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Effect of dapagliflozin on paracetamol-induced hepatotoxicity and nephrotoxicity in type 2 diabetic albino rats

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Abstract:
Introduction: paracetamol (PARA) is a worldwide antipyretic and analgesic medication. It has been extensively utilized during the last few years. PARA abuse would lead to liver and kidney injury. Dapagliflozin (DAPA)a sodium/glucose cotransporter 2 (SGLT2) inhibitor that wasapproved for treating patients with type 2 DM. In addition to its antidiabetic purpose, it has many pharmacological and biological actions, including antioxidant (radical scavenging), anti-inflammatory, and cardioprotective effects.Therefore, weaim to assess and evaluate the effect of dapagliflozin on paracetamol-induced hepatotoxicity and nephrotoxicity in rats with type 2 diabetic albino rats.
Materials and Methods: eighty Wistar male rats were grouped randomly into 8 equal groups (10 rats each). Oral distilled water was given to the control group, the paracetamol group received paracetamol 0.5 g/kg dissolved in purified water orally for 4 weeks, the dapagliflozin group received dapagliflozin 1 mg/Kg daily dissolved in distilled water for 4 weeks, the paracetamol + dapagliflozin groupreceivedparacetamol and were treated with dapagliflozin.Type 2 DM was induced in rats using by intraperitoneal Streptozotocin (STZ) single injection "50mg /kg".Rats were further subdivided into 4 groups.The control group with diabetes was given distilled water, the diabetic paracetamol group, the diabetic dapagliflozin group, and thediabetic paracetamol + dapagliflozin all received the same doses as mentioned. At the end of the experimental period, FBG, insulin, C peptide, lipid profile, liver function, fructosamine, CK-MB, TNF alpha, and IL4 were measured. Also, oxidative stress biomarkers were assessed in both kidney and liver tissues.
Results: Treatment of PARA-administered rats with Dapagliflozin significantly improved FBG, insulin, C peptide, lipid profile, liver function, fructosamine, CK-MB, TNF alpha, IL4, andOxidative stress biomarkers.
Conclusion: dapagliflozin has a protective effect against paracetamol-induced hepatotoxicity and nephrotoxicity.
Key Words:diabetes - paracetamol – Dapagliflozin – hepatotoxicity - nephrotoxicity.

Introduction:

Diabetes mellitus (DM) is a condition of hyperglycemia that occurred either due to abnormalities in insulin production, abnormal tissue resistance to insulin, or both. Chronicity of DM results in vasculature damage causing both microvascular and macrovascular complications such as atherosclerosis, and thrombosis affecting various organ systems mainly the eyes, nerves, kidneys, and heart [1].
Paracetamol / acetaminophen or N-acetyl-p-paminophenol (APAP) is an acylated aromatic amide derived from metabolism of phenacetin. It was first brought in 1893 into medicine by Von Mering as antipyretic and analgesic drug and it has been used for more than 30 years as a home analgesic.
Despite the safe usage of paracetamol in recommended doses, it may be harmful to the liver in acute overdose situations or even as an idiosyncrasy. As a result, overdose of paracetamol is one of the frequent causes of drug poisoning globally and has been identified as the primary cause of drug toxicity in the US,

UK, Australia, and Egypt [3,4]. Acute renal failure and renal tubular damage brought on by paracetamol are typically fatal in addition to hepatotoxicity, however some protectants may be able to stop them from happening [5].

It is quite challenging to determine a paracetamol hazardous dose. For healthy persons, a maximum intake of 4 grammes per day is advised. There is a plausible risk of drug toxicity when doses over 10 gm or 200 mg/kg are administered, either as a single dose or as several smaller doses over the course of 24 hours [6,7].

Paracetamol toxicity is related to the production of acetyl-para-aminophenol (APAP) and the hepatic cytochrome P450 system's reactive intermediate N-acetyl-p-benzoquinoneimine (NAPQI). Induction of CYP450 enzyme by drugs such as (INH, rifampin, anti-convulsant) increases (NAPQI) production beyond the hepatic detoxifying power. Excess NAPQI causes hepatocyte death by binding to cellular constituents [8].

Dapagliflozin (DAPA) is an antidiabetic drug that acts mainly by directly inhibition of sodium/glucose cotransporter 2 (SGLT2) on the convoluted tubule (CT) in the kidney aiming to decrease reabsorption of glucose by the kidney and increasing excretion of glucose in urine[9,10].Additional non-glycemic advantages of SGLT2 usage include decreased body weight, plasma uric acid, and blood pressure [11].

According to Wanner and his colleagues, patientswith type 2 DM treated with empagliflozin (SGLT2 inhibitor) had a decreased major cardiovascular events risk and a slower progression rate of kidney disease, including a decrease in albuminuria, a decrease in glomerular filtration rate (eGFR), and a decreased need for renal replacement therapy [12]. Serum ALT was significantly reduced in prior human studies evaluating the safety and effectiveness of SGLT2i(s) in T2DM patients [13]. Significant drops in body weight and glycated haemoglobin (HbA1c) were first thought to be the cause of this impact [14]. Nevertheless, another research revealed that the decrease in liver enzymes happens regardless of weight fluctuations [15]. Myeloperoxidase and reactive oxygen species, two indicators of oxidative stress and inflammation, are improved in patients with type 2 diabetes and fatty liver when dapagliflozin is used [16].

Materials and Methods

1- Experimental animals:

Wistar male adult rats (110–120 g weights) was acquired from the Egyptian Organization for Biological Product and Vaccine's animal care facility in Helwan, Cairo, Egypt. To exclude any concurrent and hidden infections, they were monitored for around 15 days before the start of the investigation. In the house of animal at the Zoology Department, Faculty of Science, Beni-Suef University, Egypt, the selected animals were kept in plastic cages with good, aerated covers (four per cage) at a normal temperature (25±5°) and with a normal light/dark cycle.

They are fed a regular feed with a known composition every day and have unrestricted access to water. The practical work started in November 2021. The Ethics Review Committee approved the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals for all experimental protocols detailed in this study at the Faculty of Science, Beni-Suef University, Egypt.(Ethical Approval Number: 021-213).

2- Drug and Chemicals:

Used drugs including nicotinamide (NA), Streptozotocin (STZ "2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glycopyranoside"), and Dapagliflozin powder were bought from AstraZeneca Pharmaceutical Corporation (Egypt) and suspended in distilled water. At 2 – 4°C both NA and dapagliflozin were stored, while at – 20°C Streptozotocin was kept. The other compounds were of proven quality and commercially available.

