



## THE POTENTIAL PROTECTIVE EFFECT OF STEVIA REBAUDIANA ON DEXAMETHASONE-INDUCED NONALCOHOLIC FATTY LIVER DISEASE IN RATS: ROLE OF PPAR- $\alpha$

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**Background:** Currently, using medicinal herbs has a great interest in the treatment of nonalcoholic fatty liver disease (NAFLD). This study evaluated the impact of stevia on the hallmark signs of dexamethasone-induced NAFLD in rats. **Methods:** Group I: Negative control rats received 2 ml of vehicle carboxy methyl cellulose (CMC). Group II: Rats received dexamethasone 8 mg/kg/day intraperitoneal for 6 days. Group III: Rats received oral stevia extract 400 mg/kg/day for 12 days. Group IV: Rats received dexamethasone + stevia extract. Serum ALT, AST, TC, TG and adiponectin were measured. MDA, GPx and TNF- $\alpha$  were estimated in liver homogenate. Histopathological changes and PPAR- $\alpha$  expressions in the liver were evaluated. **Results:** Dexamethasone caused a reduction in GPx and ADP levels with an elevation in ALT, AST, TC, TG, MDA and TNF- $\alpha$ . There was a decrease in immunostaining expression of liver PPAR- $\alpha$ . Stevia caused an improvement in liver enzymes, lipid profile, oxidative stress, TNF- $\alpha$  and adiponectin levels. **Conclusion:** Stevia provided some protection against dexamethasone-induced NAFLD.

**Keywords:** Dexamethasone, NAFLD, PPAR- $\alpha$  and Stevia

### INTRODUCTION

The most prevalent liver disease in the world is nonalcoholic fatty liver disease (NAFLD) that affects up to 25% of adult population<sup>1</sup>. Furthermore, several lines of evidence have shown a strong link between NAFLD and obesity, visceral adiposity, insulin resistance and metabolic syndrome which in turn leads to development of cardiovascular diseases and type 2 diabetes<sup>1-3</sup>. The lack of a specific pharmacological treatment behind this investigation for seeking a novel therapeutic approaches that could efficiently and safely control NAFLD<sup>4</sup>.

Some drugs, including dexamethasone (DEX), have been linked to NAFLD<sup>5</sup>. In clinical practice, glucocorticoids like dexamethasone are frequently utilized and over 1.5 million postmenopausal women and men over 50 years in the United States are thought to be receiving glucocorticoid therapy, with the great majority receiving long-term treatment. Numerous undesirable side effects as hypertension, glucose intolerance, increased susceptibility to infections, dyslipidemia and excessive deposition of lipid in the liver leading to non-alcoholic fatty liver disease, can emerge from the hormone's excessive and chronic use. Other negative effects include psychological disorders and osteoporosis.

Excessive short-term usage of dexamethasone can cause a number of metabolic difficulties as insulin resistance in the liver and peripheral tissues, hyperinsulinemia, hyperglycemia and high plasma triglyceride levels<sup>6</sup>. Increased fatty acid synthesis and decreased fatty acid  $\beta$ -oxidation are two ways by which glucocorticoids produce fatty liver<sup>7</sup>.

Over the past ten years, herbal medicines have gained increased attention due to their potential impact on NAFLD prevention and therapy as well as their efficiency and minimal risk of side effects<sup>8</sup>. The herbal plant "Stevia rebaudiana" is one of these. Steviol glycosides are the active components of stevia and include stevioside, rebaudioside (A, B, C, D, E, F and M), dulcoside A, dulcoside C and steviolbioside. These glycosides are 150–300 times sweeter than sugar. Stevioside and rebaudioside A are the two most prevalent sweetening molecules<sup>9</sup>. According to studies, stevia has important pharmacological effects including anti-diabetic, anti-obesity, anti-tumor, anti-microbial, anti-caries, anti-hypertensive and antioxidant effects<sup>10</sup>.

According to a report, NAFLD frequently coexists with diabetes, insulin resistance, obesity and dyslipidemia<sup>11</sup>. Researchers are attempting to assess anti-hyperlipidemic, anti-diabetic, and anti-obesity medications to treat NAFLD in light of the correlation between metabolic diseases and NAFLD. The ideal impacts of stevia on these disorders have been supported<sup>12,13</sup>. However, there is still some uncertainty regarding the direct effects of stevia on NAFLD stimulated by dexamethasone. This research investigated the potential effect of stevia in alleviating the hallmark features of dexamethasone-induced NAFLD in rats.

## MATERIALS USED AND METHODS

### *Chemicals and kits*

Dexamethasone was kindly donated in the form of powder from Egyptian International Pharmaceutical Industries Company (EIPICO). Carboxy methyl cellulose (CMC) was supplied from Sigma Aldrich company in Egypt.

Adiponectin (ADP) ELISA Kit (CAT. NO. 201-11-0759) was obtained from Sunred biotechnology, China. Tumor necrosis factor alpha (TNF- $\alpha$ ) ELISA Kit (CAT. NO. CSB-

E11987r) was purchased from CUSABIO, China. Glutathione peroxidase (GPx) (CAT. No. GP 2524) and malondialdehyde (MDA) (CAT. No. MD 25 29) kits were obtained from Biodiagnostic, Egypt.

Triglycerides (TG) (CAT. No. TR 20 30) and total cholesterol (TC) Kits (CAT. No. CH 12 20) were obtained from Biodiagnostic, Egypt. Aspartate aminotransferase (AST) (CAT. No. 260 001) and alanine aminotransferase (ALT) Kits (CAT. No. 264 001) were supplied from *Spectrum* Diagnostics, Egypt.

### *Plant materials*

*Stevia rebaudiana* (Asteraceae) leaves were collected from a farm in Assiut, Egypt, during the flowering stage. The stevia leaves were washed with water and dried to get rid of dust particles.

### *Extraction procedures*

400 g of powdered, air-dried stevia leaves were macerated in 4 L of 100% methanol for 7 days while being sometimes stirred and shaken and then the mixture was filtered. A rotary evaporator with a 40°–45°C temperature setting was used to dry the filtrate that included solvent. The residual free of solvent weighed 75 g. The extraction was done using the technique outlined by previous studies<sup>14-16</sup> at Sohag University's Department of Pharmacognosy, Faculty of Pharmacy.

### *Animals*

Adult male albino Wistar rats (weighing 170–210 g) were bought from the animal house, Faculty of Medicine, Sohag University, Egypt. Prior to testing, animals were kept in the lab room for a week. They were housed in a controlled environment (12 hrs. cycles of light and dark, constant humidity (50%) and room temperature (23  $\pm$  4 °C). Animals were given a conventional pellet diet and unlimited access to water. The work procedure has received a permission from Sohag University's Institutional Animal Care and Use Committee (approval code: 12-7-2022-01).

### *Experimental design*

This study was conducted in 4 groups of rats. Each group contained 6 animals. Group I: Negative control rats that received 2 ml of

0.5% CMC solution orally by gavage for 12 days. Group II: Rats received dexamethasone (DEX) 8 mg/kg/day for 6 days and used as positive control group. Group III: Rats received stevia extract (S) 400 mg/kg/day for 12 days. Group IV: Rats received stevia extract 400 mg/kg/day for 12 successive days and dexamethasone 8 mg/kg/day was added in the last 6 day.

