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Sitagliptin ameliorates thioacetamide-induced acute liver injury via modulating TLR4/NF-KB signaling pathway in mice

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ABSTRACT

Sitagliptin is an oral hypoglycemic drug that acts by selective inhibition of dipeptidyl peptidase-4 (DDP-4) enzyme.

Aim: This study scrutinized the hepatoprotective impact of sitagliptin) against thioacetamide (TAA)-induced liver damage in mice.

Main methods: Male mice were injected with TAA (500 mg/kg) then treated with sitagliptin (20 mg/kg) orally for 5 days.

Key findings: Histopathological results of TAA group revealed severe degree of centrolobular hepatic necrosis. Additionally, biochemical findings showed marked elevation in the serum transaminases and gamma glutamyl transpeptidase (GGT) levels in TAA group. Injection of TAA significantly disrupted oxidant/antioxidants hemostasis of the hepatic tissues. Also, TAA markedly increased the expression of nuclear factor kappa-B (NF-KB); and enhanced Toll like receptor 4 (TLR4) as well as NLPR3 inflammosome production. Moreover, there was an elevation in the hepatic levels of tumor necrosis factor-alpha (TNF-α) and interleukin -1 beta (IL-1β) besides increased immunoexpression of Bcl-2-associated X protein (Bax) as well as caspase 3. In contrast, treatment with sitagliptin significantly attenuated TAA-induced histopathological, biochemical and immunohistochemical alterations.

Significance: Our results suggest that the hepatoprophylactic impact of sitagliptin might be arbitrated via modulating TLR4 and NK-KB signaling cascade followed by depression of inflammation besides apoptosis.

1. Introduction

Liver is an essential organ that has a pivotal role in detoxification, metabolism, storage of glycogen, production of plasma proteins toxins, and regulation of cholesterol levels [6,42]. Liver diseases are diverse; out of them are viral-induced hepatitis, fibrosis, hepatocellular carcinoma and drug-induced liver toxicity. These liver diseases induce alterations in the physiological functions of the body and may result in coma and death if they are left untreated [37]. Some animal models have been established to evaluate the efficacy of drugs against liver diseases and their mechanisms of action [22].

Thioacetamide (TAA) is utilized as a fungicide and considered a source of sulfur in industrial field [20]. Mainly, it is a standard hepatotoxic compound which induces oxidative stress and inflammation associated with enhanced plasma transaminases and resulted in liver injury [14]. TAA undergoes oxidative bioactivation and transformed into its *S*-oxide (TASO) and then to *S,S*-dioxide ($TASO_2$),the chemically reactive form that eventually modify proteins and lipids leading to further systemic oxidative stress [15]. Injection of TAA in animals induced boosted lipid peroxidation, generation of reactive oxygen species (ROS) as well as enhancement of Nuclear factor Kappa B (NF-κB) production and consequently proinflammatory cytokines [33].

Sitagliptin is a drug that selectively inhibits dipeptidylpeptidase-4 (DPP4) and used in management of diabetic patients of type II [26]. DPP-4 spreads in many body organs exerting various biological activities. It stimulates disintegration of the two incretin hormones in serum; glucose-dependent insulino-tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) [21]. These hormones stimulate insulin secretion from the b-cells and inhibit secretion of glucagon secretion. The antidiabetic effect of sitagliptin may be due to the enhancement of the incretin function. Thus, inhibition of DPP4 by sitagliptin could increase the bioavailability of these hormones and subsequently decrease serum glucose level [36]. It is believed that DPP-4 is involved in the regulation of cytokines and implicates the signaling functions. Recently, sitagliptin has proven to be hepatoprotective in experimentally induced steatohepatitis [19] and methotrexate induced hepatotoxicity [3] via

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inhibition of inflammation and apoptosis.

So far, the impact of sitagliptin against TAA-induced acute liver injury has not been reported. Thus, this study was designed to explicate the probable curative effect of sitagliptin against TAA-induced hepatotoxicity and shed light on the underlying mechanisms.

2. Materials and methods

2.1. Animals

Thirty male Swiss albino mice (25–30 g) were purchased from VACSERA (Helwan, Egypt). Animals had free access to food and water throughout the whole experiment. The experimental procedures done in this study comply with laboratory animal care and use of recommendations approved by "Research Ethics Committee", Faculty of Pharmacy, Mansoura University.

2.2. Drugs and chemicals

Thioacetamide was obtained from Sigma–Aldrich (St. Louis, MO, USA). Sitagliptin phosphate under the brand name of Januvia was purchased from Merck Sharp and Dohme, a subsidiary of merck & CO. Inc. (Whitehouse station, NJ, USA). Other chemicals were purchased of best grades from available companies.

2.3. Experimental protocol

Induction of liver injury was achieved by single injection of TAA (500 mg/kg, I.P.) dissolved in normal saline [23]. Also, the tested drug was dissolved in normal saline.

