

Original Article



The Preclinical Benefit of Glutamine in bisphenol A-induced Hepatotoxicity in Wistar Rats

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ABSTRACT

Background: Oxidative stress may be a causative factor for bisphenol A (BPA) -induced hepatotoxicity. Glutamine (GM) is an amino acid with the ability to inhibit oxidative stress.

Objective: This study evaluated the ability of GM to prevent BPA-induced hepatotoxicity in rats.

Methods: Adult Wistar rats of both sexes (n=30) were used. The rats were randomly grouped into six of five rats each. Groups A (Control), B, and C were treated with normal saline (0.2 mL), GM (80 mg/kg), and BPA (50 mg/kg), respectively for 60 days. Groups D-F were treated with GM (20 mg/kg)+BPA (50 mg/kg), GM (40 mg/kg)+BPA (50 mg/kg), and GM (80 mg/kg)+BPA (50 mg/kg), respectively for 60 days. After treatment, blood and liver samples were obtained for biochemical and histological assessments, respectively.

Results: Significantly (P<0.01) decreased body weight and significantly (P<0.01) increased liver weight occurred in the BPA-administered group when compared to the control group. The BPA-administered group showed significantly (P<0.001) elevated serum total bilirubin, lactate dehydrogenase, aminotransferases, conjugated bilirubin, gamma-glutamyl transferase, alkaline phosphatase, and liver malondialdehyde concentrations when compared to the control group. Significantly (P<0.001) decreased liver superoxide dismutase, glutathione peroxidase, catalase, and glutathione levels occurred in the PBA-administered group when compared to the control group. BPA caused hepatocyte necrosis, sinusoids, and central vein congestion. BPA-induced hepatotoxicity was reversed by GM; 20 mg/kg (P<0.05), 40 mg/kg (P<0.01), and 80 mg/kg (P<0.001) in a dose-related fashion when compared to BPA.

Conclusion: GM may be effective against BPA-associated hepatotoxicity.

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Introduction

Oxidative stress results from increased production of reactive oxygen species (ROS), reduced elimination of ROS, and suboptimal antioxidant response [1]. Excess ROS has been associated with liver damage. It has been widely suggested that the covalent interaction of ROS and reactive intermediates with macromolecules contributes to severe harmful drug reactions [2]. Most hepatotoxic drugs have been linked to ROS generation [2], causing oxidative stress characterized by lipid peroxidation [3]. Oxidative stress has been associated with the initiation and progression of liver diseases, such as alcoholic liver diseases, chronic viral hepatitis, and non-alcoholic steatohepatitis [4].

Bisphenol A (BPA) is an environmental chemical agent used for the production of polycarbonate plastics and epoxy resins. It has applications in the production of packages for food and beverages; therefore, people are inevitably exposed to BPA daily [5]. This raises great concern regarding its impact on human health [6]. It can accumulate in organs, such as the liver causing toxicities thereby incapacitating organ functions [7]. The liver is a primary point of toxicity due to its involvement in BPA metabolism [8, 9]. BPA causes liver diseases, such as hepatic steatosis and liver tumors [10], and disturbs liver redox balance, through the production of BPA radicals via reaction with oxygen radicals [11]. It also generates ROS, causes oxidative stress, and induces lipid peroxidation in the liver [9, 12]. BPA can activate caspases and induce apoptotic signals, leading to apoptosis in liver tissues [9, 13].

Glutamine (GM), the most abundant amino acid in the body, has the largest free pool and one of the highest fluxes over organs of all amino acids. It is a primary vehicle that transports amino-nitrogen to organs, such as the liver and kidney, and a respiratory fuel for cells, including hepatocytes [14]. It is a rate-limiter essential for glutathione synthesis [15]. Glutathione plays a critical role in protecting cells from oxidative damage and maintaining redox homeostasis [16]. GM has immunoregulative and cell-regulative capabilities [17] and is involved in cell membrane stabilization, antioxidantation, and detoxification [18, 19]. It is essential for hepatocyte function and is a key amino acid for the production of urea in the liver [20]. GM has stabilized liver function among liver disorders, such as liver ischemia, acute liver failure, and alcohol-induced liver damage [20, 21].

Given the aforementioned, the ability of GM to protect liver function amidst BPA intoxication remains unevaluated. This study assessed the protective effect of GM against BPA-induced hepatotoxicity in Wistar rats.

Materials and Methods

Chemicals and animals

BPA (Loba Chemie Pvt. Ltd, India) and L-Glutamine (Qualikems Fine Chem Pvt Ltd, India) were used. Adult Wistar rats of both sexes (n=30) randomly grouped into six of five rats each were used. The rats were obtained from the animal breeding unit of the Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria. The rats had access to water and standard chow ad libitum and were maintained under standard temperature, light, and relative humidity. The experiment was performed in accordance with the Guide for the Use of Laboratory Animals [22]. Approval (REC/PHARM/023/2022) was obtained from the Research Ethics Committee of the Department of Pharmacology, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria.

Dose selection and animal treatment

The rats were administered with BPA (50 mg/kg) [23] and GM (20, 40, and 80 mg/kg) [24] for 60 days as follows: Group A (Control) was treated with normal saline (0.2 mL) whereas groups B and C were administered with GM (80 mg/kg) and BPA (50 mg/kg), respectively. Groups D-F were administered with GM (20 mg/kg)+BPA (50 mg/kg), GM (40 mg/kg)+BPA (50 mg/kg), and GM (80 mg/kg)+BPA (50 mg/kg), respectively.