The doses of the used compounds were calculated according to the previous studies as the following: The dose of dexamethasone was calculated in accordance with Mathai et al., 2015<sup>17</sup> and the dose of stevia (S) was calculated in accordance with Abdel-Aal et al., 2021<sup>12</sup>. Dexamethasone was given by intraperitoneal injection and was dissolved in saline. Stevia extract was dissolved in freshly made 0.5% CMC and then gavaged orally.

#### *Blood collection and serum preparation*

On the 13th day, all rats (were fasted overnight for 12 hours), sacrificed under anesthesia using diethyl ether 1.9% (0.08 ml / Liter of container volume) and blood samples were taken via cardiac puncture using 5 ml disposable syringe according to a previous research<sup>18</sup> and placed in pre-labeled centrifuge tubes. All rats Blood sugar level was determined using glucometer (Accu-Chek Performa, Germany). After blood centrifugation for 5 minutes at a speed of 5300 rpm, the serum was separated. It was quickly stored at -20°C until analysis and was used to measure ADP, ALT, AST, TC and TG levels.

#### *Tissue sampling*

After dissection of the liver, it was weighed and cleaned with ice-cold saline. Samples from each liver were kept in formalin 10% until they were ready for immunohistochemical and histopathological testing. Other specimens were taken from each liver weighed and homogenized in ice-cold potassium phosphate buffer (pH 7.4). For 10 minutes, the homogenates were centrifuged at 4000 rpm. The supernatant was taken off and kept at -80°C for use in measuring TNF- $\alpha$ , MDA and GPx levels.

#### *Biochemical analysis*

Adiponectin and TNF- $\alpha$  levels were estimated by enzyme linked immunosorbent assay Kit in accordance with instructions of

manufactures<sup>19-21</sup>. GPx was measured spectrophotometry according to the method described by manufacturers<sup>22</sup>. MDA, TG, TC, AST and ALT levels were measured by colorimetric method according to instructions of manufactures<sup>23-26</sup>.

#### *Histopathological examination*

After being fixed in formalin saline (10%) for 24 hours, specimens from each liver were trimmed for processing, dehydration with alcohol, clearing with xylene, infiltration and embedding in paraffin wax. For general histological study, slices were sectioned (5  $\mu$ m thickness) with a microtome (Leica RM 2125) and stained with Hematoxylin and Eosin<sup>27</sup>.

#### *Protocol for immunohistochemical reaction (For detection of PPAR- $\alpha$ ):*

Liver PPAR- $\alpha$  in each rat was detected via immunohistochemistry according to the instructions of manufacture using a primary polyclonal PPAR- $\alpha$  antibody from (Bioss antibodies, USA) and a two-step detection method using goat anti-mouse/rabbit HRP including chromogen DAB and peroxidase block purchased from (Quartett, Germany). Deparaffinization, rehydration and antigen retrieval were performed by boiling in 10 mmol/l citrate buffer (pH 6.2) in microwave oven for two cycles, 3 min each. After that, blocking of the endogenous peroxidase by 2% hydrogen peroxide for 5 min was performed. Then incubation overnight at 4 degrees refrigerator with the primary antibody after dilution to 1:50. On the next morning, the sections were subjected to biotinylated secondary antibody in a humid chamber. Then enzyme conjugate streptavidin was applied. The slides were stained by substrate-chromogen mixture and then counter stained using Hematoxylin reagent. The slides were rinsed in distilled water and dehydrated in ascending grades of alcohol. Finally, clearing and mounting with covers lip were done. Negative control was done with omission of primary antibody and positive reaction appeared as brown cytoplasmic deposits. The percentage of positive cells of PPAR- $\alpha$  was statistically calculated according to a previous research<sup>28</sup>.

### Statistical analysis

The results were represented as the mean  $\pm$  SE of six observations. The data were compared between groups by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison method to compare means between different groups. If  $P < 0.05$  the results were regarded as significant. Prism software program was used for the analysis (Graph-Pad Software Inc, version 8.0.2).

## RESULTS AND DISCUSSION

### Effect of the tested compounds on body weight and liver weight

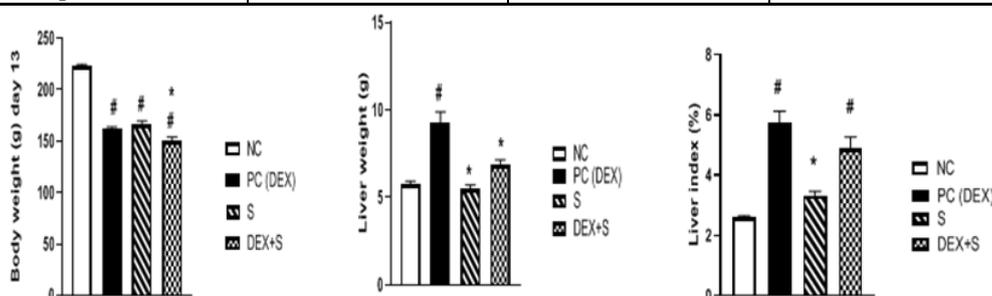
Treatment with dexamethasone (8 mg/kg IP daily for 6 days) caused a significant reduction in the body weight of the rats compared to the negative control group at  $p < 0.001$ . Also, treatment with stevia extract (400 mg/kg daily for 12 days) alone or in combination with dexamethasone (8 mg/kg IP daily for the last 6 days) caused a significant decrease in the body weight of the rats compared to the negative control group at  $p$

$< 0.05$ . The reduction in body weight was significantly higher in the group that treated by stevia extract and dexamethasone at  $p < 0.05$  compared to DEX-treated group as shown in **Fig 1**.

In DEX-treated rats, there was significant elevation in liver weight and liver index compared to the negative control group at  $p < 0.001$ . Rats treated with stevia extract alone or in combination with dexamethasone caused a significant decrease in the liver weight of rats compared to positive control group at  $p < 0.05$ . There was no significant difference in liver weight between the group that treated with stevia extract only and negative control group. Rats treated with the combination stevia extract plus dexamethasone caused a significant increase in the liver index of rats compared to negative control group at  $p < 0.001$  but rats treated with stevia extract alone caused a significant reduction in liver index compared to dexamethasone group at  $p < 0.05$  as shown in **Table 1** and **Fig 1**.

**Table 1:** Effect of daily I.P injection of dexamethasone (DEX) 8 mg/kg for 6 days, oral administration of stevia extract (S) alone 400 mg/kg for successive 12 days and the combination stevia extract 400 mg/kg orally for 12 successive days and dexamethasone that was added in the last 6 days on body weight, liver weight and liver index in dexamethasone induced fatty liver disease in rats. Negative control group received 2 ml of 0.5% CMC solution. Data represent mean  $\pm$  SE of six observations. # Significant difference at  $p < 0.001$  vs. NC group. \*Significant difference at  $p < 0.05$  vs. PC (DEX) group. NC: negative control, PC; positive control. CMC: Carboxymethyl cellulose.