Mice were randomly divided into:

1) Control group ($n = 6$) received normal saline.
2) TAA group ($n = 8$) injected with TAA only, then left untreated.
3) TAA/Sita group ($n = 8$), injected with TAA then received 20 mg/kg sitagliptin orally [3]. The first dose of sita was given 2 h after TAA injection and continued for the following four days.
4) Sita group ($n = 8$), received 20 mg/kg sitagliptin orally for five consecutive days.

At day 6, all animals were anesthetized with thiopental (50 mg/kg, ip) then sacrificed. Samples of blood were obtained, centrifuged, and then serum was collected for biochemical analysis. Liver was harvested and cut into 2 portions. The first one was used to prepare liver homogenate (10% w/v in phosphate buffer, pH 7.5) and the other portion was kept in 10% v/v buffered formalin for histopathology.

2.4. Assessment of liver functions

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, and gamma glutamyl transpeptidase (GGT) were measured by means of Human kits (Germany).

2.5. Evaluation of oxidative stress

Lipid peroxidation was indirectly measured via evaluating hepatic malondialdehyde level as defined by Siddique [35]. Briefly, sodium dodecyl sulfate, acetic acid, and thiobarbituric acid were added in a test tube. The liver homogenate was added to the reaction mixture and then heated at 90 °C for 60 min. After cooling, the resulting pink color was assessed spectrophotometrically [35].

Hepatic reduced glutathione (GSH) level was determined as demonstrated by Moron [28] with some modifications. Briefly, 50 µl of 50% (w/v) trichloro-acetic acid were mixed with 450 µl of liver homogenate then centrifuged. 250 µl of supernatant were added to 1 ml of 0.2 M Tris–HCl (pH 8.9) and 50 µl of 0.01 M Ellman reagent and kept at 25 °C for 5 min. The resultant yellow color was measured spectrophotometrically [28].

The enzymatic activity of superoxide dismutase (SOD) was assessed in accordance to the method of Marklund and Marklund [24]. In brief, 100 µl of homogenate plus 25 µl pyrogallol (24 mmol/l prepared in 10 mM HCl) were mixed with 2875 µl Tris HCl buffer (0.1 M, pH 7.8). Then, the alteration in optical density at 420 nm was monitored for 3 min at 1-min interval using spectrophotometer [24].

Nitric oxide content was evaluated using the technique of Miranda et al. [27]. 0.5 ml of liver homogenate was added to 0.25 ml 0.3 N NaOH, and incubated for 5 min at 25 °C, then mixed with 0.25 ml of 5% (w/v) $ZnSO_4$ (for deproteinization). At that time centrifugation was done and 0.3 ml Griess reagent and 0.3 ml VCl_3 (8 mg/ml) in 1 N HCl were added to 0.3 ml of the obtained supernatant. After incubation at 37 °C for 45 min, absorbance was determined spectrophotometrically at 540 nm against blank [27].

2.6. Estimation of hepatic toll like receptor 4 (TLR4) and NLRP3 inflammasome

Hepatic levels of TLR4 and NLRP3 inflammasome were measured by MyBioSource and Shanghai BlueGene Biotech CO., LTD Elisa kits according to the manufacturers' instructions.

2.7. Measurement of hepatic inflammatory markers

Hepatic levels of nuclear factor kappa B (NF-KB) and intrerleukin 1-β (IL-1β) were estimated using by MyBioSource CO Elisa kit; Tumor necrosis factor-α (TNF-α) levels were measured using Abcam Elisa kit following the guidelines provided.

2.8. Determination of hepatic Bcl-2-associated X protein (Bax)

Hepatic levels of Bax were measured by Biovision CO. Elisa kit following the manufacturers' protocol.

2.9. Hepatic histopathological evaluation

Hepatic tissues that were fixed in formalin underwent entrenching in paraffin and sectioning at 5-µm thickness. Liver specimens were stained by hematoxylin-eosin (H&E) then scrutinized by a pathologist in random manner.

2.10. Immunohistochemical (IHC) assessment of BCL2 associated X protein (Bax), caspase 3 and NF-KB

Hepatic levels of Bax, caspase 3 and NF-KB were measured by IHC evaluation as follow: Paraffin-entrenched sections were deparaffinized with xylene then dehydrated, sections were treated with H_2O_2 in methanol for 10 min. to inhibit endogenous peroxidase activity. After that, they were processed in EDTA and incubated for 1 h at 37 °C with antibodies against: Bax, caspase 3 and nuclear factor kappa B, dilution ratio (1/100). All steps were carried at 25 °C in accordance to the manufacturer's guidelines by means of HRP-DAB detection system. Binding was envisaged with 3,3′-diaminobenzidine and sections were inspected after counterstaining with hematoxylin. Slides were observed by light microscope for Bax, caspase-3 and NF-KB positive cells. Assessment of immunostaining was carried out by determining the percentage of positive areas using image J analysis software.

2.11. Statistical analysis

Values are presented as mean ± S.E. Statistical analyses were performed using one way analysis of variance (ANOVA), followed by the Tukey–Kramer multiple comparison test. Statistical analysis was performed using Instat software III and GraphPad Instat V 3.05

Table 1
Effect of TAA (500 mg/kg) and/or Sita (20 mg/kg) on liver functions.

