Research Article

Protective effect of p-coumaric acid on fructose induced-insulin resistance in albino rats

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Abstract
Background: Treatment of diabetes mellitus (DM) without any side effects is still a challenge. This leads to increase the demand for natural products with antidiabetic activity with fewer side effects. P-Coumaric acid has several pharmacological and biological actions, such as antioxidant (radical scavenging), neuroprotective, cardioprotective besides its antidiabetic effect. So we aim to evaluate the protective effect of p-coumaric acid on fructose-induced insulin resistance in rats. **Materials and Methods:** Thirty male adult white albino rats were randomly divided into 3 groups of 10 animals each. control group received normal saline orally fructose group received fructose 60% dissolved in water for 5 weeks coumaric +fructose group received p-coumaric acid 100mg/kg/day for 4 weeks orally followed by fructose 60 ‰ dissolved in water for 5 week. At the end of the experimental period, BMI , blood pressure , insulin, glucose, lipid profile, ALT and AST were measured . Oxidative stress biomarkers were measured in liver tissue and HOMA-IR test was taken as an indicator of insulin resistance. **Results** There was significant elevation of BMI, blood pressure, fasting plasma glucose, fasting plasma insulin, HOMA-IR, fasting plasma (cholesterol, triglycerides and LDL levels) and MDA with decrease of HDL, SOD and glutathione in fructose group when compared to control group. Administration of p-coumaric acid prevents these results. **Conclusion:** treatment with *p*-coumaric acid protect against high fructose diet-induced hyperglycemia, insulin resistance, dyslipidemia and oxidative stress.
Key Words: p-coumaric acid - insulin resistance – hypertension – dyslipidemia - hyperglycemia.

Introduction
Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels[(1)].

Disturbances in glucose metabolism and changes in lipid profiles are important biochemical findings in fructose-fed rats[(2)]. These metabolic alterations are attributed to disturbances in glucose uptake pathways and glucose metabolism and changes in the expression of several enzymes involved in hepatic lipid production[(3)].

Fructose-rich diet-induced impaired glucose tolerance accompanied by insulin resistance and hyperinsulinemia[(4)]. In addition, high levels of glucose and free fatty acids have been shown to promote oxidative cellular damage and exert detrimental effects by inducing the generation of free radicals as well as reducing antioxidant defenses[(5)].

Consequently, hyperglycemia and hyperlipidemia produced adverse metabolic alterations in glucose and lipid metabolism[(6)]. The mechanism underlying fructose-induced metabolic syndrome is complex but may be related to stimulation of gluconeogenesis and lipogenesis since it by-passes the major regulatory steps of glycolysis[(7)].

Currently, the available therapy for diabetes includes insulin and various oral anti-diabetic agents such as sulfonylureas, metformin, etc. These drugs are used as monotherapy or in combination to achieve better glycemic control. The synthetic antidiabetic agents can produce serious side effects. Treatment of diabetes without any side effects is still a challenge to the medical systems. This leads to increase the demand for natural products with antidiabetic activity with fewer side effects[8].

P-coumaric acid is found in a number of edible plants ingested by animals and humans[9]. It is present in plenty of foods, such as grapes, tomato, spinach, coffee, carrot, and garlic[10].

P-Coumaric acid has attracted substantial attention due to its several pharmacological and biological actions, such as antioxidant (radical scavenging) [11], cardioprotective[12],besides its antidiabetic effect[13]. Keeping the above facts in view, the purpose of this study is to evaluate the protective effect of p-coumaric acid on fructose-induced insulin resistance in rats.