Groups	Body weight (g)	Liver weight (g)	Liver index (%)
Group I NC	222.5 $\pm$ 2.25	5.78 $\pm$ 0.14	2.6 $\pm$ 0.06
Group II PC (DEX)	162.2 $\pm$ 1.78 <sup>#</sup>	9.3 $\pm$ 0.58 <sup>#</sup>	5.47 $\pm$ 0.38 <sup>#</sup>
Group III S	166.5 $\pm$ 2.92 <sup>#</sup>	5.5 $\pm$ 0.19*	3.32 $\pm$ 0.16*
Group IV DEX+S	150.3 $\pm$ 3.59 <sup>#*</sup>	6.9 $\pm$ 0.29*	4.89 $\pm$ 0.38 <sup>#</sup>



**Fig.1:** Effect of daily I.P injection of dexamethasone (DEX) 8 mg/kg for 6 days, oral administration of stevia extract (S) alone 400 mg/kg for 12 days and the combination stevia extract 400 mg/kg orally for 12 successive days and dexamethasone that was added in the last 6 days on body weight, liver weight and liver index in dexamethasone induced fatty liver disease in rats. Negative control group received 2 ml of 0.5% CMC solution. Data represent mean  $\pm$  SE of six observations. # Significant difference at  $p < 0.001$  vs. NC group. \*Significant difference at  $p < 0.05$  vs. PC (DEX) group. NC: negative control, PC; positive control. CMC: Carboxymethyl cellulose.

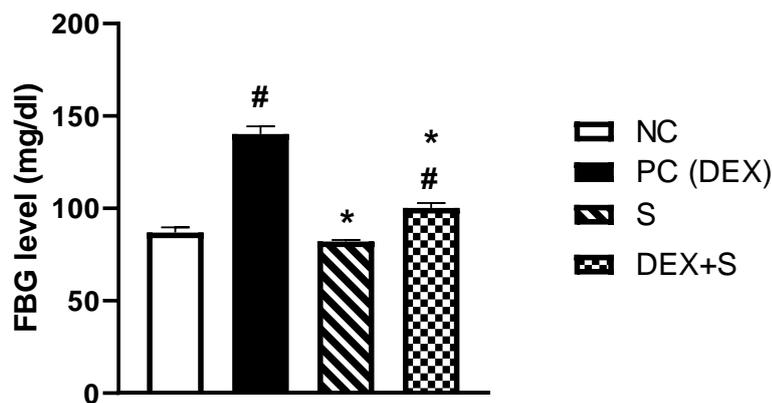
*Effect of the tested compounds on the level of fasting blood glucose (FBG)*

At the end of the experiment (on day 13), FBG level was determined. Treatment with dexamethasone caused a significant increase in FBG level of the rats compared to the negative control group at  $p < 0.05$ . Rats treated with stevia extract either alone or in combination with dexamethasone revealed a significant decrease in FBG level compared to dexamethasone treated group at  $p < 0.0001$  as shown in **Fig 2**. There was no significant difference in FBG level in the group that

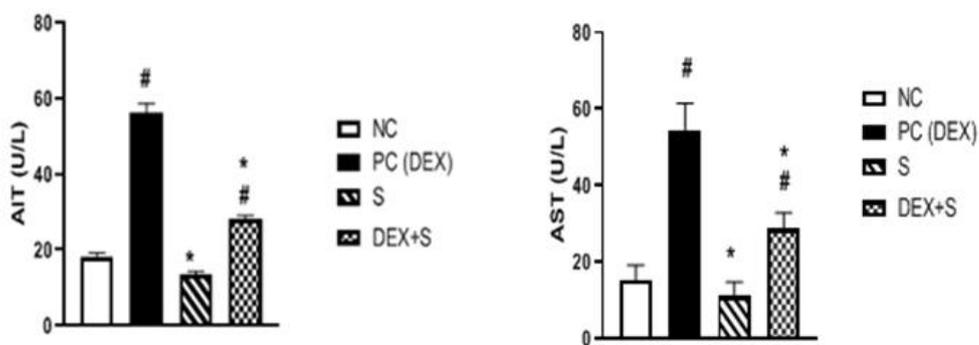
treated with stevia only and negative control group.

*Effect of the tested compounds on liver enzymes (ALT and AST)*

Treatment with dexamethasone caused significant elevation in ALT and AST levels of the rats compared to the negative control group at  $p < 0.001$  while rats treated with stevia extract alone or in combination with dexamethasone caused a significant reduction ( $p < 0.0001$ ) in ALT and AST levels compared to positive control group as shown in **Fig 3**.



**Fig.2:** Effect of daily I.P injection of dexamethasone (DEX) 8 mg/kg for 6 days, oral administration of stevia extract (S) alone 400 mg/kg for successive 12 days and the combination stevia extract 400 mg/kg orally for 12 successive days and dexamethasone that was added in the last 6 days on FBG level in dexamethasone induced fatty liver disease in rats. Negative control group received 2 ml of CMC solution. Data represent mean  $\pm$  SE of six observations. # Significant difference at  $p < 0.05$  vs. NC group. \*Significant difference at  $p < 0.0001$  vs. PC (DEX) group. NC: negative control, PC; positive control. CMC: Carboxymethyl cellulose.



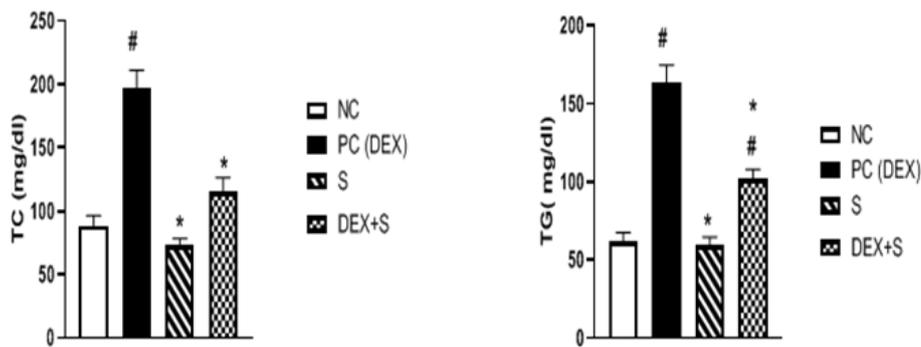
**Fig.3:** Effect of daily I.P injection of dexamethasone (DEX) 8 mg/kg for 6 days, oral administration of stevia extract (S) alone 400 mg/kg for successive 12 days and the combination stevia extract 400 mg/kg orally for 12 successive days and dexamethasone that was added in the last 6 days on liver enzymes (AST and ALT) in dexamethasone induced fatty liver disease in rats. Negative control group received 2 ml of CMC solution. Data represent mean  $\pm$  SE of six observations. # Significant difference at  $p < 0.001$  vs. NC group. \*Significant difference at  $p < 0.0001$  vs. PC (DEX) group. NC: negative control, PC; positive control. CMC: Carboxymethyl cellulose.

*Effect of the tested compounds on serum lipid profiles (TC and TG)*

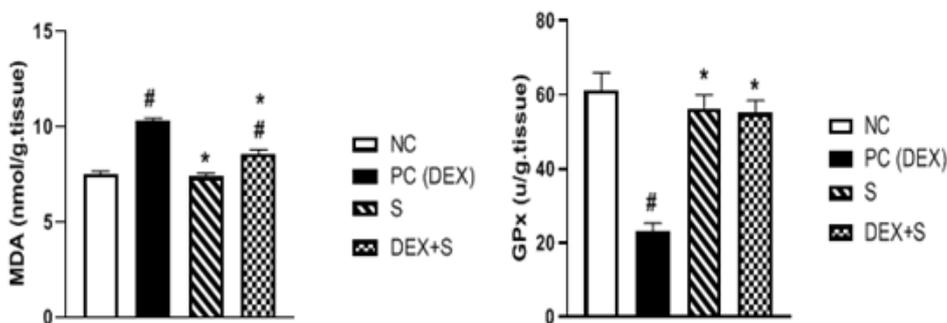
Statistically, there was significant elevations in TC and TG levels of the rats in DEX treated group compared to the negative control group at  $p < 0.001$ . However, stevia extract alone or in combination with dexamethasone could improve these elevations ( $p < 0.0001$ ) in rats compared to DEX-treated group as shown in **Fig 4**.

