver of each animal after its dissection, and then it was weighed. Liver index was tabulated based on this equation: Liver index (%) = liver weight (g)/BW (g)×100. [19]

8. Biochemical analysis

The TNF-α and ADP levels were calculated by ELISA Kit according to the method described by manufacturers.[20-22] GPx was estimated spectrophotometry in accordance with the instructions of manufactures. [23] MDA, AST, TG, ALT, and TC levels were determined by colorimetric method in the light of instructions of manufactures. [24-27]

9. Histopathological examination

Slices from hepatic tissues had been fixed in formalin solution (10%) for 24 hours so they could be processed, dehydrated with alcohol, cleared with xylene, infused and embedded in paraffin wax. Paraffin blocks were taken (5 um thickness) utilizing Leica RM 2125 microtome. Hematoxylin & Eosin (H&E) stain was used for the staining process to perform the general histological analysis. [28]

10. Protocol for immunohistochemical reaction (For detection of PRAP α)

For immunohistochemical detection of liver PPAR-α in rats, a primary polyclonal antibody of PPAR-α obtained from (Bioss antibodies, USA) and a two-step detection system goat anti-mouse/rabbit HRP including Peroxidase Block and

Chromogen DAB obtained from (quartett, Germany) were used. Deparaffinization and rehydration were done then antigen retrieval was performed by boiling in microwave oven in 10 mmol/l citrate buffer (pH 6.2) for two cycles, 3 min each. Endogenous peroxidase was blocked by 2% hydrogen peroxide for 5 min. The sections were incubated after dilution to 1:50 at 4°C overnight with the primary antibody. On the next morning, in a humid chamber, the sections were undergone biotinylated secondary antibody. Enzyme conjugate streptavidin was applied. **S**ubstrate
chromogen mixture was used to stain the slides and then counter-stained using Hematoxylin reagent. Negative control was done with the omission of primary antibody. Brown cytoplasmic deposits represented the positive reaction. According to previous research, the percentage of PPAR-α positive cells was statistically calculated. [29]

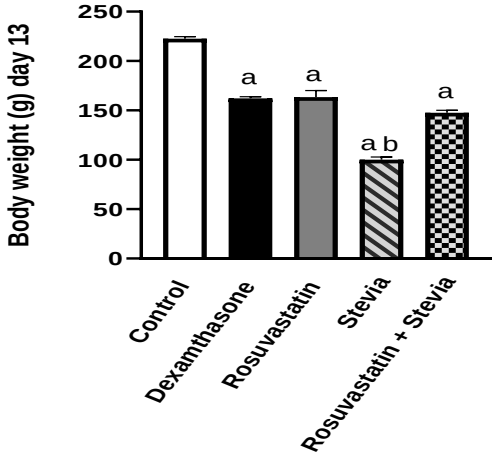
11. Statistical analysis

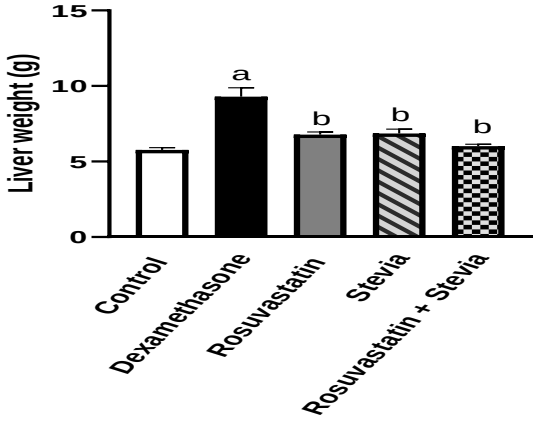
Data of 6 observations were displayed as mean ± SEM. One-way analysis of variance (ANOVA) was used to analyse the data among groups and Tukey's multiple comparison procedure was utilized to compare means between different groups. In order to conduct the analysis, Prism software was used (Graph-Pad Software Inc, version 8.0.2). The findings were deemed significant if $P < 0.05$.

Results

1. Effects of the tested agents on BW, liver weight and liver index

Treatment with DEX (8 mg/kg IP daily for 6 days), RSV (20 mg/kg) or stevia extract (400 mg/kg) alone or combined revealed a significantly reduced BW of the rats in comparison with the control group at $p < 0.01$ **(Fig. 1.a)**. DEX injection caused a statistically significant increase in the rats liver weight and liver index in comparison to the control group at $p < 0.01$. RSV group demonstrated a significantly reduced the rats liver index and liver weight compared with DEX treated group at $p < 0.01$. Combined administration of RSV and stevia revealed a significantly reduced liver weight and liver index index of the rats in comparison with DEX treated group at $p < 0.01$ **(Fig. 1.b and 1.c)**.





