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RESEARCH ARTICLE

Effects of Gibberellic Acid on Thioacetamide-Induced Acute Liver Toxicity in Sprague-Dawley Rats

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A B S T R A C T

Gibberellic acid (GBA) is a natural plant growth hormone, controlling many developmental processes. In order to explore the protective effects of GBA against Thioacetamide (TAA)-induced acute liver toxicity, thirty male Sprague-Dawley (SD) rats were randomly allocated into six groups (5 rats/group); Normal control (NC): received distilled water (DW) per os (p.o.), GBA only: received (20 mg/kg, p.o.) of GBA, TAA: received DW and TAA (350 mg/kg, i.p.), GBA 5: received GBA (5 mg/kg, p.o.) and TAA (350 mg/kg, i.p.), GBA 10: received GBA (10 mg/kg, p.o.) and TAA (350 mg/kg, i.p.), and GBA 20: received GBA (20 mg/kg, p.o.) and TAA (350 mg/kg, i.p.). Blood and plasma were collected for biochemical analysis while liver tissues were harvested and preserved for histological analysis. The results confirmed the TAA-induced acute liver toxicity by the significantly increased liver enzymes, hypoglycemia, hypoxia, polycythemia, and metabolic acidosis as compared with NC. However, pretreatment of TAA-intoxicated rats with GBA indicated the hepatoprotective effects by reducing liver enzyme levels significantly and alleviating hepatic lesions in a dose-dependent manner.

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INTRODUCTION

The liver plays a major role in the detoxification of drugs and xenobiotics by drug metabolizing enzymes (DMEs). Liver toxicity resulted from exposure to environmental toxicants, pharmaceuticals, and microbial metabolites are the causes of liver failure worldwide (Singh *et al.,* 2016).

Thioacetamide (TAA) is an organosulfur, white crystalline compound, which is used to induce an acute liver injury in rats (Zargar *et al.,* 2017). TAA is bioactivated to TA-S-oxide and TA-S, S-dioxide by hepatic microsomal enzyme cytochrome P4502E1 (CYP2E1). These TAA metabolites induce liver injury as a result of covalent binding to critical cellular components. TAA is a model hepatotoxicant, widely used to induce acute and chronic liver injuries (Hill *et al.,* 2010); it is used to induce acute liver failure, encephalopathy, hypothermia and hypoglycemia (Wallace *et al.,* 2015). Beside the liver toxicity, TAA exerts nephrotoxicity and immunotoxicity (Hill *et al.,* 2010).

Gibberellic acid (GBA) is an important member of the gibberellin family, which is a natural plant growth hormone, controlling many developmental processes, and has an effective use in agriculture, and tissue culture (Rodrigues *et al.,* 2012). GBA is a metabolic byproduct of the fungus *Gibberella fujikuroi* (Hosseinchi *et al.,* 2013). In many and different studies, GBA was used to induce hepatotoxicity (Hussein *et al.,* 2011) and nephrotoxicity (El-Mancy, 2020). Recently, Soliman *et al*. (2021) used GBA to induce hepatorenal dysfunctions (Soliman *et al.,* 2021). However, to the best of our knowledge, no studies in the literature have investigated the possible protective role of GBA against a drug-induced toxicity in the rats. Therefore, in this study, we intended to evaluate the hepatoprotective effect of low doses of GBA in a rat model of TAA-induced acute liver toxicity.

MATERIALS AND METHODS

Chemicals: Gibberellic acid (≥90% gibberellin A3 basis), G7645) and Thioacetamide (≥99.0%, 163678) were obtained from Sigma-Aldrich.

Experimental Animals and Protocols: All experimental protocols were approved according to the terms of Care of Laboratory Animal Resources, Jeonbuk National University.

Thirty male Sprague-Dawley (SD) rats weighing (200-220 g) were used in this experiment; they were obtained from Koatech, Gyeonggi, Korea. The rats were kept in cages maintained at 23±2°C and 50±5 % humidity in a 12h light/dark cycle with feed and water ad libitum.