Treatment with dexamethasone caused an increase in MDA level with a decrease in the level GPx of the rat liver homogenate. These abnormalities were statistically significant compared to the negative control group at  $p < 0.01$ . Rats treated with stevia extract alone or in combination with dexamethasone demonstrated significant correction of these abnormalities in rats compared to DEX-treated group at  $p < 0.0001$  as shown in **Fig 5**.

*Effect of the tested compounds on malondialdehyde (MDA) and glutathione peroxidase (GPx) levels in liver homogenate*



**Fig.4:** Effect of daily I.P. injection of dexamethasone (DEX) 8 mg/kg for 6 days, oral administration of stevia extract (S) alone 400 mg/kg for successive 12 days and the combination stevia extract 400 mg/kg orally for 12 successive days and dexamethasone that was added in the last 6 days on serum lipid profile (TC and TG) in dexamethasone induced fatty liver disease in rats. Negative control group received 2 ml of CMC solution. Data represent mean  $\pm$  SE of six observations. <sup>#</sup>Significant difference at  $p < 0.001$  vs. NC group. <sup>\*</sup>Significant difference at  $p < 0.0001$  vs. PC (DEX) group. NC: negative control, PC; positive control. CMC: Carboxymethyl cellulose.



**Fig.5:** Effect of daily I.P. injection of dexamethasone (DEX) 8 mg/kg for 6 days, oral administration of stevia extract (S) alone 400 mg/kg for successive 12 days and the combination stevia extract 400 mg/kg orally for 12 successive days and dexamethasone that was added in the last 6 days on malondialdehyde (MDA) and glutathione peroxidase (GPx) levels in liver homogenate in dexamethasone induced fatty liver disease in rats. Negative control group received 2 ml of CMC solution. Data represent mean  $\pm$  SE of six observations. <sup>#</sup> Significant difference at  $p < 0.01$  vs. NC group. <sup>\*</sup>Significant difference at  $p < 0.0001$  vs. PC (DEX) group. NC: negative control, PC; positive control. CMC: Carboxymethyl cellulose.

*Effect of the tested compounds on serum adiponectin (ADP) and liver homogenate tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) levels*

Statistically, there was a significant reduction in serum ADP level with an elevation in TNF- $\alpha$  level in rats treated with dexamethasone compared to the negative control group at  $p < 0.001$ . Rats treated with stevia extract alone or in combination with dexamethasone demonstrated a significant improvement in the previous disturbances compared to positive control group at  $p < 0.0001$  as shown in **Fig 6**.

Notably, using of stevia extract in DEX treated group restored the levels of TC, GPx and TNF- $\alpha$  to normal but the other chemical parameters were still higher than those of the negative control group

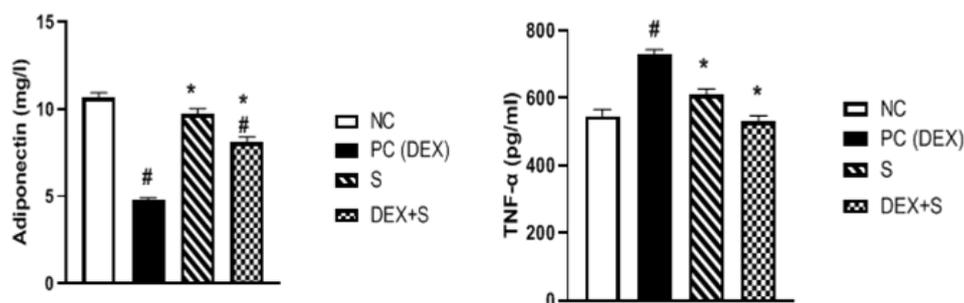
*Histopathological examination of liver tissues*

Examination of H&E stained sections of the liver in negative control group showed that hepatic parenchyma was formed of lobules. The hepatocyte plates were radiating from central vein separated by blood sinusoids. The hepatocytes were polygonal in shape with vesicular nuclei and acidophilic cytoplasm. The sinusoids were lined with endothelial cells and Kupffer cells (**Fig 7A**). Regarding to the group that treated with DEX 8 mg/kg there were hepatocytes with micro and macro steatotic changes. Other hepatocytes were apoptotic with dense nuclei and dark cytoplasm. While some others showed necrosis and ballooned hepatocytes were seen. Marked inflammatory

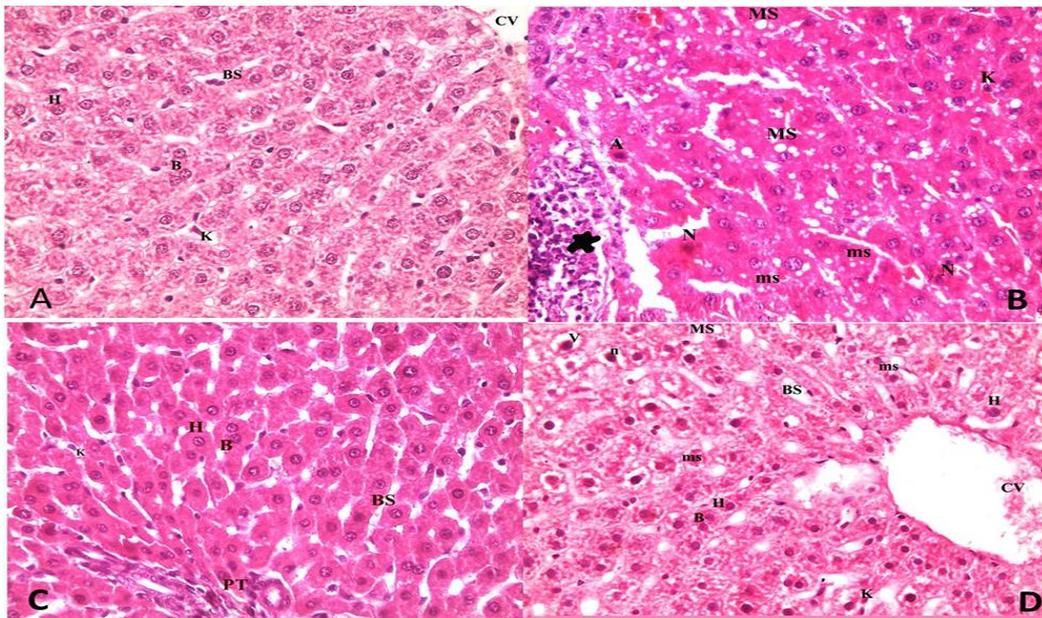
cell infiltration was also observed (**Fig 7B**). Histopathological examination of sections in liver tissues of stevia extract (400 mg/kg) treated group revealed that liver parenchyma was more or less similar to the control group (**Fig 7C**). Regarding to the group that treated with stevia extract plus DEX there were hepatocytes with vesicular nuclei and acidophilic cytoplasm. Other cells appeared with macro and microsteatosis. Few cells were enlarged, vacuolated with dense nuclei. No inflammatory cells were seen (**Fig 7D**).