Material And Methods

1. *Chemicals*:

- P-CA (CAS Number 501-98-4, formula C9H8O3), fructose, glutathione was purchased from **Sigma Aldrich (St. Louis, MO, USA)**. Commercial kits for measu-rement of glucose from **Diamond Egypt**, insulin from **Linco Research, Inc., MO**, TC from **Bio xpress U.S.A**, TG from ***Cell Biolabs, Inc U.S.A***, HDL from ***Bioo Scientific Corporation, U.S.A***. ALT and AST from **Span diagnostic reagent kit**, MDA from **Biodiagnostic Company**, SOD from **Aldrich (Steinheim, Germany)**

2. *Animals:*

- The study was carried out in 150–220 g of male albino rats obtained from Beni-Suef University, Egypt. They were residence in polypropylene cages (47cm, 34cm, 20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22 C and had free access of water and food.
- The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Beni-Suef University, Egypt ethical Committee.

3. *Experimental design:*

- In this experiment, a total of 30 male albino rats (weight ranged from 150-250gm) were divided into 3 groups and each group contains 10 rats.
  - Control group: 10 rats received normal saline for the duration of the study.
  - Fructose group: 10 rats received fructose 60% dissolved in water for 5 weeks (Song et al., 2005).
  - Coumaric +fructos., e group: 10 rats received p-coumaric acid 100mg/ kg/day for 4 weeks orally (Amalan et al., 2016) followed by fructose 60 ‰ dissolved in water for 5 weeks.
- At the end of the experimental period, all the animals were fasted overnight, and sacrificed by cervical dislocation. The ratio of 3:1 potassium oxalate and sodium fluoride containing tubes was used to collect the blood sample for the estimation of plasma glucose, insulin lipid profile and liver functions.
- Liver was immediately dissected, washed in ice-cold saline, homogenized in Tris-Hcl buffer (0.1 M pH 7.5). The homogenate was centrifuged and the supernatant was used for the estimation of oxidative stress biomarkers.

4. Procedures:

4.1. Calculation of the body mass index (BMI):

The body mass index (BMI) was calculated as the weight of rats (g)/length2 (cm2). The length of rats was measured between nasal and anal region.[14]

4.2. Method to measure hemodynamic blood pressure parameters using AD instrument Powerlab with tail-cuff apparatus

blood pressure was measured by Non-invasive Tail cuff method using pressure meter.[15]

4.3. *Collection of blood sample and preparation of serum:*

Serum is the liquid fraction of whole blood that is collected after the blood is

allowed to clot. The clot is removed by centrifugation and the resulting super-natant, designated serum, is carefully removed using a Pasteur pipette.[16].

4.4. Measurement of serum glucose :

Serum Glucose Estimation by method of [17]

4.5. Measurement of insulin level :

Radioimmunoassay:

There are many variations of the insulin RIA.[18]

4.6. Insulin resistance estimation using the homeostasis model assessment for insulin resistance (HOMA-IR) index:

using the formula HOMA-IR index = [fasting glucose (mmol/L) x fasting insulin (mU/ml)]/22.5).[19]

4.7. Lipid profile:

Cholesterol, triglycerides, and HDL-cholesterol levels were measured using kits obtained from Bio xpress U.S.A, *Cell Biolabs, Inc U.S.A, Bioo Scientific Corporation, U.S.A, respectively*.

4.8. Measurement of liver function (ALT and AST):

Alanine and aspartate aminotransferases were determined based on the colouri-metric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine[20]**.** ALT and AST assay kit supplied by (**Span diagnostic reagent kit**).the method was designed especially for use as a routine laboratory procedure. The reactions are as follows:

α-ketoglutarate + aspartate —> oxalacetate + glutamate

α-ketoglutarate + alanine —> pyruvate + glutamate

4.9. Measurement of oxidative stress biomarkers (GSH, SOD, MDA):

4.9.1. SOD measurement:

SOD Assay with Cytochrome *c*

the most frequently used assay is that developed by[21]. This method employs the xanthine-xanthine oxidase system to generate O^{-}_{2} and oxidized cytochrome c as a superoxide-trapping detection system. The reduction rate of cytochrome c by O^{-}_{2} was monitored spectrophotometrically at 550 nm. The reduction of cytochrome c was inhibited when the SOD containing sample was added because of enzymatic dismutation of superoxide anion O^{-}.