After one week of acclimatization period, the rats were divided into six groups (5 rats/group) and received drugs per os (p.o.) daily for 3 weeks as follow:

- **Normal control (NC),** which received 1 ml of distilled water (DW).
- **GBA only,** which received (20 mg/kg BW) of GBA dissolved in 1 ml DW.
- **TAA,** which received 1 ml of DW.
- **GBA 5,** which received (5 mg/kg BW) of GBA dissolved in 1 ml DW.
- **GBA 10,** which received (10 mg/kg BW) of GBA dissolved in 1 ml DW.
- **GBA 20**, which received (20 mg/kg BW) of GBA dissolved in 1 ml DW.

On the 18th day, the rats on TAA, GBA 1, GBA 5, and GBA 10 groups were injected intraperitoneally with a single dose of TAA (350 mg/kg BW) dissolved in 1ml DW to induce acute liver toxicity; TAA dosage was chosen depending the previous literature (Wallace *et al.,* 2015; Zargar *et al.,* 2017).

Seventy-two hours after TAA intoxication and three hours later to the last dose of GBA, rats were anesthetized by inhalant Isoflurane at 5% for induction and 2% for maintenance with a 500 ml/min O_2 flow rate.

Measurement of Blood Parameters, Electrolytes, and Plasma Biochemical Analysis: After anesthesia, blood was collected from the caudal vena cava using 10 ml sterilized syringe transferred to a 10 ml BD Vacutainer with Sodium Heparin (143 USP Units) as an anticoagulant.

Stat Profile pHOx Ultra (NOVA Biomedical Corp, USA) was used to test blood electrolytes (Na^+, K^+, Cl^-), hemoglobin (Hb), hematocrit (Hct), pH, bicarbonate (HCO_3^-), base excess on extracellular fluid (BE-ecf), base excess on blood (BE-b), partial pressure of oxygen (pO_2), oxygen saturation (SO_2), partial pressure of carbon dioxide (pCO_2), free-ionized magnesium (Mg^{2+}), and free-ionized calcium (Ca^{2+}).

Blood plasma was separated by centrifugation at 3000 rpm for 10 min and then stored at -20°C to be analyzed later. The plasma levels of AST, ALT, LDH, creatinine (CRE), glucose (GLU), total protein (T-PRO), albumin (Alb), total cholesterol (T-CHO), low-density lipoprotein (LDL), and triglycerides (TG) were analyzed using a Model 7020 automatic analyzer (Hitachi, Tokyo, Japan).

Histological Analysis: Each rat's liver was immediately fixed in 10% neutral buffered formalin (NBF), then small samples of the same lobe were embedded in paraffin, cut into 5–6 µm sections, stained with hematoxylin-eosin (H&E), and tested under a BX51 fluorescence microscope (40x & 100x).

Statistical Analysis: Data were analyzed appropriately using GraphPad Prism 8.4.2 (GraphPad Software Inc., San Diego, USA) with two-tailed student's t-test or one-way ANOVA followed by a Bonferroni's test. All results are expressed as the mean±SEM. P values <0.05 were considered significant.

RESULTS

Effect of GBA on Liver and Kidney Enzymes: The liver function was checked by testing the plasma levels of AST, ALT and LDH, whereas the kidney function was checked by the plasma levels of CRE. Fig. 1 shows a significant elevation of AST, ALT, LDH and CRE in TAA-treated groups compared to NC. However, GBA pretreatment revealed significantly dropped levels of AST, ALT, LDH, and CRE compared to the TAA group in a dose-dependent manner, while the GBA-only treated rats showed no difference compared to the normal rats.

Effect of GBA on Liver Histopathology: Liver sections stained with H&E displayed normal architecture of liver lobules and hepatocytes around the central veins in NC and GBA-only groups. However, liver sections of the TAA group showed off massive centrilobular and periportal necrosis accompanied by hemorrhage, vacuolization of hepatocytes, and severe inflammatory cell infiltration; meanwhile, GBA treatments meliorated these TAA-induced microscopic pathological changes gradually in a dose-depended manner in GBA 5, GBA 10, and GBA 20 groups (Fig. 2A). Necroinflammatory score (0–4) was assessed according to Scheuer's classification (Shen *et al.,* 2018). Score 0 indicated no necroinflammation, score 1 indicated inflammation without necrosis, while scores 2, 3, and 4 indicated a mild, moderate, and severe necro-inflammation respectively (Fig. 2C).