Induction of T2DM

STZ single injection (60 mg/kg) formulated in citrate buffer (pH 4.5) given intraperitoneally was used to induce experimental T2DM in overnight-fasted rats, 15 minutes following an administration of NA (120 mg/kg) melted in normal saline (0.9%). Seven days after STZ injection, animals that had fasted for 10 – 12 hours overnight were given glucose (3 g/kg) by orally, and serum glucose levels were determined in

lateral tail vein blood samples after 2 hours [17]. Rats with 200 - 300 mg/dL blood glucose levels were designated mild diabetics and included [18].

3- **Animals Grouping:**

Eighty Wistar male adult rats were grouped into 8 equal groups (10 rats / each group) as follows**:**

Group 1: (Control group): In this groups, an equivalent purified water volume was given to rats for 4 weeks every other day by oral gavage.

Group 2: (paracetamol group)administration of paracetamol 0.5 g/kg (dissolved in distilled water) for 4 weeks every other day by oral gavage.

Group 3: (dapagliflozin): rats was given dapagliflozin 1 mg/Kg daily (dissolved in distilled water) for 4 weeks by oral gavage.

Group 4:**(paracetamol + dapagliflozin)**: In this group Rats were given paracetamol as group 2 and were treated with dapagliflozin as group 3.

Group 5: (control for diabetic group): Diabetic rats were given distilled water by oral gavage for four weeks withoutfurther treatment.

Group 6: (diabetic paracetamol group) rats of this group were administrated with paracetamol in the same dose and duration as group 2.

Group 7: (diabetic dapagliflozin group) rats of this group were treated with dapagliflozin in the same dose and duration similar to group 3.

Group 8: (diabetic paracetamol + dapagliflozin) In this group Rats were given paracetamol as group 2 and were treated with dapagliflozin as group 3.

4- **Blood sampling**

Blood samples from the jugular veins of rats that had fasted overnight while under diethyl ether inhalation anesthesia were taken at the conclusion of the experiment.

After being drawn into gel and clot activator tubes, after blood clotting, the samples were centrifuged for fifteen minutes at 3000 rpm. The non-hemolyzed clear supernatant sera were quickly separated using a Pasteur pipette into Eppendorf tubes (3 tubes for every animal), they were stored at -80° C till time of need for additional parameters detection. After being put to sleep, the rats were promptly dissected.

5- **Biochemical Assay:**

A BIONIME GM100 glucometer is used to monitor serum fasting blood glucose levels every 30 minutes (at 0, 30, 60, 90, and 120 minutes)after taking glucose (3 g/kg) orally.

According to the manufacturer's protocol instructions, specific ELISA kits that was purchased from R and A Systems were used, USA, to determine levels of the following parameters: serum insulin, serum insulin C peptide, serum fructosamine, serum lipid profile (TG, total cholesterol, HDL, LDL), TNF-α, IL-4, Creatine Kinase MB (CK-MB), Serological assessment of liver functions (S. ALT, S. AST, S. ALP, and S. albumin), and levels of oxidative stress biomarkers (glutathione (GSH), lipid peroxide (malondialdehyde), and superoxide dismutase) were done.

6- **Histopathological examination:**

The paraffin blocks were serially sectioned at "3-5 μm" thickness. Afterward, stained with routine hematoxylin-eosin and were scored subjectively by pathologists using a light microscope, X 200 & X 400 HPF. The whole slide scan was done using a slide scanner (ScanScope APER10LV1-LEICA) and was analyzed using an image analysis program [19].

7- **Statistical Analysis**

By using SPSS statistical software (version 26) data collected were coded and analyzed. Findings and results were expressed using quantitative variables, mean and standard deviation. comparisons between the multiple comparisons were done using post hoc test and analysis of variance (ANOVA) [20]. P-values below 0.05 was considered statistically significant.

Results

Biochemical results:

Serum fasting blood glucose level (FBG)

Change in serum FBG level was insignificantly lower in normal para, normal dapa, and normal

mixed groups than in the control group by -11.35%, -3.43%, and -18.43% respectively ($p > 0.05$). The diabetic group exhibited significantly elevated levels of FBG, exceeding the control group by +189.97 ($p < 0.05$). In comparison to the diabetic group, paracetamol, dapagliflozin, and mixed treatment resulted in a significant reduction in fasting blood glucose (FBG), with the diabetic dapagliflozin group demonstrating a notable change of -41.22% ($p < 0.05$). **(table 1)**

S. Insulin and C – peptide levels

Insulin and C peptide serum levels were significantly higher in all normal groups compared to normal para group except the normal para group showed a statistically significant reduction by -9.82% ($p < 0.05$). In addition, the mixed normal group had significantly higher levels than the para-normal group but lower levels of c-peptide than rats in the normal dapa-group ($p < 0.05$). Also in the diabetic group, the reduction in serum insulin level and serum C-peptide was significantly lower than the normal control group by -73.41% and -80% respectively. All diabetic treatment groups revealed better results compared to the diabetic control group. The diabetic dapa-group showed the highest levels compared to other diabetic groups (p-value < 0.05). **(table 1)**

Serum lipid profile levels

The mean values of serum TG, Total Cholesterol, LDL, and VLDL were statistically significantly higher in the normal para group than in the control group by +25.96%, +27.08%, +52.61% and +25.96% respectively (p-value < 0.05). However, serum HDL was significantlylower in the normal para group than in the control group by -37.25% (p-value < 0.05).

Serum HDL was significantlyhigher in the normal dapa group than in the control group by +79.61% (p-value < 0.05), despite the nonsignificant change in serum TG, T.Cholesterol, and VLDL. In the normal mixed group, there was a statistically significant increase in serum HDL and LDL vs the control group by +53.54% and +34.97% respectively ($p < 0.05$). The normal mixed group showed better results regarding the lipid profile aspect than in the normal para group. Serum TG, T. Cholesterol, LDL, and VLDL were significantly elevated in the normal mixed group vs the normal dapa group ($p < 0.05$). And serum HDL showed a statistically significant decrease ($p < 0.05$).