Groups	ALT (U/l)	AST(U/l)	GGT (U/l)	Albumin (g/dl)
Control	58.8 ± 4.3	66.6 ± 5.3	1.8 ± 0.11	3.8 ± 0.03
TAA	442 ± 35.2*	456.3 ± 39.4*	5.9 ± 0.22*	2.7 ± 0.06*
TAA/Sita	73.1 ± 6.8$	116.5 ± 4.4$	3.3 ± 0.2$	3.4 ± 0.22$
Sita	62.2 ± 4.5$	69.5 ± 6.3$	1.9 ± 0.09$	3.7 ± 0.05$

Data expressed as means ± SEM. Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test.
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase.
* $p < 0.05$ vs. control.
$ $p < 0.05$ vs. TAA.

(GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Effect of TAA and/or Sita on liver functions

Injection of TAA produced a profound increase in serum levels of ALT, AST and GGT when compared with the control group, concomitant with a marked decline in albumin serum levels. Oral administration of Sita significantly attenuated TAA-induced alterations in serum biochemical parameters compared with TAA-treated mice (Table 1).

3.2. Effect of TAA and/or Sita on hepatic antioxidant status

TAA (500 mg/kg) significantly increased hepatic malondialdehyde (MDA) levels with profound reduction in levels of GSH and SOD in comparison with the control group. Sita administration significantly reversed TAA-induced alterations compared to TAA-treated mice. Concerning NO content, there was no significant difference between different groups (Table 2).

3.3. Effect of TAA and/or Sita on TLR4 and NLRP3

TAA-injected mice exhibited higher levels of hepatic TLR4 and NLRP3 than the control group. Mice treated with TAA and Sita presented a profound decrease in the levels of hepatic TLR4 and NLRP3 compared to the TAA-treated nice (Fig. 1A and B).

3.4. Effect of TAA and/or Sita on inflammatory markers

TAA injection produced a marked elevation in hepatic levels of NF-KB, TNF-α and IL-1β when compared to normal mice. Administration of Sita significantly decreased these elevated levels when compared to TAA group. Also, NF-KB protein expression in TAA group was higher than that of normal and Sita-treated groups (Fig. 2).

3.5. Effect of TAA and/or Sita on Bcl-2-associated X protein (Bax)

Fig. 3 illustrated that TAA significantly increased Bax immunoexpression as well as the protein levels when compared to normal mice. Treatment with Sita effectively decreased number of immunopositive cells and protein levels as well.

3.6. Effect of TAA and/or Sita on histopathological examination

Single injection of TAA significantly affected liver morphology and structure. Liver sections from rats of TAA group showed severe degree of centrolobular hepatic necrosis (Fig. 4B) when compared to normal group which showed normal hepatocytes (Fig. 4A). Sections of liver from rats of TAA/Sita group showed marked decrease of hepatic necrosis (black arrow) with marked decrease apoptosis of hepatocytes (Fig. 4C). Sita-treated group showed normal architecture of liver tissue (Fig. 4D).

3.7. Effect of TAA and/or Sita on caspase3

Fig. 5 demonstrated that liver specimens from TAA group exhibited significant elevation in number of positive cells of caspase 3 when compared to normal liver specimens. Administration of sitagliptin markedly reduced number of positive cells of caspase 3 when compared to TAA group. Sita-treated group did not significantly differ from the normal group.

4. Discussion

On a wide scale, acute liver injury intrudes in our life. Several causes can contribute to acute liver injury such as radioactive damage, drug abuse, food additives, viral infection and autoimmune attack [39,41]. Thus, it is a challenge to find and test effective strategies to treat and protect against acute liver injury.

Thioacetamide is considered as a highly useful experimental liver injury model. It has been used extensively because the lesions produced by this hepatotoxic drug resemble those seen in most cases of human liver disease, which makes it a meaningful model to study the mechanism in vivo. In previous researches, the pathological roles of high-dose TAA is mainly restricted to acute liver injury instead of direct damage to other organs, and is known to induce lipid peroxidation and oxidative stress [29], and to cause a reduction in antioxidant status [4]. As the mechanism of TAA induced hepatic damage depends on lipid peroxidation, inflammation and oxidative stress, we decided, in our present study, to make oxidative stress and inflammation as the corner stones for studying the mechanism of sitagliptin against TAA-induced acute liver injury.

In the current study, the hepatoprotective effect of sitagliptin against TAA-induced hepatotoxicity in mice was probed for the first time, and the findings revealed that sitagliptin significantly reduced serum levels of ALT, AST and GGT, markedly increased serum albumin

Table 2
Effect of TAA (500 mg/kg) and/or Sita (20 mg/kg) on hepatic antioxidant status in mice.