4.9.2. Glutathione Measurements:

***Reduced Plus Oxidized Glutathione method:*[22]**

The sum of the reduced and oxidized forms of glutathione was determined using a kinetic assay in which catalytic amounts of GSH or GSSG and glutathione reductase bring about the continuous reduction of 5,5'-dithiobis(Znitrobenzoic acid) (abbreviated DTNB) by NADPH.

4.9.3. MDA measurement:

Thiobarbituric acid Method by Spectrophotometry:

The method assayed according to[23] by using kits obtained from **Biodiagnostic Company.**

Statistics

All numerical data were examined with a test of one-way analysis of variance. Differences between the groups were evaluated using the Duncan multiple comparison test, which allows inter-comparison of all groups ($p < 0.05$). Our statistical analysis results were given as the mean ± standard deviation (SD).

Results

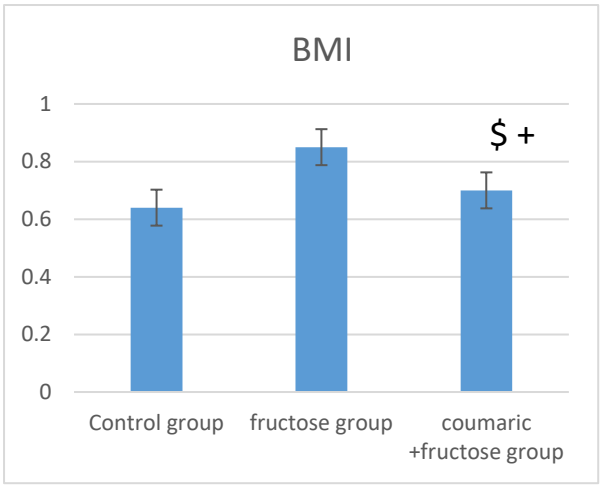
Comparison of BMI, blood pressure, fasting blood glucose, fasting insulin level and HOMA-IR among control, fructose and (coumaric+fructose) groups (Table 1 and Figure 1)

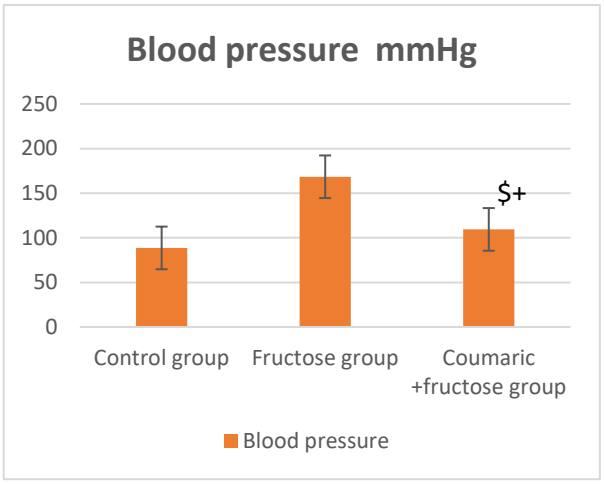
A significant increase of BMI was found in fructose group when compared to control group (p-value <0.001) there was a significant decrease of BMI in (coumaric + fructose) group when compared to fructose group (p-value <0.001) and a significant increase of BMI was found in (coumaric + fructose) group when compared to control group (p-value <0.05), a significant increase of blood pressure was found in fructose group when compared to control group (p-value <0.001) There was a significant decrease of blood pressure in (coumaric + fructose) group when compared to fructose group (p-value<0.001). A significant increase of FBG was found in fructose group when compared to control group (p-value <0.001). While FBG showed a significant