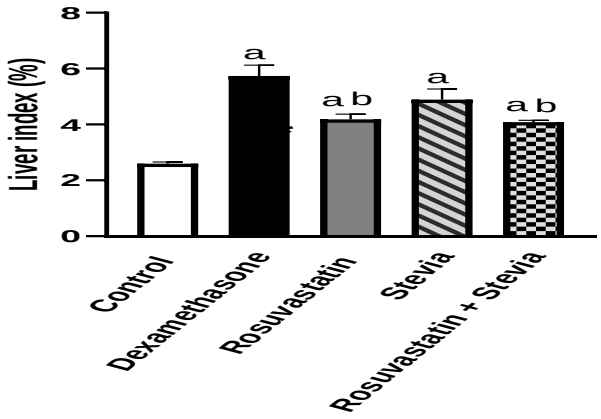


Fig. 1 Effect of the tested agents on BW, liver weight and liver index against DEX-induced NAFLD in rats. Data represent mean ± SEM of six observations. [a]Significant difference at p <0.01 vs. Control group. [b]Significant difference at p <0.01 vs. dexamethasone group

2.Effects of the tested agents on FBG level

FBG level was measured on the 13th day. Treatment with DEX caused statistically significant increase (p <0.01) in the FBG level of the rats compared to the control group. Rats treated with RSV and stevia alone or in combination revealed a significant reduction in the FBG level (p <0.0001) compared to DEX treated group **(Fig. 2)**.

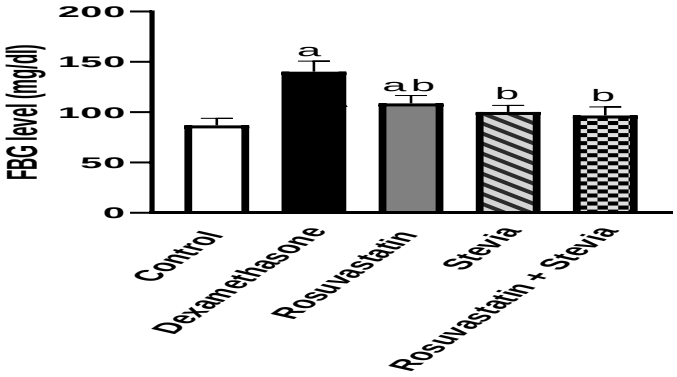


Fig.2 Effect of the tested agents on FBG level against dexamethasone-induced NAFLD in rats. Data represent mean ± SEM of six observations. [a]Significant difference at p <0.01 vs. Control group. [b]Significant difference at p <0.0001 vs. dexamethasone group

3. Effects of the tested agents on liver function tests (ALT and AST)

DEX injection significantly elevated the levels of AST and ALT of the rats in comparison with the control group at p <0.01. Oral administration of RSV and stevia alone and in combination with each other demonstrated a significant reduction in ALT and AST levels of rats (p <0.0001) compared with DEX treated group. The combination RSV + stevia significantly reduced the levels of ALT in comparison with the group treated with stevia alone at p <0.05 **(Table 1)**.

4. Effects of the tested agents on serum lipid profiles (TC and TGs)

Treatment with DEX significantly increased the TC and TG levels of the rats in comparison with the control group at p <0.01. Rats treated with RSV and stevia alone and combined with each other caused a significant decrease in the levels of TC and TG of the rats (p <0.0001) in comparison with DEX treated group. The

combination of RSV + Stevia significantly decreased the TG levels in comparison with RSV or stevia alone treated goups at p <0.05 **(Table 1)**.

Table 1: Effect of the tested agents on ALT, AST, AST/ALT, TC and TG levels level against dexamethasone-induced NAFLD in rats

	Control	Dexamethason	Rosuvastatin	Stevia	Rosuvastatin + Stevia
ALT (U/L)	18.17 ± 1.08	56.17 ± 2.54[a]	21.33 ± 1.54[bc]	28.17 ± 0.91[ab]	20.00 ± 1.57[bc]
AST (U/L)	14.67 ± 1.33	54.50 ± 2.84[a]	25.00 ± 2.27[ab]	28.67 ± 1.71[ab]	21.17 ± 1.60[ab]
AST/ALT	0.82 ± 0.08	0.98 ± 0.08	1.21 ± 0.14	0.99 ± 0.06	1.08 ± 0.12
TC (mg/dl)	88.50 ± 7.92	197.70 ± 13.17[a]	110.50 ± 6.47[b]	115.5 ±10.81[b]	84.17 ± 7.86[b]
TG (mg/dl)	61.95 ± 5.74	163.2 ± 11.72[a]	96.02 ± 4.90[ab]	101.8 ± 6.20[ab]	65.78 ± 5.21[bcd]

Data represent mean ± SEM of six observations. [a]Significant variation at p <0.01 vs. Control group. [b]Significant variation at p <0.0001 vs. DEX group. [c]Significant variation at p <0.05 vs. Stevia group. [d]Significant variation at p <0.05 vs. Rosuvastatin group.