	MDA (nmol/mg tissue)	GSH (nmol/mg tissue)	SOD (U/mg tissue)	NO (μmol/g tissue)
Control	19.6 ± 1.6	0.88 ± 0.08	48.8 ± 0.92	145.7 ± 3.8
TAA	37.5 ± 0.7*	0.04 ± 0.005*	32.4 ± 0.71*	135.5 ± 4.9
TAA/Sita	23.7 ± 1.2$	0.33 ± 0.01*,$	43.5 ± 0.25*,$	128.4 ± 2.6
Sita	20.8 ± 0.7$	0.81 ± 0.06$,&	47.6 ± 1.9$	132.1 ± 6.1

Data expressed as means ± SEM. Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test.
GSH: Reduced glutathione; MDA: Malondialdehyde; NO: nitric oxide; SOD: Superoxide dismutase.
* $p < 0.05$ vs. control.
$ $p < 0.05$ vs. TAA.
& $p < 0.0.05$ vs. TAA/Sita.

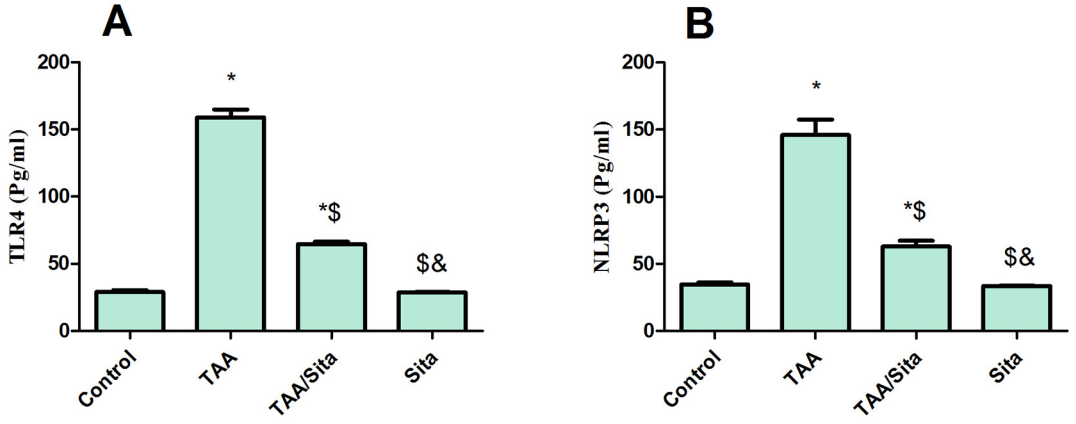

Fig. 1. Effect of TAA and/or Sita on TLR4 and NLRP3.
Data expressed as means ± SEM. Analyses performed using one-way ANOVA followed by Tukey- Kramer multiple comparisons post hoc test. NLRP3: NACHT, LRR and PYD domains-containing protein 3; TLR4: Toll-like receptor 4.
* $p < 0.05$ vs. control; \$ $p < 0.05$ vs. TAA, & $p < 0.0.05$ vs. TAA/Sita.