Effect of GBA on Protein and Lipid Metabolism: T-PRO, Alb, GLU, T-CHO, LDL and TG levels were measured on blood plasma to review the protein and lipid metabolism in TAA-induced liver toxicity. Glucose levels were significantly decreased in the TAA rats compared to NC, while GBA treatment revealed a notable rise of glucose levels in the GBA 20 group compared to the TAA. T-PRO and Alb levels showed a significant decrease in the TAA group compared to NC, however pretreatment of rats with GBA revealed a significant increase on T-PRO and Alb compared to those in the TAA group. T-CHO, LDL, and TG were markedly raised in the TAA group compared to NC, however, pretreatment of rats with GBA revealed a notable drop in the lipid profile parameters compared to the TAA group (Table 1).

Effect of GBA on Blood Parameters and Electrolytes: Hb and Hct were increased significantly in the TAA group compared to NC indicating the TAA-induced

polycythemia. However, GBA treatment showed off a significant decrease of Hbc and Hct compared to the TAA rats (Table 2). Na^+ showed no significant change in all treated groups compared to NC. K^+ and Cl^- showed a significant increase in the TAA group compared to NC. Nevertheless, GBA pretreatment showed a notable decrease of K^+ compared to the TAA rats (Table 2).

Effect of GBA on Acidosis Parameters: To explore the acidosis profile, blood pH, HCO_3^-, BE-ecf, and BE-b were measured. The result of pH and HCO_3^- revealed a significant drop in the TAA group compared to NC. Conversely, in GBA-treated rats, the levels of pH and HCO_3^- showed a significant rise compared to those of the TAA group. The levels of BE-ecf and BE-b showed a significant increase in the TAA group compared to NC, whereas the pretreatment of rats with GBA showed a significant decrease in BE-ecf and BE-b compared to the TAA group (Fig. 3).

Effect of GBA on Blood Gases: As shown in Fig. 4, the levels of pO_2, SO_2, and pCO_2 showed a significant decrease in the TAA group compared to NC. Conversely, rats in the GBA-treated groups showed a notable rise in levels of pO_2, SO_2, but slightly increased levels of pCO_2 compared to the TAA group.

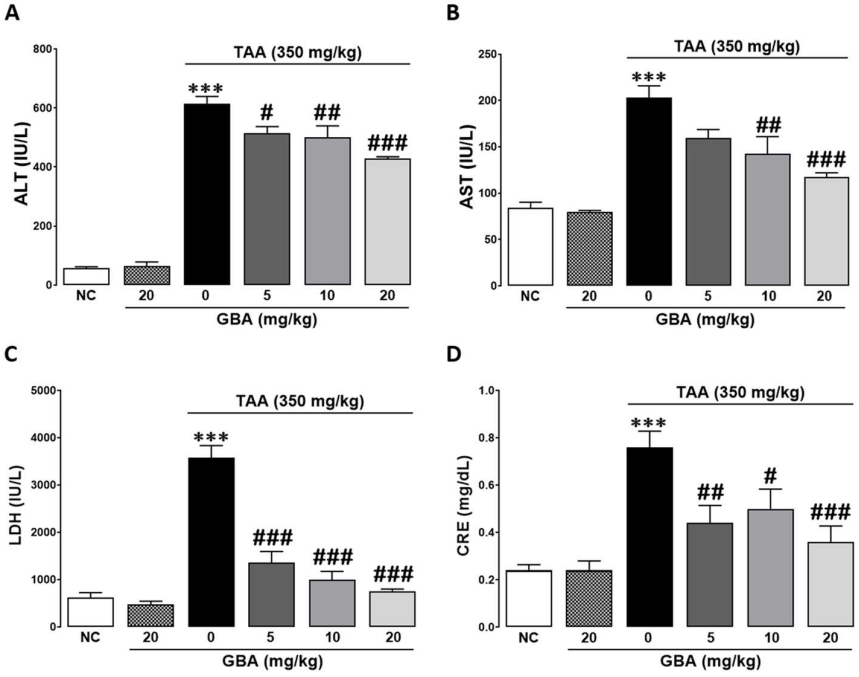

Fig. 1: Effect of GBA on liver and kidney enzyme levels; (A) ALT, (B) AST, (C) LDH, (D) creatinine (CRE). The data are shown as means±SEMs (n=5). ***: P<0.001; Two-tailed Unpaired *t* test vs. NC. #: P<0.05, ##: P<0.01, and ###: P<0.001; Bonferroni's test following one-way ANOVA vs. TAA.