5. Effects of the tested agents on malondialdehyde (MDA) and glutathione peroxidase (GPx) levels

Treatment with DEX significantly elevated MDA and reduced GPx of the rats in comparison with the control group at p <0.001. RSV and stevia alone and in combination with each other revealed a significant reduction in MDA with significant elevation in GPx levels of rats in comparison with DEX treated group at p <0.001 **(Fig. 3.a and 3.b)**. The combination RSV + stevia group caused a statistically significant reduction in MDA level in comparison to RSV or stevia alone treated groups (p <0.0001) **(Fig. 3.a)**

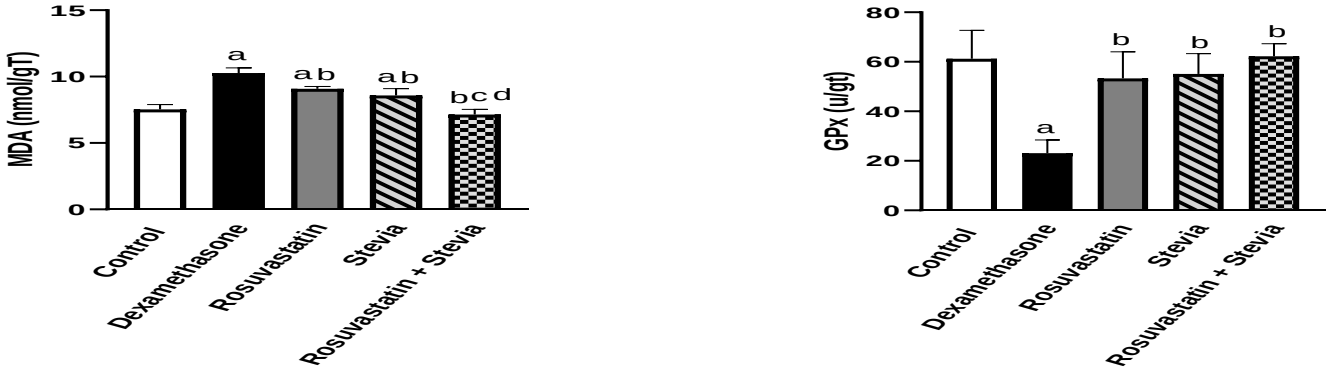


Fig.3 Effect of the tested agents on MDA and GPx levels against dexamethasone-induced NAFLD in rats. Data represent mean ± SEM of six observations. [a]Significant variation at p <0.001 vs. Control group. [b]Significant variation at p <0.001 vs. dexamethasone group. [c]Significant variation at p <0.0001 vs. Stevia group. [d]Significant variation at p <0.0001 vs. Rosuvastatin group.

6. Effects of the tested agents on serum adiponectin and liver homogenate TNF –α levels

Treatment with DEX significantly reduced the levels of adiponectin and increased the level of TNF-α of the rats in comparison with the control group at p <0.001. RSV and stevia alone and combined with each other demonstrated a significant increase in adiponectin level with decrease in the level of TNF-α in rats in

comparison with DEX treated group at p <0.0001 **(Fig. 4.a and 4.b)**. The results revealed highly significant elevation in adiponectin level in RSV and stevia + RSV treated groups in comparison with stevia treated group at p <0.001 **(Fig. 4.a)**. Addition of stevia extract to RSV group caused a high significant reduction in TNF-α level in comparison to the groups that treated with stevia or RSV alone (at p <0.001) **(Fig. 4.b)**