In diabetic groups, the lipid profile showed worse results in the diabetic para group versus the diabetic group. On the other hand, the diabetic dapa group revealed better results compared to the diabetic and diabetic para groups ($p < 0.05$). Serum TG, Total Cholesterol, LDL, and VLDL exhibited a statistically significant reduction in the diabetic mixed group vs both diabetic and diabetic para groups ($p < 0.05$). But results were non-significant compared to the diabetic dapa group ($p > 0.05$) except serum HDL showed a significant decrease as opposed to the diabetic dapa group ($p < 0.05$). **(Table 2)**

Liver function tests (ALT, AST, ALP, Albumin, and T. bilirubin)

The normal para group presented higher levels of S. ALT, S. AST, S. ALP, and T. Bilirubin than the control group by +34.96%,+26.34%, +34.23%, and+30.00% respectively ($p < 0.05$) while, serum Albumin was lower by -14.28% ($p < 0.05$). Although the decrease in the normal dapa group was nonsignificant, T. Bilirubin significantly increased by +49.99% vs the control group. While the normal mixed group revealed decreased serum Albumin levels by -20.53% compared to the control group ($p < 0.05$). Both serum AST and ALP were lower in the normal dapa group compared to the normal para group ($p < 0.05$).

Regarding diabetic groups, serum AST and T. Bilirubin were increased in the diabetic para group vs the diabetic group by +13.18% and +19.67% respectively ($p < 0.05$). However, serum ALT, ALP, and albumin results were statistically non-significant. The diabetic dapa and mixed groups reported more reduction in liver functions than the diabetic para groups ($p < 0.05$). But the difference between the diabetic dapa and mixed groups was not significant. **(Table 3)**

Serum TNF alpha, IL-4 and fructosamine levels

In normal para group, the mean values of serum TNF alpha and IL-4 were statistically significantly increased than in the control group by +77.44% and+36.35% respectively ($p < 0.05$). Nevertheless, serum TNF alpha and IL-4 were significantly lower in the normal dapa and mixed groups than in the normal para group. **(Table 4)**

On the other hand, compared to the diabetic group serum TNF alpha and IL-4 were increased in the diabetic para group by +46.66% and +40.16% respectively (p-value <0.05). The rise in serum TNF alpha and IL-4 in the diabetic dapa and mixed groups was significantly lower than noticed in the diabetic para group. Similar results were observed regards *fructosamine levels.***(Table 4)**

Liver oxidative biomarkers (GSH, MDA, and SOD)

Both liver GSH and SOD were significantly low in the normal para group in comparison to the control group by -13.12% and -7.25% respectively (p <0.05) and a higher level of liver MDA by +88.24% (p <0.05). On the other hand, when compared to the normal para group, the normal dapa and mixed groups revealed a statistically significant elevation in liver Glutathione (GSH) and superoxide dismutase (SOD) and Plasma Malondialdehyde (MDA) reduction. The normal dapa group results were preferable than those of the normal mixed group regarding liver glutathione and plasma malondialdehyde levels. **(table 5)**

The mean values of liver glutathione and superoxide dismutase were lower in the diabetic para group vs the diabetic control group by -51.81% and -50.00% respectively (p < 0.05), and higher liver MDA in the diabetic para group by +15.99% (p <0.05). the diabetic dapa and mixed groups showed a significant rise in GSH and SOD and more decrease in MDA than the diabetic para group. **(table 5)**

Table (1): Serum FBG (mg/dl), Serum insulin (mg/dl) and C-peptide (ng/ml) levels for the different studied groups:

Groups / Parameter	Control group (Group 1)	Normal para group (Group2)	Normal dapa group (Group3)	Normal mixed group (Group 4)	Diabetic Group (Group5)	Diabetic para group (Group 6)	Diabetic dapa group (group 7)	Diabetic mixed group (group8)
FBG (mg/dl)	112.21± 2.33	99.47± 1.17	108.35± 1.70	91.52± 1.43	325.38± [a] 20.02	270.21± [b] 4.88	191.23± [be] 2.54	201.59± [be] 2.84
Change %		-11.35%	-3.43%	-18.43%	+189.97%	-16.95%	-41.22%	-38.04%
Insulin (ng/ml)	1.73± 0.08	1.56± [a] 0.02	1.95± [ac] 0.00	1.81± [c] 0.01	0.46± [a] 0.03	0.68± [b] 0.01	1.19± [be] 0.02	0.88± [bef] 0.01
Change %		-9.82%	+12.71%	+ 4.62%	-73.41%	+47.82%	+158.69%	+91.30%
C-peptide (ng/ml)	1.40± 0.06	1.60± [a] 0.03	2.02± [ac] 0.01	1.87± [acd] 0.01	0.28± [a] 0.02	0.70± [b] 0.01	1.21± [be] 0.02	0.90± [bef] 0.01
Change %		+14.29%	+44.29%	+33.57%	-80%	+150%	+ 332.14%	+221.43%

** Data are expressed as mean ± SE (standard error)*
** The number of animals in each group is 6.*
** % change was calculated by comparing normal-treated rats with normal control and diabetic-treated rats with diabetic control.*
**a: statistically significant from Control group at p-value ≤0.05*
**b:statisticallysignificantfrom Diabeticgroupatp-value≤0.05*

**c:statisticallysignificantfromnormal paragroupatp-value ≤0.05 *d:statisticallysignificantfromnormal dapa groupatp-value ≤0.05 *e: statistically significant from diabetic para group at p-value ≤0.05 *f: statistically significant from diabetic dapa group at p-value ≤0.05*

Table (2): serum TG (mg/dl), Total Cholesterol (mg/dl), HDL (mg/dl),LDL (mg/dl), and VLDL (mg/dl) levels for the different studied groups:

Group / Parameter	Control group (Group 1)	Normal para group (Group2)	Normal dapa group (Group3)	Normal mixed group (Group 4)	Diabetic Group (Group5)	Diabetic para group (Group 6)	Diabetic dapa group (group 7)	Diabetic mixed group (group8)
TG (mg/dl)	97.25± 2.60	122.50± 1.30 [a]	93.55 ± 1.77 [c]	108.34± 1.02 [c d]	154.94± 4.90 [a]	198.25± 4.45 [b]	127.13± 1.67 [be]	135.41± 2.22 [be]
%change		+25.96%	-3.81%	+11.40%	+59.32%	+27.96%	-17.95%	-12.60%
Total Cholesterol (mg/dl)	107.96± 2.90	137.20± 1.46 [a]	104.77± 1.98 [c]	121.34± 1.14 [cd]	170.32± 5.91 [a]	222.05± 4.98 [b]	142.39± 1.87 [be]	152.02± 2.32 [be]
%change		+27.08%	-2.95%	+12.39%	+57.76%	+30.37%	-16.40%	-10.75%
HDL (mg/dl)	32.38± 1.36	20.32± 0.46 [a]	58.16± 1.05 [ac]	49.72± 0.49 [acd]	21.38± 0.71 [a]	15.95± 0.97 [b]	49.46± 1.02 [be]	44.19± 0.87 [bef]
%change		-37.25%	+79.61%	+53.54%	-33.98%	-25.40%	+131.34%	+106.69%
LDL (mg/dl)	56.19± 1.95	85.75± 0.91 [a]	65.49± 1.24 [c]	75.84± 0.71 [ad]	117.95± 4.50 [a]	138.79± 3.11 [b]	89.00± 1.17 [be]	95.01± 1.45 [be]
%change		+52.61%	+16.55%	+34.97%	+109.92%	+17.66%	-24.55%	-19.45%
vLDL (mg/dl)	19.45± 0.52	24.50± 0.26 [a]	18.71± 0.35 [c]	21.67± 0.20 [cd]	31.00± 0.98 [a]	39.65± 0.89 [b]	25.42± 0.33 [be]	27.08± 0.45 [be]
%change		+25.96%	-3.80%	+11.41%	+59.37%	+27.91%	-17.98%	-12.63%