*Immunohistochemical expression of PPAR- $\alpha$  in liver tissues*

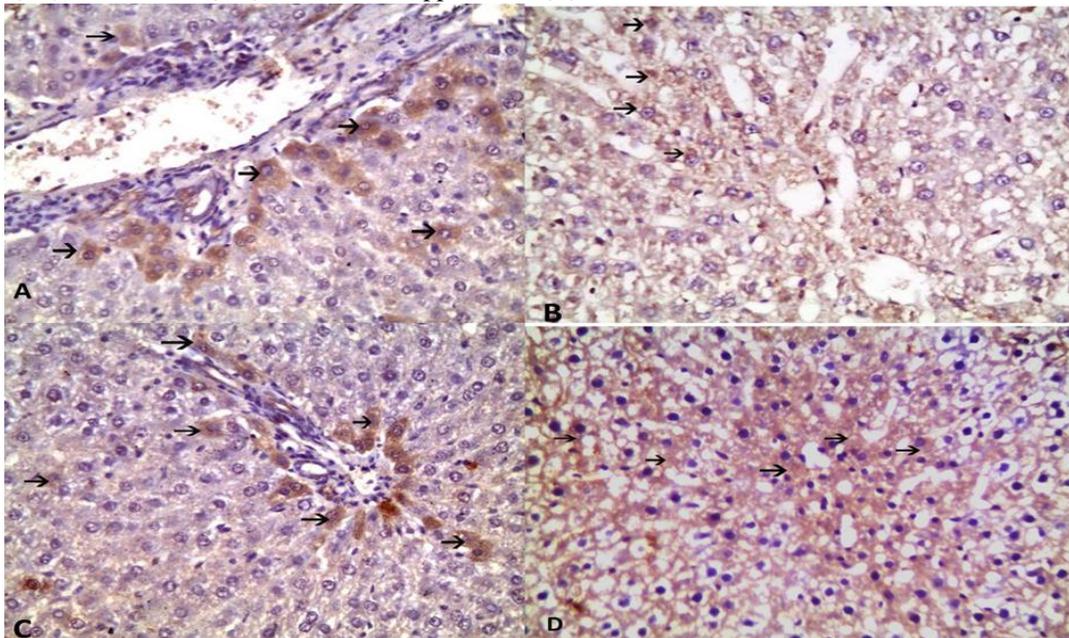
Immunohistochemical examination of the negative control group showed positive PPAR- $\alpha$  immunostaining reaction in the cytoplasm of many hepatocytes (strong PPAR- $\alpha$  protein expression) (**Fig 8A**). Sections in the liver of DEX-treated group showed a significant decrease in the number of positive PPAR- $\alpha$  immunostaining cells ( $7.75 \pm 0.43$ ) compared to the negative control group ( $16.68 \pm 0.90$ ) at ( $p < 0.0001$ ) (**Fig 8B**). Examination of sections in the liver of stevia treated group was more or less similar to the negative control group ( $16.23 \pm 0.54$ ), there was no significant difference (**Fig 8C**). Regarding to the group that treated with stevia and DEX there was a significant increase in the area percent of positive PPAR- $\alpha$  immunostaining reaction ( $13.05 \pm 0.99$ ) at ( $p < 0.01$ ) compared to positive control group (**Fig 8D**).



**Fig.6:** Effect of daily I.P injection of dexamethasone (DEX) 8 mg/kg for 6 days, oral administration of stevia extract (S) alone 400 mg/kg for successive 12 days and the combination stevia extract 400 mg/kg orally for 12 successive days and dexamethasone that was added in the last 6 days on serum adiponectin (ADP) and liver homogenate tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) levels in dexamethasone induced fatty liver disease in rats. Negative control group received 2 ml of CMC solution. Data represent mean  $\pm$  SE of six observations. <sup>#</sup> Significant difference at  $p < 0.001$  vs. NC group. <sup>\*</sup>Significant difference at  $p < 0.0001$  vs. PC (DEX) group. NC: negative control, PC; positive control. CMC: Carboxymethyl cellulose.



**Fig.7:** Photomicrographs of H&E stained hepatic sections of (A, x400) the negative control group showing central vein (CV), Hepatocytes (H), Binucleated hepatocytes (B), Blood sinusoid (BS) and Kupffer cells (K). (B, x400) section from dexamethasone-treated group showing macrosteatosis (MS) and microsteatotic changes (ms), necrotic hepatocytes (N), apoptotic hepatocytes (A), inflammatory cell infiltration (star) and Kupffer cells (K). (C, x400) section from stevia treated group showing hepatocytes (H), binucleated hepatocytes (B), blood sinusoids (BS), portal tract (PT) and Kupffer cell (K). (D, x400) section from stevia + dexamethasone treated group showing hepatocytes (H), Binucleated hepatocytes (B), macrosteatosis (MS) and microsteatosis (ms), degenerated cells (V) with dense nuclei (n). Blood sinusoid (BS), central vein (CV), some and Kupffer cells (K).



**Fig. 8:** Immunohistochemistry of PPAR- $\alpha$  in liver tissues of rats (x400). (A) Section in the liver of the negative control group showing numerous positive cells (Strong PPAR- $\alpha$  expression) with brown cytoplasmic coloration (arrow). (B) Section from dexamethasone-treated group showing few positive PPAR- $\alpha$  immunostaining reaction cells compared to the control group (arrow). (C) Section from stevia treated group showing numerous positive cells (arrow). (D) Section from stevia + dexamethasone treated group showing numerous positive PPAR- $\alpha$  immunostaining reaction in hepatocytes compared to DEX group (arrow).

## Discussion

Simple steatosis, NASH, liver cirrhosis and hepatic carcinoma are among the phases of NAFLD<sup>29</sup>. In animals, glucocorticoids such as dexamethasone have been used to induce hyperlipidemia and hepatic steatosis. By reducing fatty acid  $\beta$ -oxidation and increasing fatty acid synthesis, dexamethasone promotes fatty liver<sup>7</sup>. Low-dose dexamethasone, a pioneering treatment for COVID-19, lowers mortality risk by one-third in patients using ventilators and by one-fifth in patients using oxygen<sup>30</sup>.

Medicinal herbs have had significant positive impacts on the alleviation of inflammation and steatosis when used to treat NAFLD<sup>31</sup>. *Stevia rebaudiana* is a type of medicinal plants<sup>13</sup>. It has a variety of biological effects that promote health. Stevia extract, which has secondary metabolites such stevia glycosides (rebaudioside A, rebaudioside C and stevioside) and polyphenols causes these biological effects<sup>32</sup>.

In this study, there was a decrease in body weight, liver GPx and serum adiponectin level with an elevation in liver weight, liver index, liver enzymes, TC and TG, liver MDA and TNF- $\alpha$  in positive control group that administered dexamethasone 8 mg/kg/ day for 6 days. Altered liver histopathology including steatosis, inflammation and fibrosis were also observed with a decrease in immunostaining expression of PPAR- $\alpha$  receptors. Stevia extract that was combined with dexamethasone could correct most of these abnormalities.