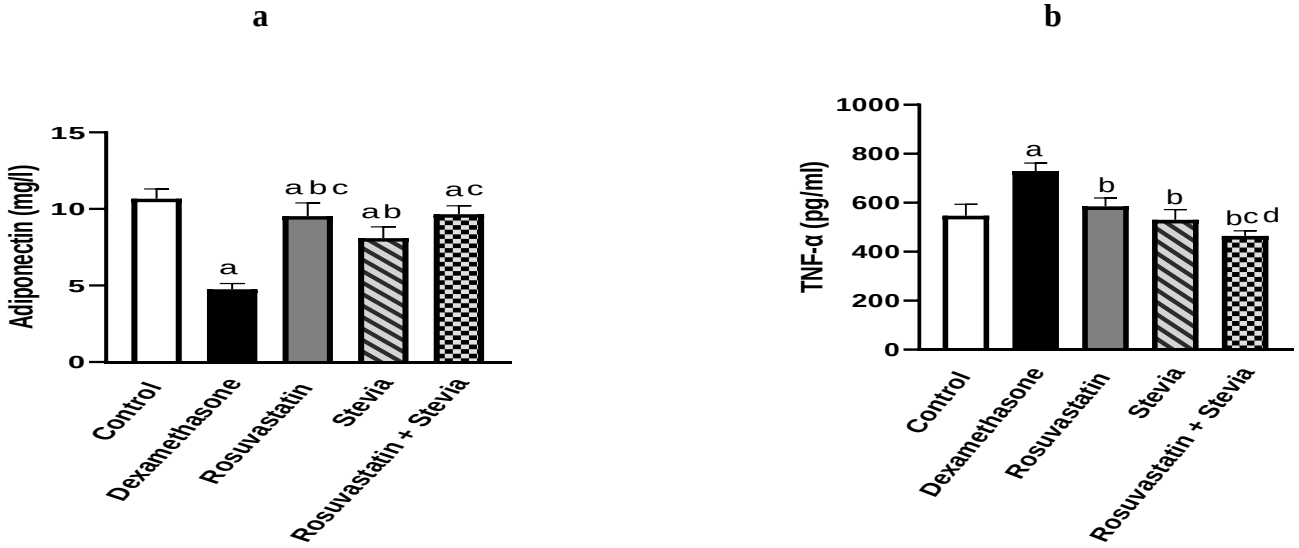


Fig.4 Effect of the tested agents on adiponectin and TNF-α levels against dexamethasone-induced NAFLD in rats. Data represent mean ± SEM of six observations. [a]Significant variation at p <0.001 vs. Control group. [b]Significant variation at p <0.0001 vs. dexamethasone group. [c]Significant variation at p <0.001 vs. Stevia group. [d]Significant variation at p <0.001 vs. Rosuvastatin group.

7. Histopathological examination

Examination of the sections stained with H&E of the control group showed normal hepatocytes **(Fig. 5A)**. Histopathological examination of DEX group revealed that most of hepatocytes with micro and macro steatotic changes. Some hepatocytes were apoptotic with dark acidophilic cytoplasm and dense nuclei while others were ballooned and necrotic. Inflammatory cell infiltration has been also observed **(Fig. 5B)**. Examination of RSV group showed an apparent decrease in steatotic changes compared to DEX group. Some hepatocytes appeared as the control group with homogenous cytoplasm **(Fig. 5C)**. Regarding to the group that treated with stevia extract, there were hepatocytes with vesicular nuclei and acidophilic cytoplasm. Other cells appeared with macro and microsteaosis. Few cells were enlarged, vacuolated with dense nuclei. No inflammatory cells were seen **(Fig. 5D**). Also, examination of the DEX group that was given with RSV + stevia extract revealed a marked reduction in the steatotic changes in comparison with DEX group. Most of hepatocytes appeared with vesicular nuclei and homogenous cytoplasm. Some binucleated cells were seen. Few hepatocytes appeared vacuolated and ballooned in addition to micro-steatosis in others **(Fig. 5E)**.