** Data are expressed as mean ± SE (standard error)*
** The number of animals in each group is 6.*

** % change was calculated by comparing normal-treated rats with normal control and diabetic-treated rats with diabetic control.*
**a: statistically significant from the Control group at p-value ≤0.05.*
**b: statistically significant from the Diabetic group at p-value ≤0.05.*
**c: statistically significant from the normal para group at p-value ≤0.05.*
**d: statistically significant from normal dapa group at p-value ≤0.05.*
**e: statistically significant from the diabetic para group at p-value ≤0.05.*
**f: statistically significant from diabetic dapa group at p-value ≤0.05.*

Table (3): serum ALT(U/L), AST(U/L), ALP(U/L), Albumin(g/dl) and T. Bilirubin(mg/dl) levels for the different studied groups.

Group / Parameter	Control group (Group 1)	Normal para group (Group2)	Normal dapa group (Group3)	Normal mixed group (Group 4)	Diabetic Group (Group5)	Diabetic para group (Group 6)	Diabetic dapa group (group 7)	Diabetic mixed group (group8)
ALT (U/L)	30.97± 1.01	41.79±[a] 1.11	30.61± 0.29	36.01± 0.89	98.95±[a] 6.03	103.30± 3.32	83.52±[be] 1.70	89.77±[be] 0.71
%change		+34.96%	-1.15%	+16.30%	+219.55%	+4.39%	-15.60%	-9.28%
AST (U/L)	43.01± 1.39	54.33±[a] 1.44	39.79±[c] 0.37	46.82± 1.16	118.65±[a] 2.27	134.29±[b] 4.31	108.57±[be] 2.21	116.70±[e] 0.92
%change		+26.34%	-7.47%	+8.87%	+175.89%	+13.18%	-8.50%	-1.65%
ALP (U/L)	68.81± 2.22	92.36±[a] 2.45	67.66±[c] 0.63	79.60±[ab] 1.98	219.85±[a] 3.64	228.29± 7.33	184.58±[be] 3.77	198.39±[be] 1.57
%change		+34.23%	-1.67%	+15.68%	+219.50%	+3.84%	-16.04%	-9.76%
Albumin (g/dl)	3.73± 0.19	3.20±[a] 0.04	3.47± 0.06	2.97±[ad] 0.04	2.40±[a] 0.07	2.33± 0.06	2.93±[be] 0.06	2.63± 0.06
%change		-14.28%	-7.14%	-20.53%	-35.71%	-2.78%	+22.22%	+9.72%
T. Bilirubin (mg/dl)	0.67± 0.08	0.87±[a] 0.02	1.00±[a] 0.04	0.80± 0.04	2.03±[a] 0.11	2.43±[b] 0.06	1.70±[be] 0.04	1.77±[e] 0.06

%change		+30.00%	+49.99%	+19.99%	+204.98%	+19.67%	-16.39%	-13.11%

** Data are expressed as mean ± SE (standard error)*
** The number of animals in each group is 6.*
** % change was calculated by comparing normal-treated rats with normal control and diabetic-treated rats with diabetic control.*
**a: statistically significant from Control group at p-value ≤0.05*
**b:statisticallysignificantfrom Diabeticgroupatp-value≤0.05*
**c:statisticallysignificantfromnormal paragroupatp-value ≤0.05*
**d:statisticallysignificantfromnormal dapa groupatp-value ≤0.05 *e: statistically significant from diabetic para group at p-value ≤0.05*

Table (4): TNF alpha(pg/ml) and IL-4(pg/ml) levels for the different studied groups:

Group / Parameter	**Control group (Group 1)**	**Normal para group (Group2)**	**Normal dapa group (Group3)**	**Normal mixed group (Group 4)**	**Diabetic Group (Group5)**	**Diabetic para group (Group 6)**	**Diabetic dapa group (group 7)**	**Diabetic mixed group (group8)**
TNF alpha (pg/ml)	53.33± 1.64	94.63±[a] 2.02	53.72±[c] 1.93	74.42±[acd] 1.99	113.28±[a] 5.75	166.14±[b] 3.74	110.44±[e] 2.57	130.37±[bef] 3.29
%change		+77.44%	+0.74%	+39.56%	+112.43%	+46.66%	-2.51%	+15.08%
IL-4 (pg/ml)	22.34± 0.25	30.46±[a] 0.42	21.21±[c] 0.24	23.54±[c] 0.18	32.25±[a] 0.95	45.20±[b] 0.27	24.47±[be] 0.39	31.36±[ef] 0.27
%change		+36.35%	-5.06%	+5.37%	+44.36%	+40.16%	-24.12%	-2.76%
fructosamine (µmol/L)	134.68± 2.80	218.76±[a] 5.19	138.75±[c] 5.07	192.39±[a] 5.16	488.07±[a] 30.04	429.58±[b] 9.67	285.58±[be] 6.63	337.09±[be] 8.51
%change		+62.43%	+3.02%	+42.85%	+262.39%	-11.98%	-41.49%	-30.93%

** Data are expressed as mean ± SE (standard error)*
** Number of animals in each group is 6.*
** % change was calculated by comparing normal treated rats with normal control and diabetic treated rats with diabetic control.*
**a: statistically significant from Control group at p-value ≤0.05 *b:statisticallysignificantfrom Diabeticgroupatp-value≤0.05 *c:statisticallysignificantfromnormal paragroupatp-value ≤0.05 *d:statisticallysignificantfromnormal dapa groupatp-value ≤0.05 *e: statistically significant from diabetic para group at p-value ≤0.05 *f: statistically significant from diabetic dapa group at p-value ≤0.05*