Table 1: Effect of GBA on protein and lipid profile

Parameters	NC	GBA (20 mg/kg)	TAA (350 mg/kg)			
			TAA	GBA 5	GBA 10	GBA 20
GLU (mg/dL)	219.8±11.1	254.2±9.8	142.6±10.7 ***	181.4±5.3	171.2±6.9	200.2±16.7 ##
T-PRO (g/dL)	7.8±0.3	6.8±0.2	5.6±0.2 ***	6.9±0.2 #	7.2±0.2 ##	7.2±0.3 ###
Alb (g/dL)	4.6±0.1	4.7±0.1	3.3±0.1 ***	4.5±0.1 ###	4.5±0.1 ###	4.4±0.1 ###
T-CHO (mg/dL)	112.2±4.3	110.6±3.9	140.8±2.7 ***	132.4±2.3	134.0±5.0	122.6±2.8 ###
LDL (mg/dL)	16.2±0.8	16.4±0.9	51.4±4.3 ***	36.6±2.0 ###	22.2±1.8 ###	24.2±1.7 ###
TG (mg/dL)	66.4±3.2	56.0±7.0	112.2±2.1 ***	83.8±6.5 ##	77.2±8.1 ##	80.2±4.6 ##

GLU: Glucose, T-PRO: Total protein, Alb: Albumin, T-CHO: Total cholesterol, LDL: Low-density lipoprotein, TG: Triglycerides. The data are shown as means±SEMs (n=5). ***: P<0.001; Two-tailed Unpaired t test vs. NC. #: P<0.05, ##: P<0.01 and ###: P<0.001; Bonferroni's test following one-way ANOVA vs. TAA group.

Table 2. Effect of GBA on blood parameters and electrolytes.

Parameter/Group	NC	GBA (20 mg/kg)	TAA (350 mg/kg)			
			TAA	GBA 5	GBA 10	GBA 20
Hb (g/dL)	12.4±0.2	13.1±0.4	16.4±0.2 ***	15.6±0.1	14.4±0.7 ###	14.4±0.2 ###
Hct (%)	38.2±0.4	38.3±0.4	51.1±0.7 ***	46.8±0.4 #	45.3±1.7 ##	44.4±1.5 ###
Na^+ (mM/L)	139.2±0.5	140.0±0.7	139.0±0.9	139.1±0.4	139.7±0.8	139.3±0.6
K^+ (mM/L)	4.1±0.1	5.0±0.4	6.7±0.6 **	5.9±0.6	4.9±0.4 #	4.5±0.3 ##
Cl^- (mM/L)	103.1±0.3	102.6±0.2	105.7±0.9 *	104.9±1.0	104.3±0.7	104.9±0.7

Hb: Hemoglobin concentration, Hct: hematocrit, Na^+: Sodium ions, K^+: Potassium ions, Cl^-: Chloride ions. The data are shown as means±SEMs (n=5). *: P<0.05, **: P<0.01, and ***: P<0.001; Two-tailed Unpaired *t* test vs. NC. #: P<0.05, ##: P<0.01, and ###: P<0.001; Bonferroni's test following one-way ANOVA vs. TAA.