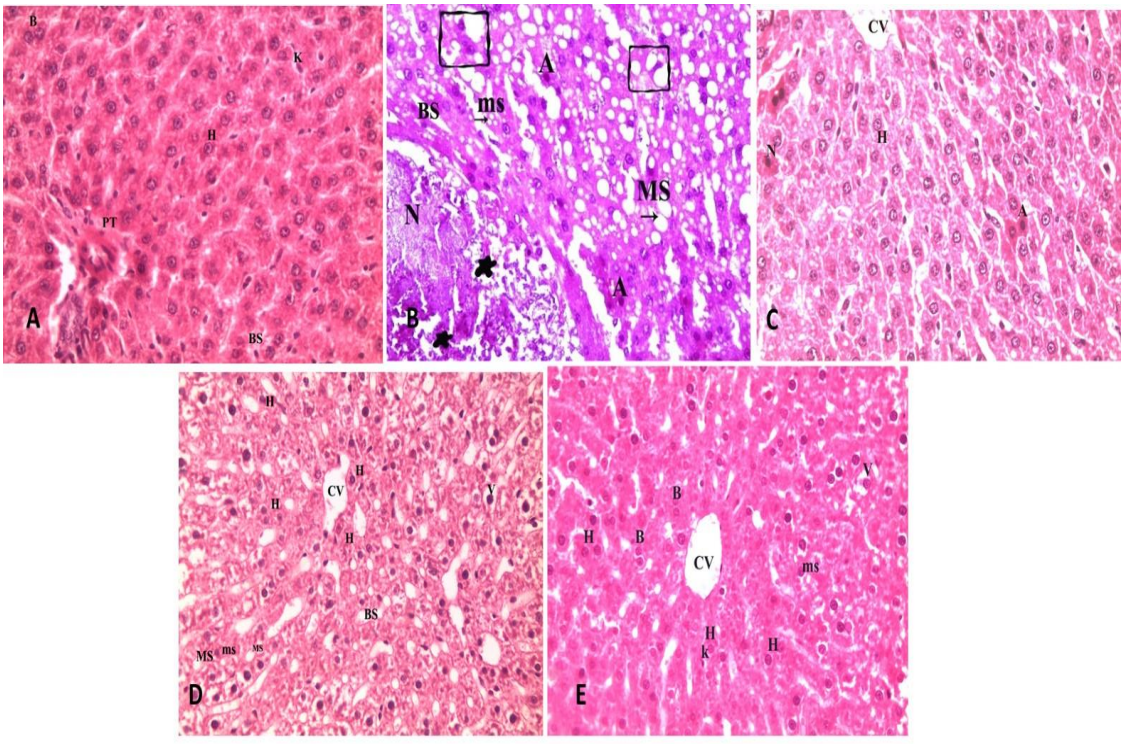


Fig. 5; Photomicrographs of H&E (x400) stained liver sections of: (A: control group) showing portal tract (PT), Kupffer cells (K), blood sinusoid (BS), binucleated hepatocytes (B) and Hepatocytes (H). (B: DEX group) showing macro and micro steatotic changes (MS, ms), necrotic hepatocytes (N), apoptotic hepatocytes (A), ballooned hepatocytes (box) and inflammatory cell infiltration (*). (C: RSV group) showing central vein (CV), negative hepatocytes (H), necrotic hepatocytes (N) and apoptotic hepatocytes (A). (D: Stevia group) showing binucleated hepatocytes (B), hepatocytes (H), blood sinusoids (BS), Kuppfer cell (K) and portal tract (PT). (E: RSV + Stevia group) showing central vein (CV), negative hepatocytes (H), binucleated hepatocytes (B), Kupffer cells (K), ballooned vacuolated hepatocytes (V) and micro-steatosis in some hepatocytes (ms).

8. Immunohistochemical results and statistical analysis

Immunohistochemical analysis of hepatic tissues of the control group revealed strong expression of PPAR-α protein (24.06 ± 0.53) **(Fig. 6A)**. DEX-treated group showed a significantly reduced number of positive PPAR-α immunostaining cells as well as the cytoplasmic area of positive reactions (13.32 ± 0.27) compared to the negative control group at ($p < 0.05$) **(Fig. 6B)**. Examination of RSV treated group revealed significant increase in the percentage of the area of positive PPAR-α immunostaining hepatocytes (20.84 ± 0.34) compared to DEX group at ($p < 0.05$) **(Fig. 6C)**. Regarding to the group that treated with stevia, a significant elevation in the percentage of the area of positive PPAR-α immunostaining reaction was reported (22.22 ± 0.24) at ($p < 0.05$) in comparison with DEX group **(Fig. 6D)**. Immunohistochemical examination of the group that administered RSV and stevia extract revealed significant elevation in the percentage of the positive reaction area (23.18 ± 0.15) compared to DEX treated group at ($p < 0.05$) **(Fig. 6E).**