Dexamethasone administration in 8 mg/kg/day for 6 days caused a reduction in body weight with an increase in the liver weight, liver index and fasting blood glucose level. These results were similar to previous researches<sup>17,33</sup>.

Mathai et al., 2015 investigated the effect of sitagliptin in combination with pioglitazone on dexamethasone-induced NAFLD in rats. They injected dexamethasone daily for 6 days (8 mg/kg i.p.) and there was a significant weight reduction with an increase in the liver weight and fasting blood glucose level in DEX-treated group in comparison to the negative control group<sup>17</sup>.

Mahmoud et al., 2023 studied the effect of coriander oil in reversing dexamethasone induced insulin resistance in rats. They found

that the liver index of DEX-treated group was higher than the negative control group<sup>33</sup>.

The observed body weight loss in DEX-treated rats could be attributed to an increase in serum insulin levels, which is the main component responsible for body weight loss by favoring food intake suppression and acting on the hypothalamus<sup>34</sup>. Furthermore, a previous study showed that the reducing effect in body weight is related to suppression of synthesis of muscle proteins and enhancement of protein catabolism<sup>35</sup>.

In contrast, in 2019, Luijten et al suggested that high doses of glucocorticoids can lead to obesity development in both humans and mice, but they did not show the mechanism for this suggestion<sup>36</sup>. Furthermore, Zakrzewska et al.1999 showed that dexamethasone can induce obesity in rats by acting centrally not peripherally. They compared the effects of intraperitoneal and intracerebroventricular dexamethasone infusion (5  $\mu$ g/day for 3 days) on body weight. Intraperitoneal infusion of dexamethasone resulted in a reduction in food intake and body weight with a decrease in neuropeptide Y level in hypothalamus, whereas intracerebroventricular infused dexamethasone caused marked increase in food intake and body weight with an elevation in neuropeptide Y level relative to the negative control group<sup>37</sup>.

Several factors contribute to hyperglycemia following dexamethasone administration, including increased hepatic gluconeogenesis, decreased pancreatic  $\alpha$ - and  $\beta$ - cell activities and diminished insulin sensitivity<sup>38</sup>.

Daily oral administration of the combination stevia extract 400 mg/kg orally for 12 successive days and dexamethasone that was added in the last 6 days caused a reduction in body weight compared to DEX-treated group. These results were in harmony with a recent research that was done to estimate the effect of stevia either alone or combined with saxagliptin in diabetic rats. The study reported that oral administration of stevia 400 mg/kg/day reduced the diabetic rats body weight significantly<sup>12</sup>. A previous research had identified a number of factors for the decrease in body weight caused by stevia, including its ability to lower rat food intake, glucose levels,

fat absorption and lipogenic enzymes as well as to promote fat excretion<sup>39</sup>.

The present study showed that, daily oral administration of stevia extract plus DEX injection caused statistically significant reduction in the liver weight of rats compared to dexamethasone treated rats. The results were in accordance with a recent study which was carried out to evaluate the effects of stevia extract on liver steatosis in mice. Stevia extract in both doses (200 and 500 mg/kg daily for 21 days) significantly reduced liver weights of mice compared to the negative control group<sup>32</sup>.

However, when we calculated the liver index in the group treated with stevia alone, there was no significant difference compared to the negative control group and in case of the combination (stevia extract + dexamethasone) there was a decrease in liver index compared to DEX-treated group but it was not significant.

The present study showed that daily oral administration of stevia plus dexamethasone statistically caused significant decrease in blood glucose level compared to dexamethasone group and this was in agreement with a previous study that was done to investigate the effect of stevia on serum visfatin and omentin levels (novel adipocytokines) in diabetic rats. Stevia was gavaged daily at doses of 250 and 500 mg/kg for 30 days. At the end of the study there was significant decrease in fasting blood sugar in both groups in comparison diabetic group<sup>40</sup>. The improvement in fat catabolism, bile acid metabolism, glucose metabolism, lipid storage, and transport in the liver of obese mice with insulin resistant is the proposed mechanism of stevia for lowering blood glucose levels<sup>41</sup>.

Administration of stevia extract 400 mg/kg/day alone for 12 days caused a reduction in body weight compared to the negative control group with a decrease in weights of liver tissues and FBG level compared to dexamethasone-treated group.

ALT and AST are indicators in the detection of hepatic injury as discharged into the bloodstream following hepatocellular injury<sup>42</sup>. It is well recognized that ALT is only released by the liver, whereas AST is released by a variety of organs, including the liver and the heart<sup>43</sup>. Elevated liver enzymes levels in DEX-treated group in this study were in

agreement with a previous research that was carried out to estimate the antihyperglycemic and antioxidant effect of *Alstonia boonei* extract in dexamethasone-induced hyperglycemia in rats. Dexamethasone (0.4 mg/kg) that injected daily for 30 days to induce hyperglycemia caused a significant elevation in ALT and AST<sup>44</sup>.

In comparison to the DEX-treated group, stevia extracts in combination with DEX reduced levels of AST and ALT. These results were in accordance with a research that reported the effect of stevia leaves on streptozotocin induced diabetic rats. Stevia leaves powder incorporated in diet (4.0 g leaf powder in 96 g dry diet) for 5 weeks in STZ-induced diabetic rats. At the end of the study there was a reduction in ALT and AST levels<sup>45</sup>.

With regard to hyperlipidemia markers, the DEX model group caused significant increase in TC and TG levels compared to the negative control group. These findings were consistent with those reported by Ahmed et al., 2020 who estimated the potential protective effect of canagliflozin compared with atorvastatin and the combination of both drugs on DEX-induced dyslipidemia and hepatic steatosis. Significant elevations in TC and TG were observed in DEX (8 mg/kg intraperitoneally for 6 days) treated group compared to negative control group<sup>7</sup>. The increased lipid levels are due to diminished insulin sensitivity of tissues, particularly the liver, as well as decreased TG hydrolysis due to decreased lipoprotein lipase activity<sup>46</sup>.

Administration of stevia extract with dexamethasone improved serum levels of TC and TG that were in harmony with a previous research that was done to investigate the anti-hyperglycemic, antioxidative and anti-dyslipidemic properties of methanolic stevia extract in diabetic mice. The daily oral treatment of stevia extract (300 mg/kg) for 3 weeks significantly reversed TC and TG levels towards normal<sup>47</sup>. Stevia extract ameliorated liver dysfunction by controlling hyperglycemia, lowering elevated liver enzymes, providing an antioxidant effect and improving insulin resistance<sup>48</sup>.

Administration of stevia extract 400 mg/kg/day alone for 12 days caused a reduction in the level of liver enzymes and lipid profile

parameters compared to dexamethasone-treated group.

It is thought that oxidative stress is a key factor in the pathophysiology of NAFLD. Induced oxidative stress by the oxidation of cytotoxic free fatty acids has been shown to increase cytokine levels while decreasing levels of liver antioxidants<sup>49</sup>. Furthermore, increased lipid peroxidation produces byproducts like MDA, which have been proven to further promote cytokine production<sup>50</sup>. The oxidation of accumulated lipids in the liver could release free radicals such reactive oxygen species (ROS)<sup>51</sup>. ROS increases lipid peroxidation through destruction of unsaturated fatty acids in cell membranes and reduces endogenous antioxidants, resulting in liver damage<sup>52</sup>.