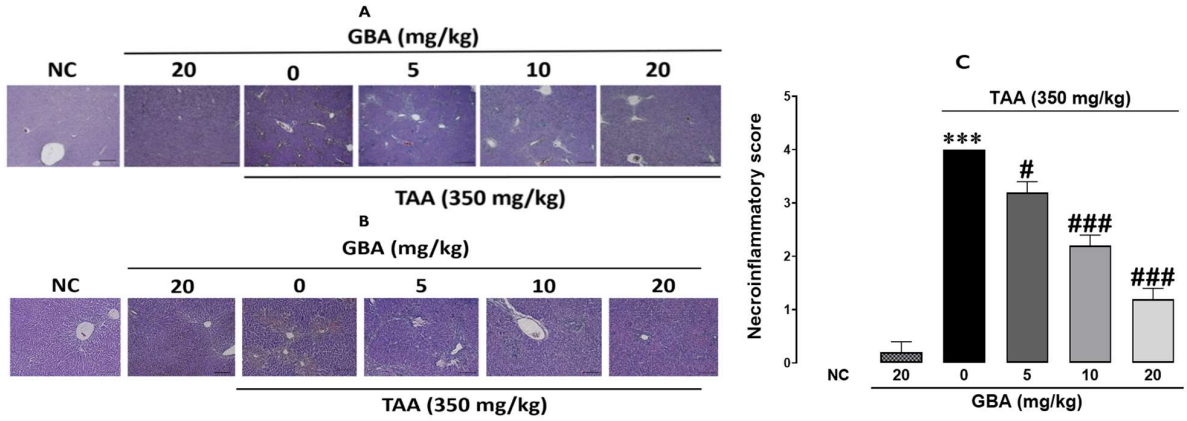

Fig. 2: Microscopic features of H&E-stained liver sections; Magnification: (A) 40x. (B) 100x, scale bar: 50 µm. (C) Necroinflammatory score of the liver histopathology. The data are shown as means ± SEMs (n=5). ***: P<0.001; Two-tailed Unpaired *t* test vs. NC. #: P<0.05, ###: P<0.001; Bonferroni's test following one-way ANOVA vs. TAA group.

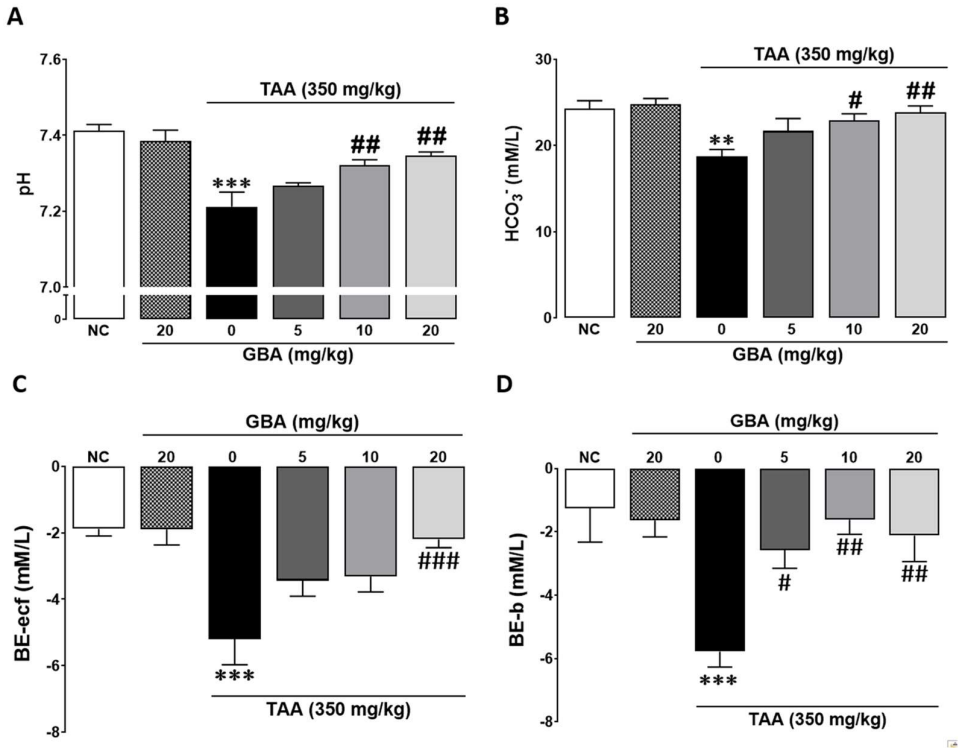

Fig. 3: Effect of GBA on blood acidosis parameters; (A) pH, (B) bicarbonate (HCO_3^-), (C) base excess on extracellular fluid (BE-ecf), (D) base excess on blood (BE-b). The data are shown as means ± SEMs (n=5). ***: P<0.001; Two-tailed Unpaired *t* test vs. NC. #: P<0.05, ##: P<0.01, and ###: P<0.001; Bonferroni's test following one-way ANOVA vs. TAA.