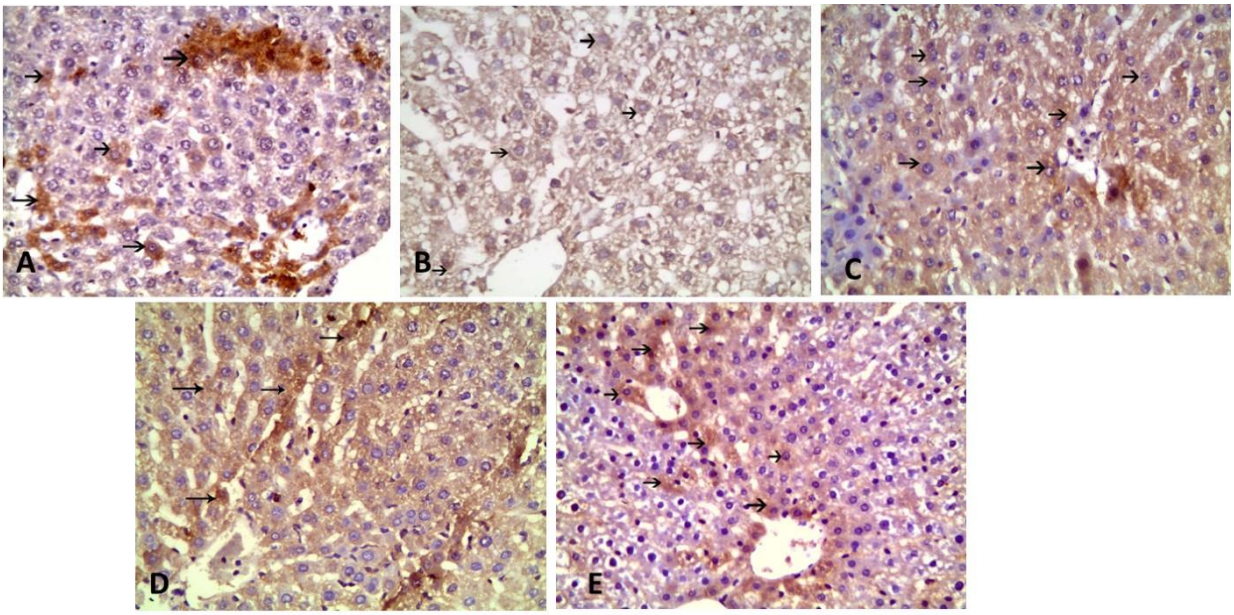


Fig. 6: PPAR- α immunohistochemical photomicrographs of stained liver sections of: (A: Control group) showing numerous positive cells (Strong PPAR-α expression) with brown cytoplasmic coloration (arrow). (B: DEX group) showing few positive PPAR-α immunostaining cells compared to the control group (arrow). (C: RSV group, D: Stevia group and E: RSV + Stevia group) showing numerous positive hepatocytes compared to DEX group (arrow). (Anti PPAR- α immunostaining x 400).

Discussion

All stages of the pathophysiology of NAFLD have been linked to glucocorticoids. [30] DEX is among the most powerful synthetic glucocorticoids with minimal or no mineralocorticoid action. [31-33] The data revealed a significant elevation in the FBG level, liver weight and liver index with a decrease in the BW of rats that administered DEX in comparison with the control group, Mathai et al. who examined the effect of pioglitazone with sitagliptin on DEX-induced NAFLD in albino rats reported similar results. [18] The reason for the reduction in the BW that caused by DEX is due to various factors involving inhibition of the synthesis of muscle protein, increased the catabolism of protein and energy expenditure. [34] Contradirectory to the results, it was reported that intracerebroventricular infusion of DEX resulted in an increase in BW whereas intraperitoneal infusion of DEX caused a reduction in BW than the control group. [35] The explanation for DEX-induced insulin resistance is that DEX may induce hepatic gluconeogenesis, inhibit hepatic glucose oxidation and inhibit hepatic hexokinase activity. [36]

Circulating liver enzymes; especially, ALT and AST, are sensitive liver injury biomarkers as they are released during the damage of the hepatocyte membranes into the blood stream. [37] The data of this research revealed a high significant elevation in the serum concentrations of ALT and AST in DEX model group than the control group, Hasona et al. reported similar findings. [38] With respect to the markers of hyperlipidemia, DEX administration caused significant elevation in the serum TC and TG levels than the control group. Mahmoud et al. who studied coriander oil effect on DEX-induced insulin resistance in rats reported similar findings. [39] The increased levels of lipids are related to reduced insulin sensitivity in tissues, primarily the liver, as well as reduced TG hydrolysis caused by reduced lipoprotein lipase activity. [40]

An imbalance between the production of reactive oxygen species (ROS) and the antioxidant system's capacity to scavenge them results in oxidative stress. Increased hepatic lipids cause oxidant overproduction through affecting several mechanisms of ROS-generation. At high levels,

ROS cause oxidative changes to cellular macromolecules (proteins, lipids, DNA, etc.) resulting in the damaged macromolecules accumulation and causing hepatic damage. [41] The activity of GPx is believed to be the primary protective reaction necessary for regulating H_2O_2 concentrations during physiological settings and after oxidative damage. [42] The findings of this research demonstrated an elevation in the level of MDA in liver homogenate in DEX-treated group than the control group, and [39] reported similar findings. Furthermore, DEX injection resulted in a decrease in GPx level, and Lv et al. reported similar findings as they showed that the administration of DEX lowered glutathione peroxidase levels in broiler liver. [43]

Adiponectin, is a hormone that generated from adipose tissue. It has several actions such as anti-diabetic, anti-inflammatory, insulin-sensitization, and anti-atherogenic. [44] According to a previous research, adiponectin stimulate glucose uptake in skeletal muscle, decreases hepatic gluconeogenesis and enhances oxidation of fatty acid in liver and skeletal muscles. [45] The findings of this study showed that DEX administration decreased serum adiponectin level, that were consistent with the results of a previous research which was done to examine the effect of DEX injection in rats treated with fatty die. [46]