Table (5): Liver oxidative biomarkers GSH (mmol/g), MDA (nmol/g) and SOD (U/g) levels for the different studied groups

Group / Parameter	Control group (Group 1)	Normal para group (Group2)	Normal dapa group (Group3)	Normal mixed group (Group 4)	Diabetic Group (Group5)	Diabetic para group (Group 6)	Diabetic dapa group (group 7)	Diabetic mixed group (group8)
GSH (mmol /g)	2.62± 0.08	2.27±[a] 0.04	2.87±[ac] 0.02	2.64±[cd] 0.02	0.83±[a] 0.03	0.40±[b] 0.02	1.74±[be] 0.03	1.28±[bef] 0.02
%change		-13.12%	+9.81%	+0.76%	-68.28%	-51.81%	+109.64%	+54.22%
MDA (nmol / g)	1.02± 0.04	1.92±[a] 0.03	0.77±[ac] 0.03	1.07±[cd] 0.03	2.94±[a] 0.05	3.41±[b] 0.05	1.60±[be] 0.04	1.88±[bef] 0.05
%change		+88.24%	-24.51%	+5.23%	+187.91%	+15.99%	-45.63%	-35.98%
SOD (U/g)	2.34± 0.06	2.17±[a] 0.03	2.74±[ac] 0.02	2.51±[acd] 0.01	0.70±[a] 0.02	0.35±[b] 0.02	1.66±[be] 0.03	1.22±[bef] 0.02
%change		-7.25%	+16.79%	+7.11%	-70.13%	-50.00%	+136.67%	+74.29%

** Data are expressed as mean ± SE (standard error)*
** The number of animals in each group is 6.*
** % change was calculated by comparing normal-treated rats with normal control and diabetic-treated rats with diabetic control.*
**a: statistically significant from Control group at p-value ≤0.05*
**b:statisticallysignificantfrom Diabeticgroupatp-value≤0.05*
**c:statisticallysignificantfromnormal paragroupatp-value ≤0.05*
**d:statisticallysignificantfromnormal dapa groupatp-value ≤0.05 *e: statistically significant from diabetic para group at p-value ≤0.05 *f: statistically significant from diabetic dapa group at p-value ≤0.05*

Histological Examination:

***Pancreas*:**

In the control group, the section in normal pancreatic tissue showed normal pancreatic acini, islets of Langerhans, dilated duct, and vascular channel **Fig. (1)**. However, diabetic rats' pancreatic tissue showed vacuolar degeneration and destructed islets of Langerhans by its infiltration with chronic inflammatory cells and fibrosis. **Fig. (2)**.

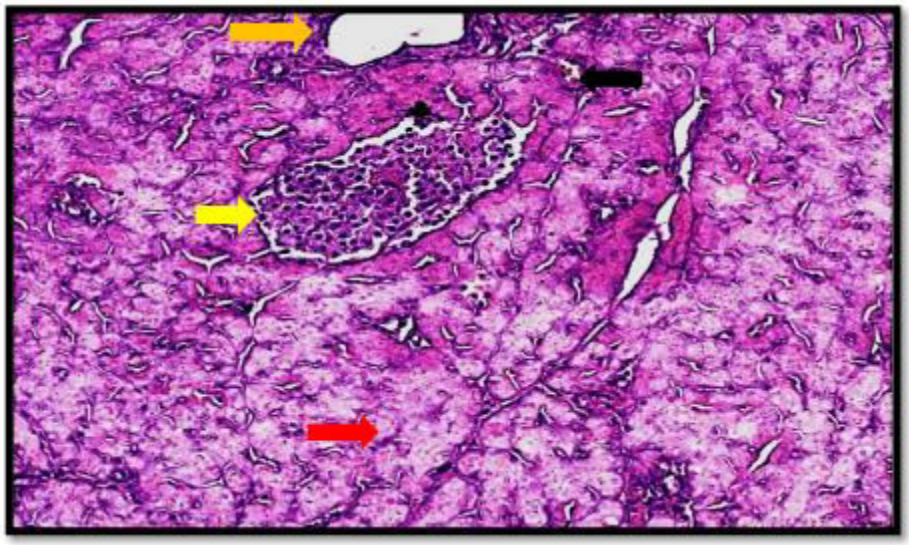

Fig. (1): Section in normal pancreatic tissue (hematoxylin & eosin x200) showing normal pancreatic acini (red arrow), islets of Langerhans (yellow arrow), dilated duct (black arrow), and vascular channel (orange arrow).

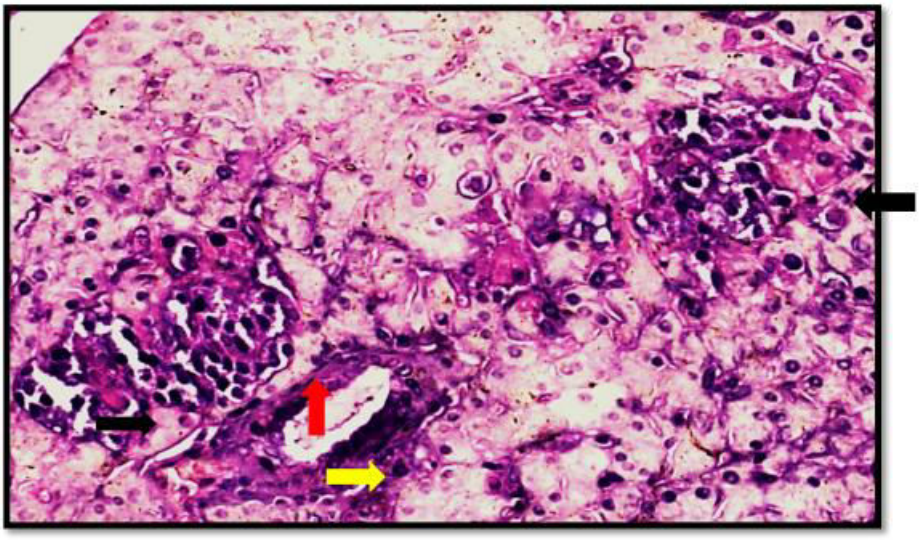

Fig. (2): Section in untreated diabetic rat (hematoxylin & eosin x400), pancreatic tissue showing vacuolar degeneration and destructed islets of Langerhans (dark arrow) characterized by its infiltration with chronic inflammatory cells (red arrows) and periductal fibrosis (yellow arrow).