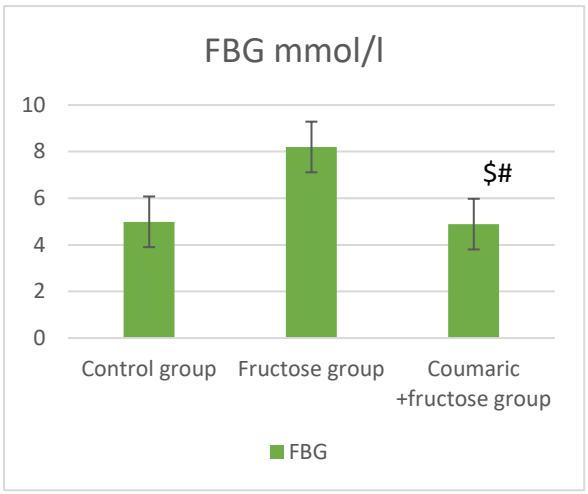
decrease in (coumaric + fructose) group when compared to fructose group (p-value <0.001). A significant increase of insulin in fructose group was found in comparison to control group (p-value<0.001). also, a significant increase of HOMA-IR was found in fructose group when compared to control group (p-value<0.001). A significant decrease of insulin level in (coumaric + fructose) group when compared to fructose group (p-value<0.001) and a significant decrease of HOMA-IR in (coumaric + fructose) group when compared to fructose group (p-value <0.001).

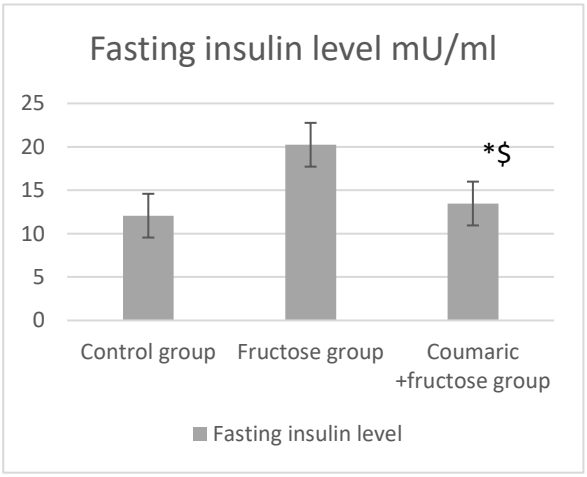
Table (1): Comparison of BMI, blood pressure, fasting blood glucose, fasting insulin level and HOMA-IR among control, fructose and (coumaric +fructose) groups

	Control group (n=10) ***mean±SD***	**Fructose group (n=10)** ***mean±SD***	**Coumaric+fructose group (n=10)** ***mean±SD***
BMI (g/cm2)	0.64±0.01	0.85 ± 0.01*****	0.70 ± 0.02$+
Blood pressure (mmHg)	88.60± 5.441	168.4± 4.03*****	109.4± 10.63$+
Fasting blood glucose (mmol/l)	4.99 ± 0.22	8.2± 0.31*****	4.89 ±0.17$#
Fasting insulin level (mlu/ml)	13.1 ± 0.74	20.24 ± 0.70*****	14.8± 0.79*****$
HOMA-IR (mmol/L)	2.68 ± 0.132	7.3± 0.394*****	2.93± 0.125$#









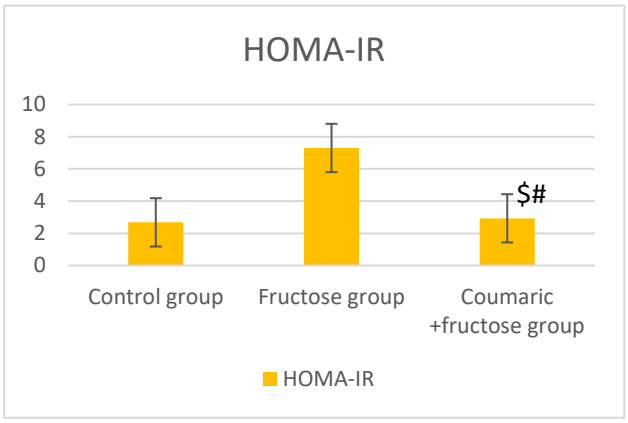


Figure (1): Comparison of BMI, blood pressure, fasting blood glucose, fasting insulin level and HOMA-IR among control, fructose and (coumaric +fructose) groups

Comparison of lipid profile (TC, TG, HDL and LDL) among control, fructose and (coumaric +fructose) groups (Table 2 and Figure 2)

a significant increase of TC, TG and LDL and a significant decrease of HDLwere found in fructose group when compared to control group (p-value<0.001). On the other hand, a significant decrease of TC, TG and LDL and a significant increase of HDL were found in (coumaric + fructose) group when compared to fructose group (p-value<0.001).