Inflammation is a key element in the pathophysiology of fatty liver disease. [47] When the liver is injured or inflamed, the hepatocyte itself and the inflammatory cells release cytokines such ROS and TNF-α. [48] TNF-α has been linked to insulin resistance development, obesity and T2DM acting via immune and inflammatory processes. [49] The findings revealed that treatment with DEX elevated TNF-α level and this was in harmony with a previous study which was performed to assess the crocetin's effect on rats with insulin resistance that was induced by DEX. [50] Also, similar findings were obtained in a recent research that was done to investigate the effect of Didymin (active component in Mentha spicata) on DEX and high-fat diet induced NAFLD in mice. Compared to the control group, TNF-α level in hepatic tissues was elevated significantly in DEX and high-fat diet group. [47] Conversely, it was reported that DEX might maintain the equilibrium between the inflammatory and anti-inflammatory responses by increasing the anti-inflammatory factor (IL-10) expression and decreasing the expression inflammatory factor levels (TNF- α) in serum. [51]

All the previous findings were confirmed with immunohistochemical and histopathological results where the hepatic tissues in DEX-treated group showed steatosis, inflammation, apoptosis and necrosis. These results were in harmony with a recent research published by Ahmed et al. [52] Steatosis is important in the occurrence and NAFLD development which can be triggered by dysregulation of PPAR-α. PPAR-α is an important regulator of lipid metabolism in the liver. Overexpression of PPAR-α promotes fatty acids uptake, utilization, and catabolism. [53] PPAR-α is mainly over expressed in tissues with high capacity to fatty acid oxidation, including heart, liver, and skeletal muscles. [54]

Interestingly, increased expression of glucocorticoid receptors in hepatic tissues in mice decreased PPAR-α protein on a normal diet. [55] Immunohistochemical examination of PPAR-α in the liver tissues of DEX-treated group in this research revealed a significant reduction in positive PPAR-α immunostaining cells than the control group, Sun et al reported similar findings [56] Moreover, It was found that the expression. of mRNA of PPAR-α was inhibited by prenatal administration of DEX. [57]

RSV has been shown to have significant organ protective properties as well as many pharmacological effects including antioxidative and anti-inflammatory properties. RSV decreases oxidative stress via mediating several antioxidant effects, such as suppression of uncoupling of endothelial nitric oxide synthase, decreased NADPH oxidase, inhibition of hydrogen peroxide-induced DNA damage and increased antioxidant enzymatic defense mechanisms. [58]

The current research findings revealed that daily oral administration of RSV in DEX treated group significantly reduced the BW than the control rats. These were similar to the results that were reported by Al-Kuraishy & Al-Gareeb, [59] Furthermore,

administration of RSV significantly reduced liver index and liver weight in comparison with DEX group similar to the findings of El-Din et al. [60] RSV significantly reduced the level of FBG, Salunkhe et al. reported similar findings. [61]

The current research findings revealed that administration of RSV significantly reduced the levels of liver enzymes (ALT and AST). Similarly, Rajangam et al. explored the potential protective RSV effect against doxorubicin induced cardiac toxicity in rats and they reported similar results. [58] Moreover, treatment with RSV significantly reduced the levels of TG and TC. This was in harmony with the study of Fahmy et al. who found that, RSV can decrease TG by decreasing very low-density lipoprotein synthesis and by elevating its clearance. [62]

The present study reported that pretreatment with RSV minimized significantly the increase in MDA, Sultan et al. reported similar findings. [63] Furthermore, the results revealed that administration of RSV caused an increase in GPx level; and this agreed with the trial of Abdullah et al**.** [64]

Data from the present investigation noted that daily oral administration of RSV increased adiponectin level significantly compared to DEX group and this was similar to the research that was published by similar findings of El-Din et al. [60] However**,** De las Heras et al., and Valero-Muñoz et al. reported that the levels of adiponectin was not modified by RSV in treated HFD rats. [65, 66]. Furthermore, oral administration of RSV reduced the level of TNF-α and this was similar to the findings of Garg et al**.** who described that inflammatory measures levels, IL-6, TNF-α were significantly reduced after treatment with RSV. [67]

Conversely, De las Heras et al. reported that RSV administration did not affect the increased TNF-α in adipose tissue expression of the HFD rats. [65]

The study that reported by Ramadan et al. demonstrated that RSV treatment could improve the degenerative changes and lipid deposition in hepatocytes induced by NAFLD as it has anti-oxidant and anti-inflammatory effects. [68] These findings were similar to the histopathological results of the current research that reported an apparent decrease in steatotic changes compared to DEX treated group. Some hepatocyte appeared as the control group with homogenous cytoplasm. Furthermore, immunohistochemical examination of PPAR-α in the liver of RSV treated group found a statistically significant elevation in the area percentage of positive immunostaining PPAR-α in hepatocytes compared to DEX treated group, Marinho et al, reported similar findings. [69]