Liver:

The liver tissue in the control group had a central vein, normal hepatocytes organized in cord plates, and normal architecture **Fig. (3)**. However, in normal rats administered paracetamol, liver tissue showed marked pericentral and peri portal inflammations with congested dilated veins **Fig. (4)**. The normal rats managed by dapagliflozin exhibit mild peri portal inflammations in their liver tissue with mildly dilated

congested veins, the hepatocyte showed normal pattern and architecture **Fig. (5)**. In normal rats treated with paracetamol + dapagliflozin, liver tissue had moderate pericentral inflammations with moderately dilated congested central vein, and the hepatocyte showed vacuolar degeneration and interface hepatitis **Fig. (6)**.

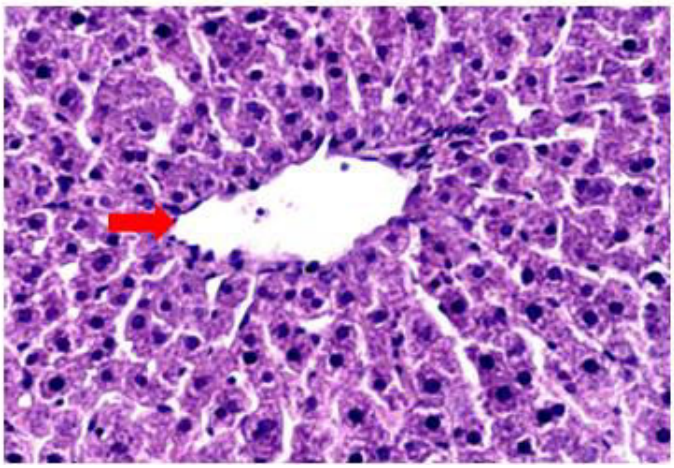

Fig. (3): Control group, normal liver tissue architecture with normal hepatocytes arranged in cord plates, (red arrow) shows a central vein lined by regular endothelial cells (H&E X 400).

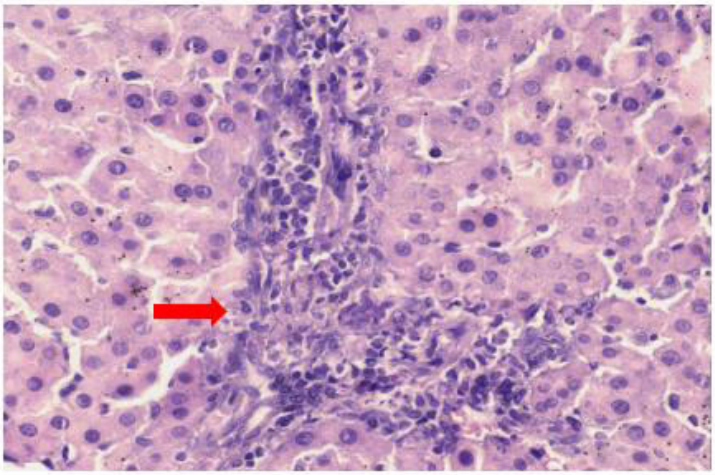

Fig. (4): NP group, liver tissue shows marked portal and peri portal inflammations with interphase hepatitis, hepatic cell necrosis, and degeneration (red arrow) (H&E X 400).

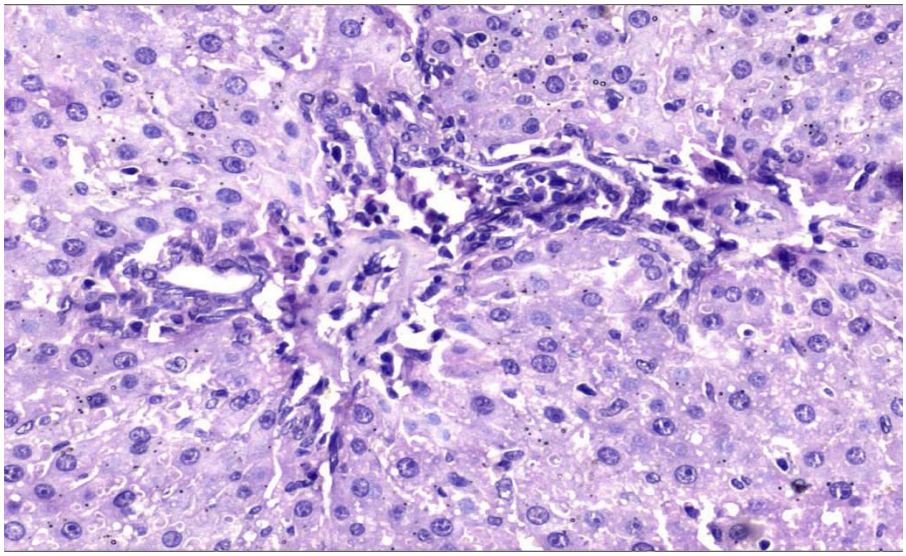

Fig. (5): Dapa group, liver tissue shows mild peri portal inflammations with mildly dilated congested vein, the hepatocyte showed normal pattern and architecture (H&E X 400).

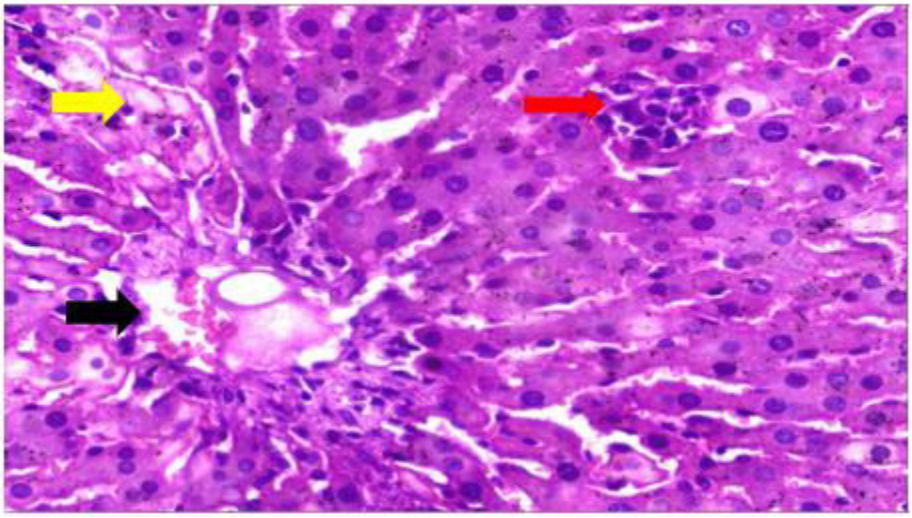

Fig. (6):mn group, liver tissue shows moderate peri central inflammations with moderately dilated congested central vein (black arrow), the hepatocyte showed vacuolar degeneration (yellow arrow) and interface hepatitis (red arrow) (H&E X 400).