Table (2): Comparison of lipid profile (TC, TG, HDL and LDL) among control, fructose and (coumaric +fructose) groups

	Control group (n=10) *mean±SD*	Fructose group (n=10) *mean±SD*	Coumaric+fructose group (n=10) *mean±SD*
TC (mg/dl)	84.7 ± 4.32	133.9 ± 5.78*	73.6 ± 3.95*$
TG (mg/dl)	71.1 ± 3.07	127.1 ± 4.63*	65.3 ± 3.89+$
HDL (mg/dl)	38.2 ± 1.75	20.4 ± 1.27*	42.7± 1.64*$
LDL (mg/dl)	23 ± 2	64.7 ± 2.58*	18.8± 1.55*$

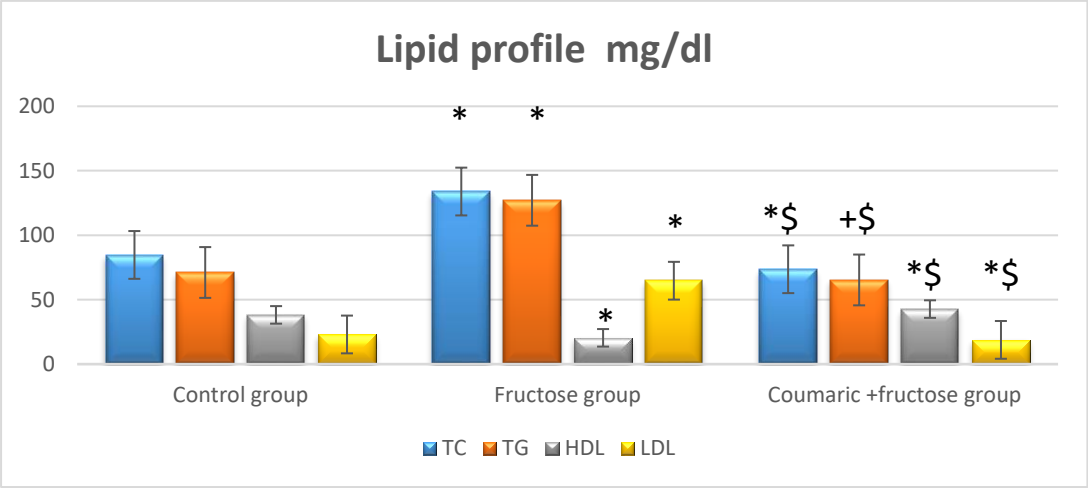


Figure (2): Comparison of lipid profile (TC, TG, HDL and LDL) among control, fructose and (coumaric +fructose) groups

Comparison of liver enzymes (ALT and AST) among control, fructose and (coumaric +fructose) groups (Table 3 and Figure 3)

There A significant increase of ALT and AST were found in fructose group when compared to control group (p-value<0.001). On the other hand, a significant decrease of ALT and AST were found in (coumaric + fructose) group when compared to fructose group (p-value<0.001). Also, a significant decrease of ALT and AST were found in (coumaric + fructose) group when compared to control group (p-value<0.001) for ALT and (p-value<0.05) for AST.