The first line of defense against free radicals is glutathione (GSH) which serves as a substrate for glutathione peroxidase (GPx) and glutathione S-transferases (GST). It is responsible for replenishing GPXs which detoxify H<sub>2</sub>O<sub>2</sub> and lipid hydroperoxides<sup>53</sup>. In this study, the DEX-treated group had higher levels of liver MDA than the negative control group. Similar findings were reported by a previous study that was done to estimate the antihyperglycemic and antioxidant effect of *Alstonia boonei* extract in dexamethasone-induced hyperglycemia in rats. Dexamethasone (0.4 mg/kg) injected daily for 30 days to induce hyperglycemia caused a significant elevation in elevation in the level of MDA<sup>44</sup>. Administration of dexamethasone caused a reduction in GPx level. In accordance with these results, Lv et al., 2018 reported that DEX treatment reduced glutathione peroxidase levels in broiler liver<sup>54</sup>.

These results demonstrated that administration of stevia extract with dexamethasone caused a significant increase in liver GPx and a significant decrease in liver MDA compared to DEX treated group. These findings were in agreement with the results of previous studies<sup>47,48</sup>.

Assi et al., 2020 explored effect of stevia extract alone and in combination with glimepiride in diabetes rats. The diabetic groups that treated with stevia extract (300 mg/kg) for 21 days reduced MDA level compared to control diabetic rats

In 2013, Singh et al investigated the anti-hyperglycemic, antioxidative and anti-

dyslipidemic properties of methanolic stevia extract in diabetic mice. The daily oral treatment of stevia extract (300mg/kg) for 3 weeks significantly increased GPx levels, implying that it plays a compensatory role in decreasing H<sub>2</sub>O<sub>2</sub> production, thereby reducing the toxic effects of the free radicals that produced by it in different secondary reactions<sup>47</sup>.

Many experimental studies have revealed that stevia extract has a powerful antioxidant action, consistent with the current work, because the plant contains high amounts of phenols and flavonoids<sup>55, 56</sup>. Stevia leaves were found to have superior antioxidant properties including scavenging of free radicals and inhibiting lipid peroxidation<sup>45</sup>.

Stevia extract has antioxidant properties due to the presence of stevioside, rebaudioside A, rebaudioside C, and dulcoside. Stevioside increases antioxidant capacity by upregulating nuclear erythroid factor 2 (Nrf2). The potential mechanism of action of stevioside is associated with its ability to inhibit beta-adrenergic and G-protein-coupled receptor kinases<sup>57</sup>.

Adiponectin is a pleiotropic hormone generated from adipose tissue. It has anti-atherogenic, anti-diabetic, anti-inflammatory and insulin-sensitizing actions<sup>58</sup>. Adiponectin has a considerable impact on the metabolism of free fatty acids and its main role is endogenous insulin sensitizer<sup>59</sup>. According to a previous study, adiponectin enhances skeletal muscle glucose uptake, lowers hepatic gluconeogenesis and promotes fatty acid oxidation in both skeletal muscles and liver<sup>60</sup>. Glucocorticoids and adiponectin control the metabolism of energy in opposing ways. Adiponectin acts mainly as an insulin sensitizer while glucocorticoids primarily contributes to insulin resistance and activation of catabolic processes<sup>61,62</sup>.

This study indicated that DEX administration resulted in a decrease in serum adiponectin levels, which was consistent with the findings of a previous study that was done to investigate the effect of dexamethasone administration in rats treated with fatty diet on the level adiponectin. Subsequently, DEX (0.2 mg/100 g) was given twice per day by subcutaneous injections after complete bilateral adrenal gland removal. Dexamethasone

resulted in decreased serum adiponectin levels<sup>63</sup>.

Administration of stevia extract plus dexamethasone has a significant effect on decreasing MDA and increasing serum adiponectin levels. This was in line with Assi et al., 2020 who explored the effect of stevia extract alone and in combination with glimepiride in diabetes rats. The diabetic groups that treated with stevia extract (300 mg/kg) for 21 days reduced MDA level and increased serum adiponectin level compared to control diabetic rats<sup>48</sup>.

Inflammation is one of the important factors in the pathogenesis of fatty liver disease<sup>64</sup>. When the liver is injured or inflamed, the hepatocyte itself and the inflammatory cells release cytokines such ROS and tumor necrosis factor (TNF- $\alpha$ )<sup>65</sup>. These mediators can lead to the peroxidation of mitochondrial and plasma membranes which results in necrosis or apoptosis resulting in cell death<sup>66,67</sup>. TNF- $\alpha$  has been implicated as a key factor in the development of obesity, T2DM and insulin resistance acting through the processes of immunity and inflammation<sup>68</sup>.

The major interleukins (ILs) which have been investigated in the pathogenesis of NAFLD include IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and IL-18<sup>69</sup>. The main role of ILs in the immune system is to mediate intercellular communication which include cell migration, proliferation, maturation, and adhesion that are crucial for the inflammatory response. Interleukins are involved in both chronic and acute inflammation<sup>70</sup>.

Both IL-1 $\alpha$  and active IL-1 $\beta$  play a crucial role in the inflammatory response by inducing numerous other cytokines. Multiple pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF), IL-1 $\alpha$ , IL-1 $\beta$ , and IL-6, are involved in inflammation, steatosis, fibrosis, and the development of cancer in many pathologies associated with liver diseases. In addition to NAFLD, researches from the past years revealed that various IL-1 type cytokines affect adipose tissue inflammation, insulin resistance and atherosclerosis<sup>71</sup>. In the studies that histologically confirmed NAFLD, most authors reported higher levels of IL-6 in NAFLD<sup>72</sup>. IL-6 enhances hepatic fibrogenesis<sup>5</sup>.

The present study revealed that administration of DEX increased TNF- $\alpha$  level

and this was in agreement with a previous research that was done to investigate the effect of crocetin on the insulin resistance induced by DEX in rats. Daily subcutaneous dexamethasone (0.08 mg/kg) for six weeks caused significant increase in TNF- $\alpha$  level<sup>73</sup>.

Furthermore, similar results were published in a recent study that was done to explore the protective action of Didymin (active constituent in *Mentha spicata*) on dexamethasone and high-fat diet induced NAFLD in C57BL/6J mice. Compared with normal control mice, TNF- $\alpha$  concentration in hepatic tissues was increased significantly in dexamethasone and high-fat diet group<sup>64</sup>.

Conversely, in 2019, Qin and Qiu demonstrated that dexamethasone can maintain the balance between anti-inflammatory response and inflammatory response through inhibiting expression levels of inflammatory factors (TNF- $\alpha$ , IL-6 and VEGF) and promoting the expression of anti-inflammatory factor (IL-10) in serum thus alleviating lung tissue injury<sup>74</sup>.

The findings in this study showed that administration of stevia extract plus dexamethasone significantly reduced the level of TNF- $\alpha$  and this was similar to the results that was reported in a previous report to investigate how the natural sweetener stevia rebaudiana and its component stevioside affected kidney damage caused by cisplatin. Mice were gavaged with 10, 20 and 50 mg/kg stevia extract or stevioside 50 mg/kg, 48 h after intraperitoneal administration of cisplatin (13 mg/kg). Treatment with stevioside and stevia extract reduced expression of TNF- $\alpha$  that was elevated due to cisplatin injection. This indicated that the inflammatory action was due to stevioside<sup>75</sup>.