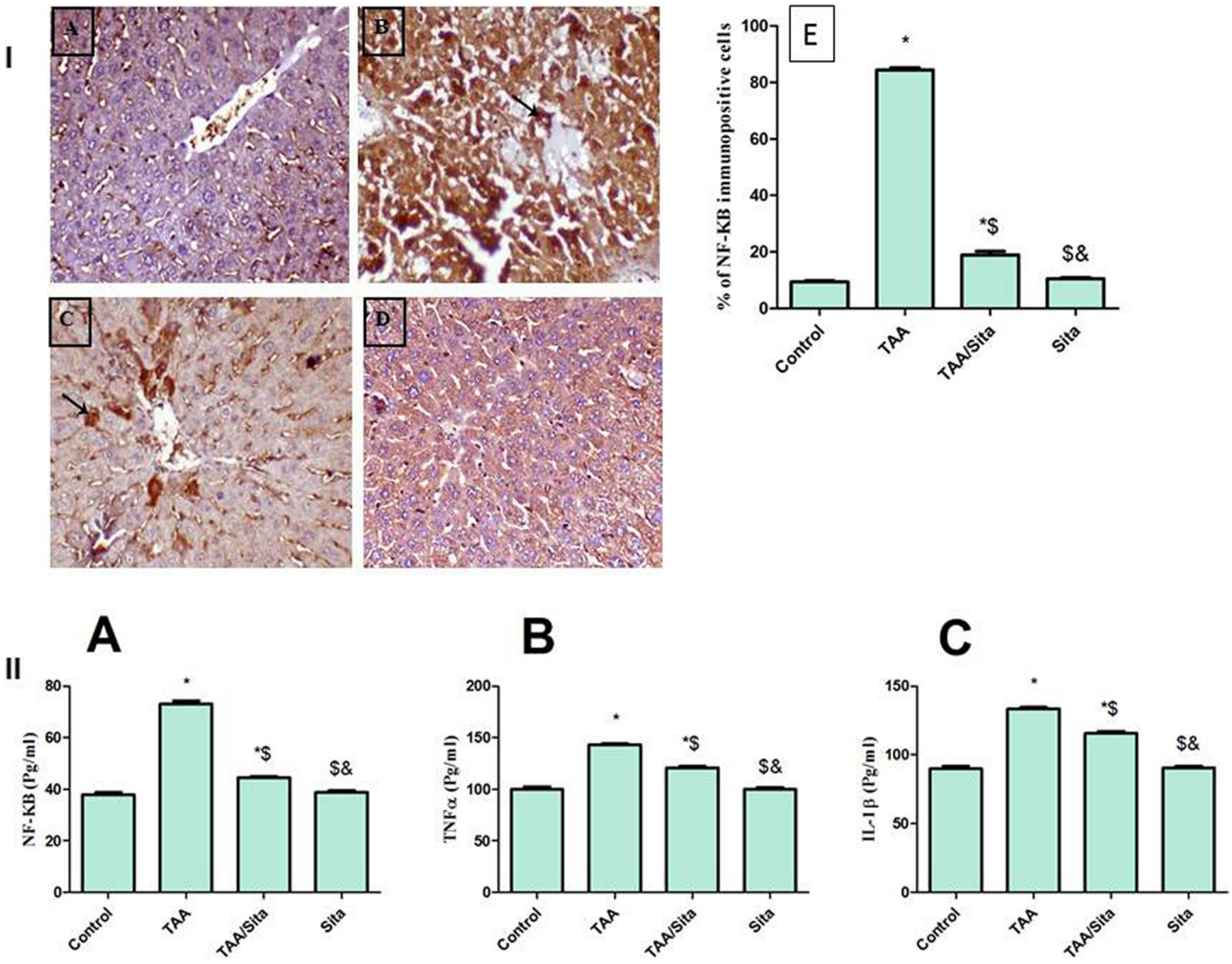

Fig. 2. Effect of TAA and/or Sita on inflammatory markers.
I. Effect of TAA and/or Sita on NF-KB p65 immunostaining (IHC X 200). A) Liver of control animal showed mild expression of NF-KB-p65; B) Liver of diseased animal exhibited marked increase of positive panlobular expression of NF-KB-p65 (arrow); C) Liver of animals treated with TAA and Sita showed marked decrease of positive hepatocytes (arrow); D) Liver of animals treated with Sita only showed mild expression of NF-KB-p65 and E) Semi-quantitative analysis of NF-κB immunostaining results in hepatic tissues expressed as % of NF-κB immunopositive cells.
II. A) Levels of NF-KB; B) TNF-α; C) IL- β.
Data expressed as means ± SEM. Analyses performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. IL-1β: Interleukine-1β; NF-KB: Nuclear factor kappa B; TNF-α: Tumor necrosis factor-alpha
* $p < 0.05$ vs. control; \$ $p < 0.05$ vs. TAA, & $p < 0.0.05$ vs. TAA/Sita.