Table (3): Comparison of liver enzymes (ALT and AST) among control, fructose and (coumaric +fructose) groups

	Control group (n=10) *mean±SD*	Fructose group (n=10) *mean±SD*	Coumaric+fructose group (n=10) *mean±SD*
ALT (IU/L)	64.2 ± 4.52	104.9 ± 3.9*	53.6 ± 3.09*$
AST (IU/L)	53 ± 2.79	91.4 ± 4.95*	42.3 ± 4.74+$

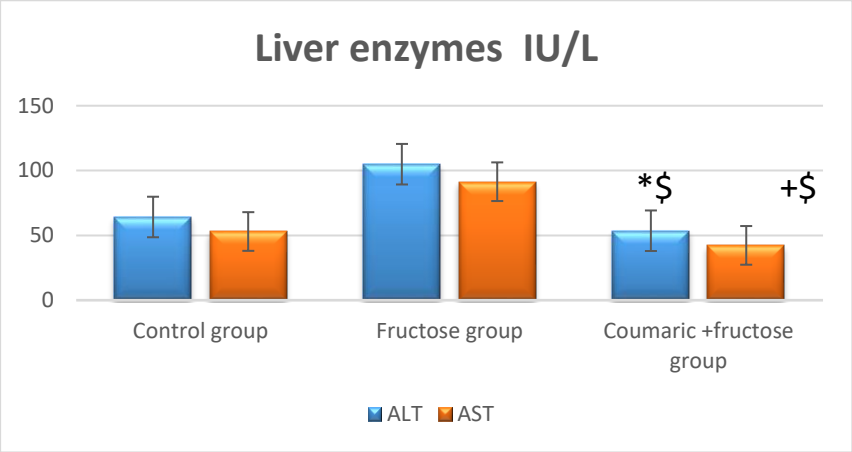


Figure (3): Comparison of liver enzymes (ALT and AST) among control, fructose and (coumaric +fructose) groups

<u>Comparison of oxidative stress biomarkers (glutathione, MDA and SOD) among control, fructose and (coumaric +fructose) groups:</u> (Table 4 and Figure 4)

A significant decrease of glutathione and SOD and a significant increase of MDA were found in fructose group when compared to control group (p-value<0.001).

On the other hand, a significant increase of glutathione and SOD and a significant decrease of MDA were found in (coumaric + fructose) group when compared to fructose group (p-value<0.001). While a significant increase of SOD and MDA and a significant decrease of glutathione were found in (coumaric + fructose) group when compared to control group (p-value<0.001) for glutathione and MDA, and (p-value<0.05) for SOD.

Table (4): Comparison of oxidative stress biomarkers (glutathione, MDA and SOD) among control, fructose and (coumaric +fructose) groups.

	Control group (n=10) ***mean±SD***	**Fructose group (n=10)** ***mean±SD***	**Coumaric+fructose group (n=10)** ***mean±SD***
Glutathione (nmol/100mg tissue)	27.4 ± 2.07	16.4 ± 1.17*	22.9 ± 1.66*$
MDA (nmol/100mg tissue)	1.5 ± 0.71	4 ± 0.82*	1.6 ± 0.69*$
SOD (U/g tissue)	10.9 ± 0.88	4.4 ± 0.84*	13.7 ± 1.25+$

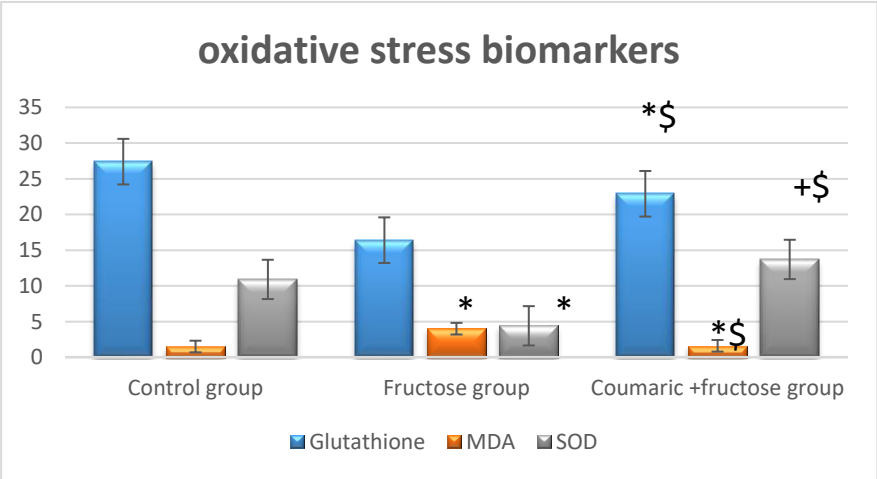


Figure (4): Comparison of oxidative stress biomarkers (glutathione, MDA and SOD) among control, fructose and (coumaric +fructose) groups .