Diabetic rat's liver tissue showed marked interface hepatitis with Porto-portal inflammations and fibrosis, the hepatocyte showed marked vacuolar degeneration **Fig. (7)**. In paracetamol-administrated diabetic rats, liver tissue had a marked periportal inflammation with dilated congested veins and sinusoids, the hepatocyte showed vacuolar degeneration **Fig. (8)**. The liver tissue of dapagliflozin-treated diabetic rat showed moderate peri portal inflammation with moderately dilated congested veins and the hepatocyte showed multiple scattered necrotic foci **Fig. (9)**. In paracetamol+ dapagliflozin treated diabetic rats, liver tissue showed normal architecture with normal hepatocytes arranged in cord plates and showed mildly dilated congested central vein **Fig. (10)**.

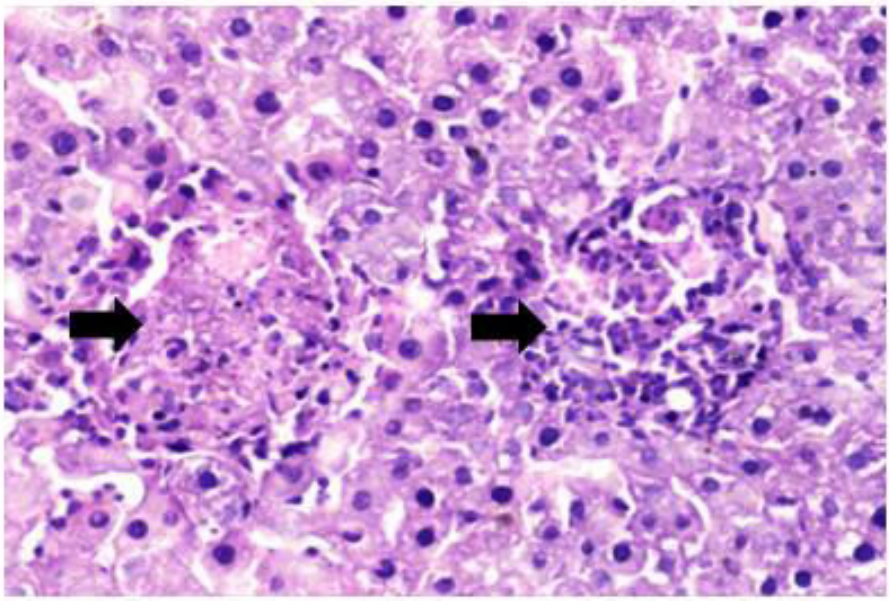

Fig. (7): Dc group, liver tissue shows marked interface hepatitis, the hepatocyte showed focal areas of necrosis and degeneration (H&E X 400).

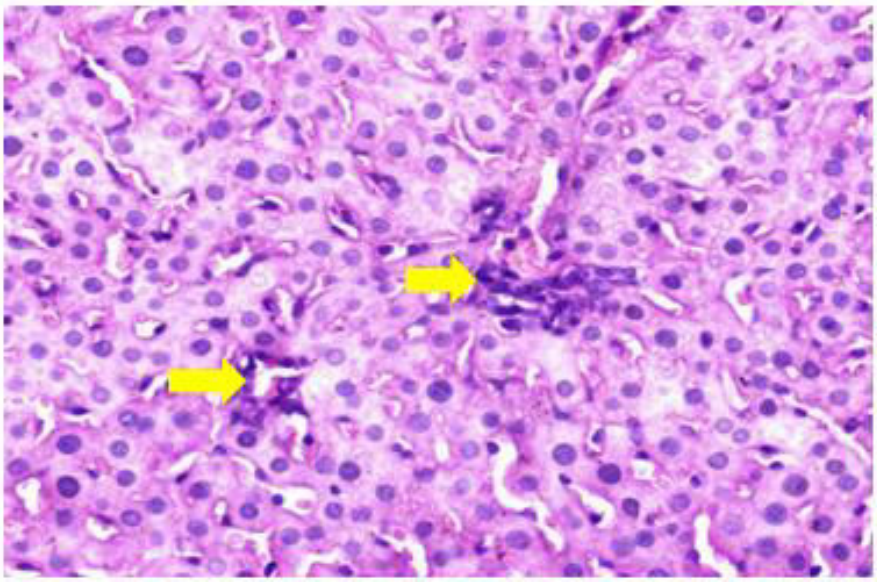

Fig. (8):DP group, liver tissue shows marked peri portal inflammations with dilated congested veins and sinusoids, the hepatocyte showed vacuolar degeneration (H&E X 400).

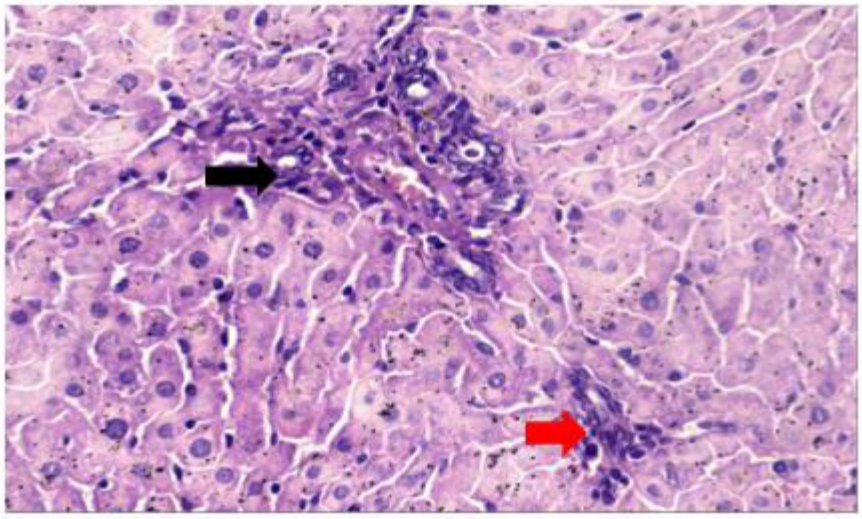

Fig. (9):dd group, liver tissue shows moderate peri portal inflammations with moderately dilated congested vein (black arrow), and the hepatocyte showed interface hepatitis (red arrow) (H&E X 400).