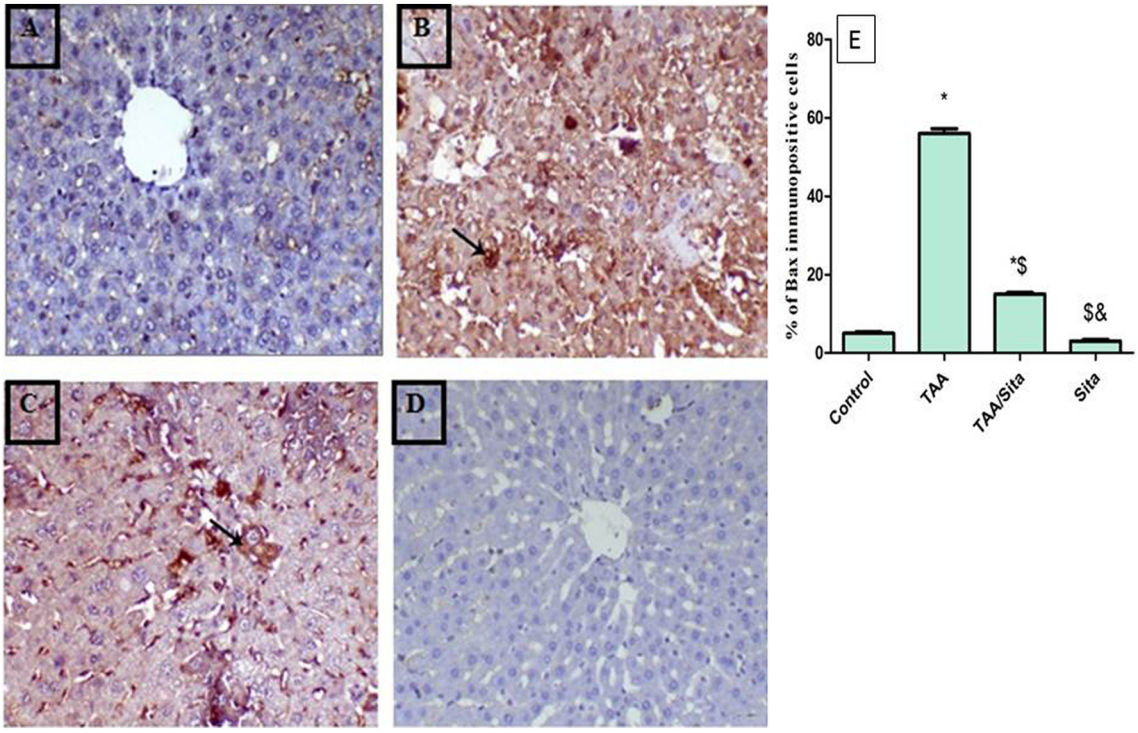

Fig. 3. Effect of TAA and/or Sita on Bax immunostaining (IHC X 200). A) Liver of control animal showing few positive hepatocytes; B) Liver of diseased animal showing marked increase in Bax immunostaining within the hepatocytes (arrow); C) Liver of animals treated with TAA and Sita showing decrease in the positive-immunostained hepatocytes (arrow); D) Liver of animals treated with Sita only showed minimal number of positive hepatocytes and E) Semi-quantitative analysis of Bax immunostaining results in hepatic tissues expressed as % of Bax immunopositive cells. Bax: Bcl-2-associated X protein.
* $p < 0.05$ vs. control; $ $p < 0.05$ vs. TAA, & $p < 0.0.05$ vs. TAA/Sita.

levels comparing to TAA mice serum levels. These cytosolic enzymes are the best indicator of liver necrosis. Their elevation in serum reflects a leakage in cell membrane, which is linked to the death of hepatocytes (Abo-Haded, Elkablawy, 2017). These biochemical alterations were confirmed by histopathological observations that showed obvious liver injury in TAA group. These functional and structural alterations were significantly mitigated by sitagliptin proposing its efficacy to counter TAA -induced liver injury.

Additionally, sitagliptin significantly normalized the hepatic levels of MDA, SOD and GSH. This observation came in line with an earlier study which showed that sitagliptin reduced oxidative stress in ovalbumin-induced asthma model via decreasing MDA content and restoring normal levels of SOD and GSH [31]. Therefore, sitagliptin might reduce oxidative burden through decreasing reactive oxygen species (ROS) generation. From the first look, sitagliptin efficiently ameliorated TAA-induced acute liver injury, suggesting that this drug may be one of the most powerful strategies for the treatment of acute liver toxicity.

Toll like receptors are a group of receptors that are implicated in innate immunity as well as inflammation. Toll like receptors are involved in liver fibrosis resulting from pathogens such as viruses, parasites and toxins [7]. TLR4 signals activate cascade of mitogen-activated protein kinase (MAPK), PI3K and NF-kB. These pathways control the expression of proinflammatory genes and cytokines which affect both survival and death of cells [5]. Also oxidative stress has a role in activation of NF-κB which in turn stimulates the subsequent inflammatory cytokines [10]. Stimulation of TLR4 signal pathway starts a series of events, comprising transfer of NF-kB p65 to the nucleus, results in stimulation as well as generation of inflammatory cytokines (TNF-a, INF-g and IL-6) [38]. TNF-α is secreted by Kupffer cells and activated T cells, and acts as a major mediator in inflammation [13]. IL-1β is released from activated macrophages and has a vital role in hepatocytes necrosis [8].

Our results clearly illustrated that sitagliptin significantly decreased the hepatic expression level of TLR4, and NF-kB production which consequently inhibited TNF-α and IL-1β release. These results agreed with the previous study which declared that sitagliptin had significant inhibitory effects on TLR4 mRNA expression in a model of choline-deficient L-amino-acid defined diet induced liver fibrosis [34]. Also, the inhibitory effect of sitagliptin on NF-kB production confirmed the results obtained by (Abo-Haded, Elkablawy, 2017) who demonstrated that the hepatoprotective impact of sitagliptin is promoted via modulating NF-κB signaling pathway with consequent inhibition of inflammatory mediators such as TNF-α and IL-β, in addition to apoptosis. Owing to its strong suppression of TLR4 expression as well as inhibition of NF-kB signaling cascade and inflammatory cytokines release, we postulated that sitagliptin shows anti-inflammatory activity and this effect may accredit the hepatoprotective effect of sitagliptin. Interestingly, sitagliptin was previously reported to inhibit inflammatory macrophage activation and alleviate oxidative stress, inflammation and fibrosis in liver fibrosis and nonalcoholic steatohepatitis models [40].

NLRP3 inflammasome is responsible for processing and secretion of the mature IL-β [16]. Many studies elucidated that the oxidative stress has a pivotal role in NLRP3 inflammasome [1,16,43]. Therefore, inhibition of oxidative stress could avert NLPR3 inflammasome activation. The effect of sitagliptin on TAA-induced NLRP3 inflammasome activation was observed in this work. Sitagliptin markedly inhibited the protein expression of NLRP3 and this finding is parallel to results obtained by [18] who confirmed that NLRP3 inflammasome was effectively repressed by treatment with sitagliptin in a doxorubicin induced nephropathy model. These results referred that the anti-inflammatory effect of sitagliptin could be arbitrated by inhibiting NLRP3 signaling pathway.