Discussion

In diabetes mellitus, hyperglycemia with biochemical changes in glucose and lipid metabolism cause an increased production of reactive oxygen species[24].
reactive oxygen species play a major role in the pathogenesis of diabetes and its complications. Hyperglycemia causes tissue damage through multiple mechanisms including increased flux of glucose and other sugars through the polyol pathway, increased intracellular formation of advanced glycation end products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms, and over-activity of the hexosemine pathway[25]

World Health Organization (WHO) has given sufficient stress in utilizing traditional plants and plant products for diabetes, since they are non-toxic, efficient, with less or no side effect. There is an inverse association between dietary phenolic compound intake and mortality from various diseases. Phenolic compounds are a group of phenolic acids that are widely distributed in whole grains, fruits, pears, vegetables and beverages such as tea, coffee, wine and chocolate[26].

p-Coumaric acid (3-[4-hydroxyphenyl]-2-propenoic acid) is a phenolic compound, abundantly present in pineapple. *p*-Coumaric acid is a ubiquitous plant metabolite possess antioxidant, antiinflammatory, anticancer, and hepatoprotective effect[27], besides its antidiabetic effect.

This study was undertaken to evaluate the protective effect of p coumaric acid on fructose-induced insulin resistance including hyperglycemia, hyperinsuli-neamia, best described, hypertension, hyperlipideamia, altered liver function and reactive oxygen species.

Body mass index is an important in assessing the progression of obesity[28]. a significant increase of BMI was found in fructose group when compared to control group and these results were in accordance with previous studies[29]. High-fructose diet-mediated increase in BMI of rats were significantly reversed following four weeks administration coumaric acid as there was a significant decrease of BMI in (coumaric + fructose) group when compared to fructose group. And a significant increase of BMI was found in (coumaric + fructose) group when compared to control group. This indicates anti-obesity activity of coumaric acid and could help in the treatment of high-fructose diet related obesity.

a significant increase of blood pressure was found in fructose group when compared to control group, in agreement with[30].
as observed that oral administration of coumaric acid partially improve sensitivity to insulin so decreasing level of insulin. There was a significant decrease of blood pressure in (coumaric + fructose) group when compared to fructose group. This indicates that coumaric acid prevents development of hypertension in association with improvement the sensitivity to insulin.

In agreement with[31] study in which cardioprotective function of p-CA was evaluated on experimental rats and it showed that natural dietary phenolic compounds, such as p-coumaric acid, could cause endothelium-dependent vasorelaxation in thoracic aorta, so decreasing blood pressure.

a significant increase of FBG was found in fructose group when compared to control group. While FBG showed a significant decrease in (coumaric + fructose) group when compared to fructose group. From these results, we evaluated that fructose diet attributes in induction of hyperglycemia, but coumaric acid has the ability to partially improve this condition.

Elevated blood glucose, a component of metabolic syndrome, as observed in this study has been documented in high-fructose diet-fed rats and is associated with insulin resistance[32], P-coumaric acid prevents this action,

This is in agreement with[33] study which found that diabetic rats exhibited increased levels of plasma glucose when compared to normal control rats. Oral administration of *p*-coumaric acid to diabetic rats improved the glycemic status.

In agreement with Amalan et al., (2016) study in which the effect of p-CA was evaluated on changes in the levels of plasma glucose in normal and experimental rats. The glucose level significantly increased in STZ treated groups. Diabetic rats treated with oral supplementation of p-CA significantly reversed glucose level. There were no changes in plasma glucose and insulin levels were observed between normal and p-CA alone treated rats.