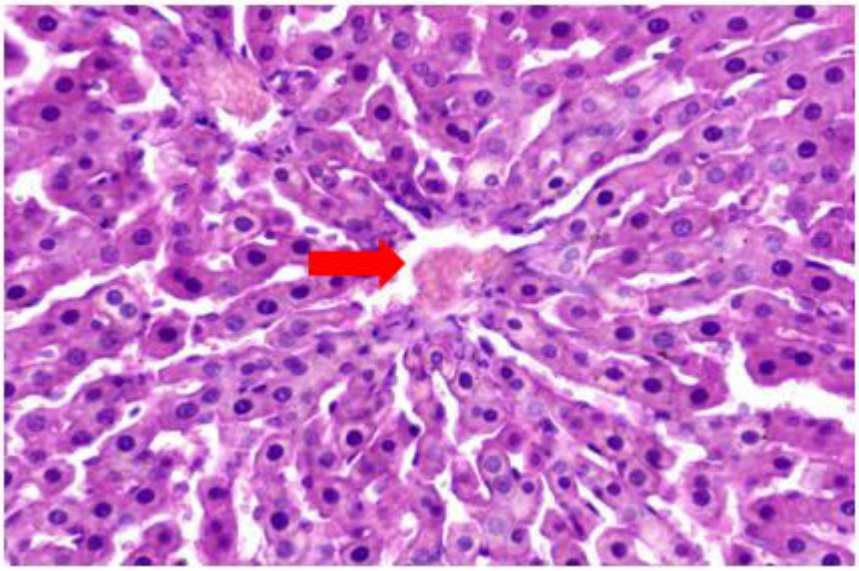

Fig. (10): md, liver tissue showed normal architecture with normal hepatocytes arranged in cord plates, (red arrow) show mildly dilated congested central vein (H&E X 400).

Discussion:

Our goal is to evaluate the dapagliflozin effect on paracetamol-induced hepatotoxicity and nephrotoxicity in type 2 diabetic albino rats. In agreement with**Songping*et al***, we found that serum FBG was significantly lower in the diabetic mixed group compared to both the diabetic and diabetic para groups [21]. This is mediated by inhibition of SGLT2 receptors -presented in the PCT- leading to renal threshold reduction and more loss of glucose in the urine [22,23]. Also, parallel with **Wei *et al***, both S. insulin and C – peptide was significantly increased in the diabetic mixed group vs in diabetic and diabetic para groups. Dapagliflozin increased islet and beta cell numbers indicated by the C-peptide level. Additionally, under high glucose levels, it increased the expression of markers unique to beta cells and pancreatic endocrine progenitors [24].

In the present study, paracetamol administration exhibited a significantlyelevatedS. TG, T. Cholesterol, LDL, and VLDL and reduction in HDL vs control groups. This was in parallel with what is reported in the literature [25,26].It is also observed that dyslipidemia was aggravated in diabetic rats due to pre-

existing lipid profile disturbance mediated by increased lipolysis, increased TG production, and reduced FFA absorption by adipocytes [27,28]. On the other hand, similar to **Piccirillo *et al***, dapagliflozin-treated rats revealed favorable lipid profile results [29]. Dapagliflozin has an antioxidant effect through lipid peroxidation reduction and downregulation of NADPH, ROS, and MDA levels [30,31]. Also, the produced glucosuria decreases systemic glucotoxicity, enhances insulin sensitivity, and decreases oxidative stress and cellular lipotoxicity by transferring the metabolic energy to lipids through beta-oxidation [32,33].In contrast, **El Medany**and his colleague reported that treatment of diabetic rats with dapagliflozin increased LDL levels [34]. In the present study, this effect only appeared in normal rats treated with dapa and not in diabetic rats. By promoting VLDL-C conversion to LDL-C and reducing LDL receptor-mediated LDL clearance, **Basu et al.** provided an explanation for the potential elevated LDL-C level[35].

In harmony with **Marzouk *et al.,*** we found that paracetamol-administrated rats had a significantlyhigh liver enzymes (S.ALT and S.AST),S.ALP, and T.Bilirubin, and decreased Albumin compared to the control group [25]. This indicates paracetamol-induced liver impairment and cellular injury [36,37]. This injury may be mediated by the oxidative paracetamol metabolites (PAPA, NAPQI, etc..), nitric oxide overproduction, and subsequent depression of intracellular GSH [38].Consistent with **Arise &Oguntibeju**reported that in streptozotocin-induced diabetic rats, changes in blood enzymes are strongly related to changes in the metabolic activities of S. AST, S. ALT, S. albumin, and bilirubin [27]. Increased production of glucose “gluconeogenesis” and ketone bodies “ketogenesis” during diabetes have been linked to elevated aminotransferase activity during insulin insufficiency [39].There was significant increase in liver enzymes “AST, ALT”, alkaline phosphatase and bilirubin in Rats treated with Dapagliflozincompared to paracetamol-administrated rats. thiswas in line with those of Wang et al., who found that a 4-week treatment of dapagliflozin dramatically decreased the elevated levels of fat, oxidative stress, fibrosis, and liver enzyme activity [40].

Regarding serum TNF alpha and IL-4, our results are in parallel with **Bourdi et al.,** reporting that the serum TNF alpha, interleukin 4, 13, and notably10, were increased after paracetamol treatment [41]. Oxidative stress activates pro-inflammatory genes leading to overproduction od ROS by inflammatory cells. Numerous diseases start and worsen as a result of this vicious cycle [42]. There is accumulating evidence that paracetamol intoxication can increase the blood levels of many proinflammatory cytokines. Signal transduction pathways, like NF-κB and AP-1, are promoted by oxidative stress [43]. The administration of paracetamol activates the NF-κB protein, leading to increased activity of TNF-α, iNOS, and COX-2 [44]. Serum TNF alpha and IL-4 levels were considerably lower in rats treated with Dapa than in rats treated with paracetamol. These findings are one with **Saleh et al.,** who found that dapagliflozin causes significant reduction of serum MDA and TNF-α and suppression of mitochondrial respiration causing an increase in the activity of antioxidant enzyme and diminishing production of ROS in diabetic rats [45, 46]. In our study, the histological alterations in the treated rats' liver and pancreas were closely associated with reduced IL4 following dapagliflozin administration..

Conclusion:

It could be concluded that treatment with dapagliflozin protects against paracetamol-induced hepatotoxicity. The possible mechanism for the observed preventive effects of p-coumaric acid is due to its hypoglycemic properties and significant protective effect against oxidative stress and inflammation. According to our study, dapagliflozin improves liver functions. Also, it is beneficial for defense against diabetes and its complications. Thus, dapagliflozin could help in shielding diabetic patients from paracetamol-induced hepatotoxicity.

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