According to the literature findings, apoptosis might play an important role in the progression of TAA-induced liver injury through increased oxidative stress and an imbalance between proapoptotic and antiapoptotic proteins in Bcl-2 family [9].

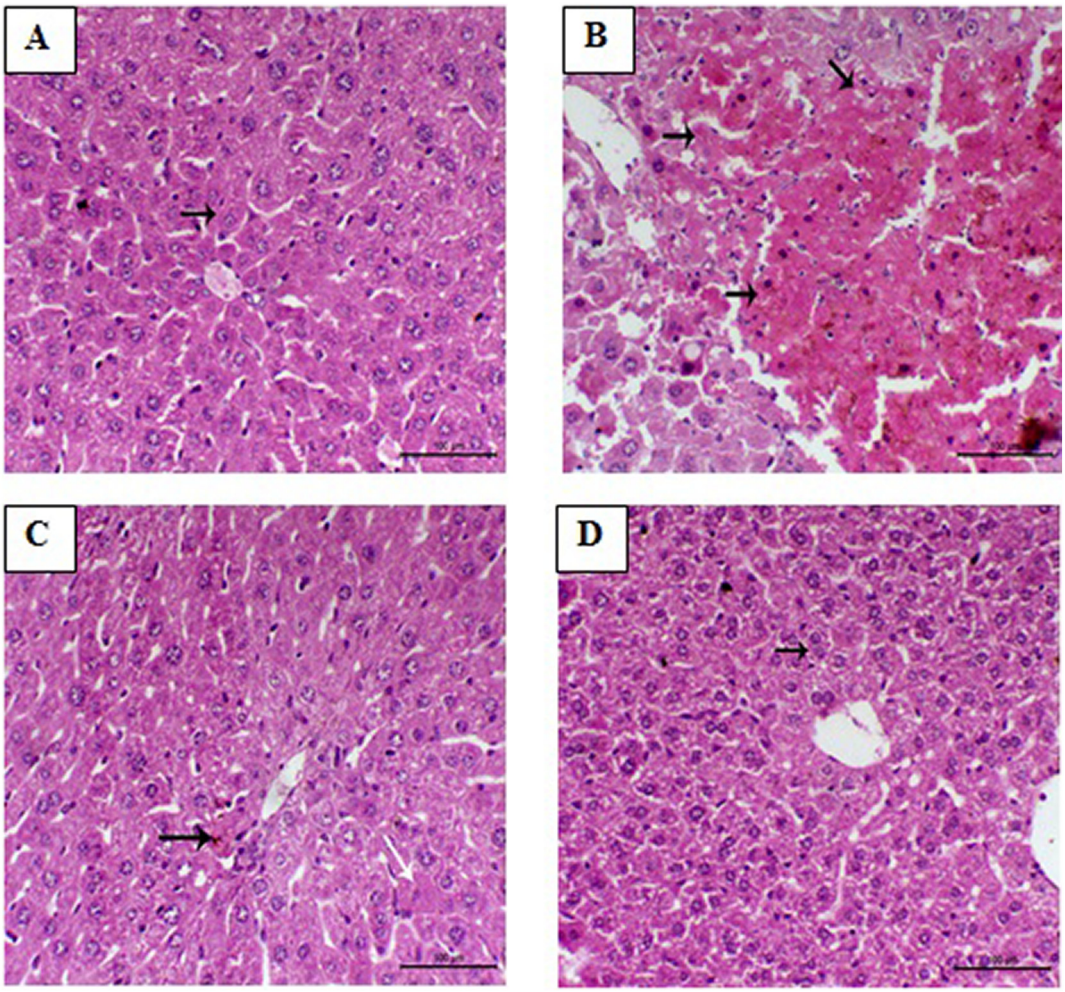

Fig. 4. Effect of TAA and/or Sita on histopathological examination (HE X 200, bar = 100 μm). A) Liver of control animal showing normal hepatocytes with normal vesicular nucleus; B) Liver of diseased animal showing severe degree of centrolobular hepatic necrosis (arrows); C) Liver of animals treated with TAA and Sita showing marked decrease of hepatic necrosis (black arrow) with marked decrease apoptosis of hepatocytes (white arrow) and D) Liver of animals treated with Sita only showed normal hepatocytes arranged in cords around the central vein.