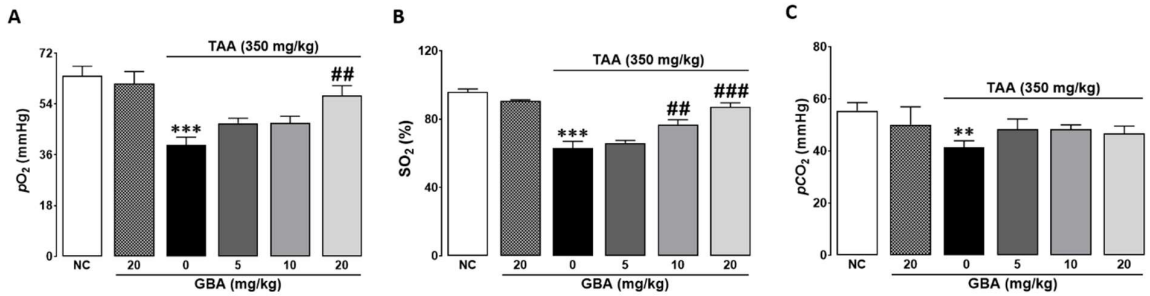

Fig. 4: Effect of GBA on blood gases; (A) partial pressure of oxygen (pO_2), (B) oxygen saturation (SO_2), (C) partial pressure of carbon dioxide (pCO_2). The data are shown as means ± SEMs (n = 5). *: P<0.05, **: P<0.01 and ***: P<0.001; Two-tailed Unpaired *t* test vs. NC. ##: P<0.01, and ###: P<0.001; Bonferroni's test following one-way ANOVA vs. TAA.

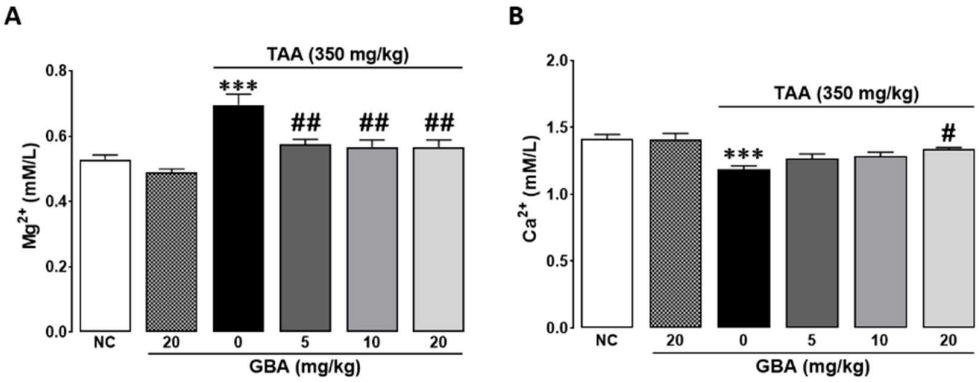

Fig. 5: Effect of GBA on blood levels of (A) free-ionized magnesium (Mg^{2+}) and (B) free-ionized calcium (Ca^{2+}). The data are shown as means ± SEMs (n = 5). ***: P<0.001; Two-tailed Unpaired *t* test vs. NC. #: P<0.05 and ##: P<0.01; Bonferroni's test following one-way ANOVA vs. TAA.

Effect of GBA on blood levels of ionized magnesium and ionized calcium: Mg^{2+} revealed a remarkable increase with a significant decrease of Ca^{2+} levels in the TAA group compared to NC. However, GBA pretreatment significantly lowered the levels of Mg^{2+} and elevated the Ca^{2+} levels compared to the TAA (Fig. 5).

DISCUSSION

Acute liver failure is the most clinical issue in humans over the world as the liver plays a crucial role in the detoxification of chemicals and drugs (Staňková *et al.,* 2010). Recently, TAA has been used at a single dose to cause acute liver injury in rats (Hussein *et al.,* 2021). In this study, we examined the hepatoprotective effect of GBA in low doses against TAA-induced acute liver toxicity in SD rats. The results showed off that TAA induces acute liver injury by significant elevation of liver biomarker enzymes (AST, ALT, LDH) (Fig. 1) and histopathological changes (Fig. 2) accordant with previous studies (Talluri *et al*., 2016; Hussein *et al.,* 2021). These changes occur due to the injured liver cells resulting in leakage of liver enzymes into serum (Ahmad *et al.,* 2002). Meanwhile, GBA treatments revealed the hepato-protective effect by lowering the levels of liver enzymes on the blood plasma of TAA-intoxicated rats. It has been reported that subacute and subchronic treatment of SD rats with GBA reduced the serum levels of liver enzymes (Tuluce and Celik, 2006); as, accordingly, our results showed a slight decrease in these enzyme levels in the GBA-only group compared to NC (Fig. 1). These results and our data could provide evidence that administration of GBA in low doses exhibits protective effects against the TAA-induced hepatotoxicity.