A significant increase of insulin in fructose group was found in comparison to control group. also, a significant increase of HOMA-IR was found in fructose group when compared to control group indicating development of hyperinsulineamia and insulin resistance as in agreement with[34]

Fructose-fed rats also display impairments in whole-body insulin sensitivity. Hyperinsulinemic-euglycemic clamp experiments demonstrated that fructose-fed rats had decreased glucose infusion rates as compared to control rats[35], hyperinsulineamia and insulin resistance were significantly reversed by coumaric acid administration as there was a significant decrease of insulin level in (coumaric + fructose) group when compared to fructose group and a significant decrease of HOMA-IR in (coumaric + fructose) group when compared to fructose group, this indicates that oral administration of coumaric acid partially improve sensitivity to insulin so decreasing level of insulin.

Regarding lipid profile, a significant increase of TC, TG and LDL and a significant decrease of HDLwere found in fructose group when compared to control group.

On the other hand, a significant decrease of TC, TG and LDL and a significant increase of HDL were found in (coumaric + fructose) group when compared to fructose group.

Elevated levels of TC, TAG, LDLc and VLDLc with concomitant reduction in HDLc reported in high-fructose diet-fed rats were consistent with previous studies**.**

Phenolic acids have been shown to regulate lipid metabolism in diabetic models[36]. Thus, Oral administration of *p*-coumaric acid to rats improved the lipid profile of Coumaric +fructose group and also could improve coronary artery and cardiovascular diseases.

Also, in agreement with Amalan et al., (2016) study which found that the concentrations of plasma lipids (TC and TGs) were increased in diabetic rats as compared to the normal control rats. Co-administartion of p-CA significantly reduced the concentrations of plasma lipids (TC and TGs). Liver and kidney of diabetic rats showed significant marked elevation in the levels of cholesterol and TGs when compared with normal rats. Upon oral supplementation of p-CA there was a significant reduction in the content of cholesterol and TGs in both the tissues,

In the present study, there A significant increase of ALT and AST were found in fructose group when compared to control group.

On the other hand, a significant decrease of ALT and AST were found in (coumaric + fructose) group when compared to fructose group.

Also, a significant decrease of ALT and AST were found in (coumaric + fructose) group when compared to control group.

Oral administration of *p*-coumaric acid to rats improved the hepatic functions of (coumaric +fructose) group.

In the current study, A significant decrease of glutathione and SOD and a significant increase of MDA were found in fructose group when compared to control group.

On the other hand, a significant increase of glutathione and SOD and a significant decrease of MDA were found in (coumaric + fructose) group when compared to fructose group.

While a significant increase of SOD and MDA and a significant decrease of gluta-

thione were found in (coumaric + fructose) group when compared to control group.
The administration of p-coumaric acid to rats significantly improved in the antioxidant status.
Free radical scavenging enzymes such as SOD and GPx are the first line of cellular defense against oxidative injury which is involved in the disposal of superoxide anions and H2O2 [37].
Increased oxidative stress biomarkers, such as MDA as noted in this study have been reported in high-fructose diet-fed rats[38].

The reversal of high-fructose diet-mediated increase in these parameters is linked to their antioxidant properties.
CAT and GPx activities were bought to near normal indicating the efficacy of *p*-coumaric acid in attenuating the oxidative stress in liver of diabetic rats.
Previous studies have also shown that phenolic compounds had free radical scavenging properties and reduced the oxidative stress associated with diabetes mellitus[39].

Abdel Reheim et al., (2018) study results also revealed that daily administration of GA and PCA to diabetic rats significantly increased the activities of antioxidant enzymes (SOD, CAT, GPx, and GST) and the levels of total thiols and GSH in the liver of diabetic rats. This could be due to decreased oxidative stress as evidenced by decreased LPO.

Contribution to authorship
All authors were involved in the writing of the manuscript and all authors made the decision to submit for publication.

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