Stevia's anti-inflammatory properties are attributed to stevioside and steviol. Stevioside inhibited the upregulation of genes involved in liver inflammation in vitro. In silico studies revealed that stevioside inhibited two proinflammatory receptors: Toll-like receptor (TLR)-4 and tumor necrosis factor receptor-1<sup>57</sup>.

Administration of stevia extract 400 mg/kg/day alone for 12 days caused a significant reduction in the level of MDA and TNF- $\alpha$  with an increase GPx and ADP levels compared to dexamethasone-treated group.

All the previous results were confirmed with histopathological and immunohistochemical findings where the severe damage and fatty degeneration in liver tissues that developed by DEX administration were resolved by administration of stevia extract. Histopathological examination of the liver tissues in DEX-treated group revealed steatosis, inflammation, necrosis and apoptosis. These findings were similar to that reported by a recent research in which there was hepatic histopathological changes including steatosis, inflammation and fibrosis in DEX-treated group in their study<sup>7</sup>. The hepatocytes in rats treated with stevia extract plus dexamethasone were smaller in size and had reduced fat deposition compared to dexamethasone treated group.

Steatosis is a critical event in the incidence and development of NAFLD that can be triggered by PPAR- $\alpha$  dysregulation. PPAR- $\alpha$  is a transcription factor and an important regulator of lipid metabolism in the liver. Increased PPAR- $\alpha$  expression promotes fatty acid uptake, utilization and catabolism<sup>76</sup>. PPAR- $\alpha$  is abundantly expressed in tissues with high fatty acid oxidation capacity, including heart, liver and skeletal muscles<sup>32</sup>. Interestingly, overexpression of glucocorticoid receptors in mice livers reduced PPAR- $\alpha$  protein and mRNA expression on a normal diet<sup>77</sup>.

Furthermore, immunohistochemical examination of PPAR- $\alpha$  in the liver of DEX treated group showed an apparent decrease positive PPAR- $\alpha$  immunostaining reaction cells as well as the cytoplasmic area of positive reactions compared to the control group. These results were in harmony with a previous study which showed that ingestion of ethanol raised endogenous the levels of glucocorticoid in humans and rodents. They explored the mechanistic relationship between the elevated glucocorticoids and alcoholic fatty liver in mice. Mice fed with ethanol for 2 weeks with or without dexamethasone treatment. There was a reduction in liver PPAR- $\alpha$  in rats administered with ethanol and dexamethasone. These results suggested that elevated glucocorticoid levels may play a role in the development of alcoholic fatty liver via liver PPAR- $\alpha$  inactivation<sup>78</sup>. In 2019, Tsai et al found out that the expression of mRNA of

PPAR- $\alpha$  was inhibited by prenatal administration of dexamethasone<sup>79</sup>.

Furthermore, immunohistochemical examination of PPAR- $\alpha$  in the liver of stevia plus dexamethasone treated group showed an apparent increase in the area percent of positive PPAR- $\alpha$  immunostaining reaction compared to DEX treated group. This was consistent with the study of Park et al., 2022 who studied the effects of stevia and stevioside on liver steatosis in db/db mice. stevia and stevioside elevated the levels PPAR- $\alpha$ <sup>32</sup>. The ability of stevia to activate PPAR- $\alpha$  has been documented and researchers discovered this characteristic as a potential mechanism underlying the stevia's hypotriglyceridemic action<sup>40</sup>.

### Conclusion

The study had shown that stevia extract had a modest antiinflammatory and antioxidant effects that may be responsible for its effectiveness in the treatment of dexamethasone-induced NAFLD. Stevia extract was effective in ameliorating the abnormalities caused by dexamethasone administration in liver enzymes, lipid profile, blood glucose and serum adiponectin levels. PPAR- $\alpha$  may play an important role in mediating stevia extract beneficial effects in NAFLD. Further studies in human are needed to confirm the potential use of stevia extract in dexamethasone-induced NAFLD.

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### Conflicts of interest

There aren't any competing interests.

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## نشرة العلوم الصيدلانية جامعة أسيوط



### التأثير الوقائي المحتمل لمستخلص نبات ستيفيا على مرض الكبد الدهني غير الكحولي الناجم عن استخدام عقار الديكساميثازون في الجرذان: دور PPAR- $\alpha$

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على مدى العقد الماضي ، جذبت الأدوية العشبية مزيداً من الاهتمام بسبب آثارها المحتملة في الوقاية من مرض الكبد الدهني غير الكحولي وعلاجه ، فضلاً عن فعاليتها وانخفاض مخاطر الآثار الجانبية. فالتأثيرات المباشرة لمستخلص نبات ستيفيا على مرض الكبد الدهني غير الكحولي الناجم عن استخدام عقار ديكساميثازون لم يتم توضيحه بشكل كافٍ ، لذلك هدفت هذه الدراسة إلى تقييم التأثير المحتمل لنبات ستيفيا على أعراض مرض الكبد الدهني الناجم عن استخدام عقار ديكساميثازون في الجرذان. تم إجراء هذه الدراسة على أربعة مجموعات من الجرذان ، تحتوي كل مجموعة على ٦ حيوانات لمدة ١٢ يوماً. المجموعة الأولى: تم إعطاء الجرذان محلول ٠,٥% كاربوكسي ميثيل سيليلوز عن طريق الفم لمدة ١٢ يوم. المجموعة الثانية: تم حقن الجرذان بعقار ديكساميثازون ٨ مجم / كجم يومياً ف التجويف البطنى لمدة ٦ أيام . المجموعة الثالثة : تم إعطاء الجرذان يومياً مستخلص نبات ستيفيا ٤٠٠ مجم / كجم عن طريق الفم لمدة ١٢ يوماً . المجموعة الرابعة: تم حقن الجرذان بعقار ديكساميثازون ٨ مجم / كجم يومياً ف التجويف البطنى لمدة ٦ أيام واعطائها مستخلص نبات ستيفيا ٤٠٠ مجم / كجم عن طريق الفم لمدة ٦ أيام قبل حقن عقار ديكساميثازون و ٦ أيام أثناء الحقن بعقار ديكساميثازون. تم قياس مستويات السكر و إنزيمات الكبد والدهون ف الدم . و ايضا تم قياس مستويات بعض من مضادات الاكسدة و مضادلت الالتهابات ف انسجة الكبد. و قد أظهرت الدراسة أن مستخلص نبات ستيفيا له تأثيرات متوسطة مضادة للالتهابات ومضادة للأكسدة قد تكون مسؤولة عن فعاليته في علاج مرض الكبد الدهني غير الكحولي الناجم عن استخدام عقار ديكساميثازون. وايضا كان له تأثيراً فعالاً في التقليل من ارتفاع مستويات السكر و إنزيمات الكبد و الدهون في الدم. ربما يكون ل PPAR- $\alpha$  دوراً مهماً في التأثيرات الناتجة عن استخدام مستخلص نبات ستيفيا في مرض الكبد الدهني غير الكحولي. لذلك لابد من إجراء المزيد من الدراسات على الإنسان لتأكيد مدى فاعلية مستخلص نبات ستيفيا في الوقاية من مرض الكبد الدهني غير الكحولي الناجم عن عقار ديكساميثازون.