A previous study found that fat deposition in liver tissues can be stimulated by statins, and after stopping high doses of statins. As a result, in order to avoid adverse effects and improve statin efficacy, interventions of natural products and statins have gained popularity. [70]

Herbal medicines have had significant beneficial effects on the reduction of inflammation and steatosis when used to treat NAFLD. [71] Stevia rebaudiana is a type of medicinal herbs. [4] Stevia extract, that has secondary metabolites including stevia glycosides (stevioside, rebaudioside A and rebaudioside C) and polyphenols, has several biological effects that improve health. [54]

Administration of stevia extract alone or combined with RSV significantly reduced in the BW of rats, Abdel-Aal et al. reported similar findings. [16] It was reported that the reduced BW caused by stevia extract is due to the ability of stevia in reducing the food intake as it is low-caloric sweetener which may not elevate calorie intake and don't stimulate appetite. [72] Our research revealed that oral administration of stevia extract alone or in combination with RSV significantly reduced the level of FBG in comparison to DEX-treated group. This was similar to the results that reported by Akbarzadeh et al. [73] The suggested mechanisms of stevia for decreasing blood glucose levels is an improvement in glucose metabolism, bile acid metabolism, fat catabolism, storage and transport of lipids in the obese mice hepatic tissues with insulin resistant. [74]

The findings of the current research revealed that administration of stevia extract alone or combined with RSV significantly decreased the hepatic enzymes (AST and ALT) in the serum, and this was similar to the trial of Latha et al. [75] The improvement of liver damage caused by stevia extract was mediated by lowering elevated hepatic

enzymes, controlling hyperglycemia, having an antioxidant effect and improving insulin resistance. [76] Stevia extract also significantly reduced the TC and TG levels in comparison with DEX-group that were similar to the finding of Ahmad et al. [72] Furthermore, the antihyperlipidemic effect of stevia may be due to an interaction between the ingestion of stevia extract and PPARs activation. PPARs are regulatory factors in the lipogenesis process. They increase of the apo C-II genes and lipoprotein lipase expression, in addition to the etherification and hepatic uptake of free fatty acids as well as enhancing mitochondrial free fatty acid oxidation. [73]

Stevia leaves were found to have antioxidant properties including scavenging of free radicals and lipid peroxidation inhibition. [77] Multiple experimental trials revealed that the antioxidant effect of stevia extract is due to various amounts of total phenols and flavonoids contained in the plant. [75, 78] Stevia contains stevioside that can increase antioxidant effect by enhancing expression of nuclear erythroid factor 2 (Nrf 2). The mechanism of action of stevioside is that, it has the ability to inhibit beta-adrenergic and G-protein-coupled receptor kinases. [79] Administration of stevia extract alone or in combination with RSV demonstrated a significant reduction in MDA level with an elevation in the level of serum adiponectin compared to DEX group, similar results reported by Assi et al. [76] Additionally, Stevia extract administration significantly elevated GPx level compared to DEX-group, this agreed with the results of Singh et al. [80] They revealed that stevia extract plays a compensatory role in reducing H_2O_2 formation, thereby reducing the toxic effects of free radicals produced by it in multiple secondary reactions. Stevia extract has important anti-inflammatory effects as when it added to RSV caused significant reduction in TNF-α, these findings were in harmony with Potočnjak et al. [81] The stevia extract anti-inflammatory effects are due to steviol and stevioside. Stevioside can inhibit the over expressions of genes involved in liver inflammation in vitro. [75]

All the previous findings were confirmed with immunohistochemical and histopathological findings where administration of RSV alone or in combination with stevia extract resolved the severe damages and fatty liver tissue degeneration that was caused by the administration of DEX. There was a marked decrease in the steatotic changes, vacuolated and ballooned hepatocytes compared to DEX treated group. Immunohistochemical examination of PPAR-α in the hepatic tissues of stevia revealed an apparent elevation of positive PPAR-α immunostaining reactions compared with DEX-treated group. This was in harmony with the study of Park et al. who evaluated the effects of stevioside and stevia on hepatic steatosis in db/db mice. [54] Activation of PPAR-α by stevia has been documented and this characteristic was discovered as a potential mechanism of the hypotriglyceridemic action of stevia. [73]

Conclusion

In the light of findings; DEX can cause toxic effects on liver tissues, RSV and stevia extract may protect against NAFLD induced by DEX. Accordingly, in this investigation, the combined administration of RSV and stevia extract modulated the biochemical and histological markers induced by DEX more effectively than either medication alone. Their synergistic anti-inflammatory, antioxidative, and lipid-lowering effects may account for these results.

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