Necrosis occurs due to toxicant-mediated oxidative stress in the mitochondria or other intracellular organelles, and cytosolic Ca^{2+} overload (Orrenius *et al.,* 2011; Galluzzi and Vitale, 2018). Mangipudy *et al*. (1995) reported that histopathology of rat liver sections after 72 hours of TAA intoxications represented extensive centrilobular necrosis and diffused periportal necrosis with active mitosis (Mangipudy *et al*., 1995). These findings correlated with our results of histological analysis (Fig. 2).

It has been reported that acute TAA-induced toxicity in rats revealed significant drop in levels of serum albumin and glucose with respect to the normal untreated rats (Chen *et al.,* 2008; Fatima *et al*., 2020). However, intoxication of rats by TAA remarkably increased the plasma levels of T-CHO, TG, and LDL compared to the normal untreated rats (Mustafa, 2013; Balamash *et al.,* 2018). This TAA-prompted hyperlipidemia was reported due to the lipid peroxidation of cell membranes (Fatima *et al.* 2020). These previous results are correlated to our results of lipid profile that represented in table 1. In contrast, our results revealed that pretreatment of rats with GBA exhibited a protective effect against TAA-induced hypoalbuminemia, hypoglycemia and hyperlipidemia (Table 1).

Hyperkalemia occurs due to acute injury of the renal collecting duct cells that responsible for K^+ secretion (Palmer and Clegg, 2018). Our results confirmed the TAA-induced acute kidney injury by the elevated plasma creatinine and hyperkalemia in the TAA group compared to NC; these results correlated with the results of Sirag who referred these changes to the damaged cell membrane permeability (Sirag, 2007). However, pretreatment of TAA-intoxicated rats with GBA improved the TAA-induced kidney injury and its consequent hyperkalemia as shown in Fig. 1 and Table 2. Acute TAA-induced toxicity in rats resulted in a significant decrease in Hct (Pluta and Albrecht, 1986; Abbasi *et al.,* 2013), but a significant increase in Hb compared to the normal rats (Abbasi *et al.,* 2013). These observations partly go in parallel with our results of Hb and Hct in TAA-intoxicated rats (Table 2). However, pretreatment of TAA-intoxicated rats with GBA significantly improved the TAA-induced polycythemia as shown above on Table 2.

Metabolic acidosis may occur due to low (more negative) value of the base excess (Verma and Roach, 2010) with bicarbonate deficit in plasma or hyperchloremia; it is majorly depends on the level of renal function (Horacio, 2014). We suggested that TAA-induced acute toxicity resulted in the primary metabolic acidosis which has been treated by GBA in a dose-dependent manner as shown in Fig. 3.

Pluta and Albrecht (1986) reported that intoxication of rats by TAA significantly decreased the venous blood pO_2 and O_2 saturation while increased the venous blood

pCO_2 compared to normal rats, our results were partly in agreement with their findings as shown in Fig. 4. Conversely, pretreatment by GBA showed to relieve the TAA-induced hypoxia (Fig. 4).

Hypermagnesemia is a rare electrolyte issue that is occurred due to an impaired function of the renal loop of Henle, which is responsible for Mg^{2+} reabsorption (Cascella and Vaqar, 2021). It has been reported that TAA affects the membrane permeability of the liver cell and the endoplasmic reticulum Ca^{2+} pump, resulting in Ca^{2+} release and elevation of cytosolic Ca^{2+} which impaired the mitochondrial respiration (Díez-Fernández *et al*., 1996). But Ahmad and Alkreathy stated that TAA-intoxicated rats showed no significant decrease of the serum Ca^{2+} and Mg^{2+} as compared to the normal rats. Our results revealed that GBA meliorated the TAA-induced hypocalcemia and hypermagnesemia (Fig. 5).

Conclusions: Considering all these results, we could confirm the hepatoprotective features of the GBA against a rat model of TAA-induced acute liver toxicity by ameliorating the elevated liver biomarkers and the histological necroinflammation. Further studies should be made to figure out the underlying mechanisms of action.

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Authors contribution: Shang-Jin Kim conceptualized and designed the study. Haytham Mohamedelfatih Mohamed Makki and Gareeballah Osman Adam equally contributed in doing the experiments and writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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