Apoptosis is mainly mediated by the activation of p53 and caspase cascade, resulting in cellular shrinkage and chromatin condensation [32]. In this study, TAA significantly increased the expression of both Bax and caspase-3. During TAA hepatotoxicity, increased oxidative stress drives the transition of Bax to the outer mitochondrial membrane leading to increasing permeability of mitochondria and cytochrome *c* release into the cytosol. This stimulates caspase 9 and other downstream caspases such as caspase-3, prompts consequent apoptosis in caspase-dependent manner [11,12,14,17]. The results obtained from this study illustrate that TAA induced marked hepatocellular apoptosis as there was a rise in Bax expression and caspase-3 activity as well in the hepatic tissue. These alterations were significantly mitigated by sitagliptin treatment. Our results are in harmony with the results obtained by Abbas et al. [2] who stated that sitagliptin, on the molecular level, decreased caspase-3 activity and downregulated the mRNA levels of Bax, and upregulated that of Bcl2. Moreover, Nader et al. [30] reported that sitagliptin treatment reduced the expression of Bax and casapase-3 level in a PTZ model of epilepsy. Results obtained from this study suggested that sitagliptin protected hepatocytes via significant inhibition of hepatic Bax and caspase 3. Sitagliptin effectively reduced the expression of Bax; this finding came in line with an earlier study which reported that sitagliptin repressed β-cell dysfunction and pancreatic tissue mutilation via reducing Bax/Bcl2 ratio and minimizing overexpression of IL-1β [25].

5. Conclusion

In light of all the previous findings, sitagliptin demonstrated significant hepatoprotective effect against TAA-induced liver injury. The effect might be mediated through a combination of several mechanisms, such as antioxidant defense, potent anti-inflammatory as manifested by significant reduction in hepatic NF-KB, TNF-α, IL-1β, NLRP3 and TLR4; and anti-apoptotic activity as evidenced by marked reduction in expression of hepatic Bax and caspase3. Further investigations are required to investigate other molecular pathways implicated in the hepatoprotective effect of sitagliptin and to be confirmed clinically.

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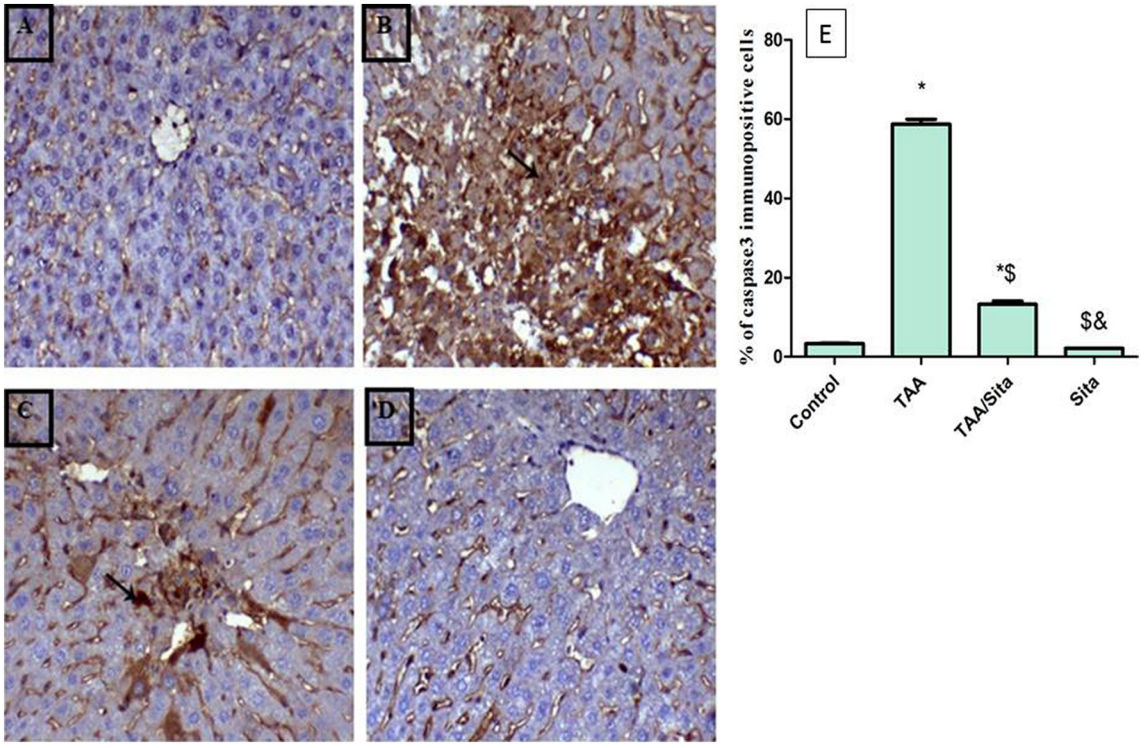

Fig. 5. Effect of TAA and/or Sita on caspase 3 immunostaining (IHC X 200). A) Liver of control animal showed mild expression of caspase 3; B) Liver of diseased animal exhibited marked increase of positive centrolobular expression of caspase 3 (arrow); C) Liver of animals treated with TAA and Sita showed marked decrease of positive hepatocytes (arrow) and D) Liver of animals treated with Sita only showed mild expression of caspase 3.
* $p < 0.05$ vs. control; $ $p < 0.05$ vs. TAA, & $p < 0.0.05$ vs. TAA/Sita.

Conflict of interest statement

The authors declare no conflict of interest.

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