Animal sacrifice

At the end of the experiment, the rats were weighed and anesthetized. Blood samples were obtained, and the serum samples were extracted and analyzed for biochemical markers. The rats were dissected, and liver samples were collected, weighed, and rinsed in physiological saline. Liver samples were homogenized in 0.1 M Tris-HCl buffer (pH 7.4) and centrifuged (1500g for 20 minutes). The supernatants were decanted for biochemical assessments of oxidative stress markers.

Evaluation of biochemical markers

Serum and Liver Alkaline Phosphatase (ALP), Total Bilirubin (TB), Alanine Aminotransferases (ALT), Conjugated Bilirubin (CB), aspartate aminotransferases (AST), Gamma-Glutamyl Transferase (GGT), and lactate dehydrogenase (LDH) were measured using standard laboratory test kits.

Evaluation of liver oxidative stress markers

Liver glutathione (GSH) was measured according to Sedlak and Lindsay (1968) [25]. Superoxide dismutase (SOD) was estimated as described by Sun and Zigman (1978) [26].

Glutathione peroxidase (GPx) was analyzed as explained by Rotruck et al. (1973) [27]. Catalase (CAT) was assayed as explained by Aebi, (1984) [28]. Malondialdehyde (MDA) was measured according to Buege and Aust (1978) [29].

Assessment of liver histology

Liver tissues were collected, cleaned, and placed in 10% formalin saline for 24 h. Liver tissues were dehydrated in alcohol solutions of graded concentrations and routinely processed and embedded in blocks of paraffin. Using a microtome, the liver tissues were sectioned (3µm thickness) and stained with Haematoxylin and Eosin on slides, and examined using a light microscope [30].

Statistical analysis

Values are expressed as Mean±SEM (Standard error of the mean). Values were analyzed with one-way analysis of variance (ANOVA) using GraphPad Prism software, version 5.0 (GraphPad Software Inc., La Jolla, CA, USA). P<0.05, P<0.01, and P<0.001 were considered significant.

Results

Effects of glutamine on body and liver weights of bisphenol A-treated rats

Body and liver weights were normal (P>0.05) in the GM-administered group (80 mg/kg) when compared

Table 1. Effects of glutamine on body and liver weights of bisphenol A-treated rats

Dose (mg/kg)	Mean±SD		
	FBW (g)	ALW (g)	RLW (%)
Control	280.6±16.9	6.00±0.76	2.14±0.05
GM 80	285.2±18.4	6.12±0.11	2.15±0.08
BPA 50	151.8±15.6 [#]	11.80±0.32 [#]	7.77±0.78 [#]
GM 20+BPA 50	208.3±17.1 ^a	9.61±0.71 ^a	4.46±0.16 ^a
GM 40+BPA 50	259.6±16.8 ^b	6.31±0.43 ^b	2.43±0.33 ^b
GM 80+BPA 50	275.5±14.3 ^b	6.20±0.55 ^b	2.25±0.41 ^b

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Abbreviations: GM: Glutamine; BPA: Bisphenol A; FBW: Final body weight; ALW: Absolute liver weight; RLW: Relative liver weight. Data are expressed as Mean±SEM, n=5, [#]P<0.01 compared to the control group, ^aP<0.05 and ^bP<0.01 compared to the BPA group. Data were analyzed by analysis of variance (ANOVA).

Table 2. Effect of glutamine on serum liver biochemical markers of bisphenol A-treated rats

Dose (mg/kg)	Mean±SD						
	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (g/dL)	CB(g/dL)	LDH (U/L)	GGT (U/L)
Control	40.12±3.65	39.01±3.67	30.94±3.90	6.56±0.40	3.77±0.34	25.84±3.98	0.31±0.01
GM 80	38.92±2.79	38.73±3.39	30.62±4.72	6.30±0.23	3.59±0.54	25.67±4.70	0.29±0.06
BPA 50	166.02±15.0 [#]	155.92±14.8 [#]	110.65±10.9 [#]	19.96±2.55 [#]	14.74±1.66 [#]	119.73±17.8 [#]	1.31±0.04 [#]
GM 20+BPA 50	101.31±12.8 ^a	110.35±13.00 ^a	78.34±5.27 ^a	15.73±1.40 ^a	10.31±1.00 ^a	76.05±6.76 ^a	0.87±0.09 ^a
GM 40+BPA 50	70.05±5.87 ^b	68.73±6.75 ^b	57.17±4.54 ^b	9.28±0.36 ^b	7.00±0.53 ^b	53.93±4.61 ^b	0.59±0.05 ^b
GM 80+BPA 50	46.47±3.63 ^c	47.95±4.47 ^c	36.86±3.61 ^c	6.03±0.28 ^c	4.01±0.45 ^c	31.52±3.54 ^c	0.36±0.08 ^c

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Abbreviations : GM: Glutamine; BPA: Bisphenol A; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; TB: Total bilirubin; CB: Conjugated bilirubin; GGT: Gamma-glutamyl transferase; LDH: Lactate dehydrogenase, n=5. Data are expressed as Mean±SEM, [#]P<0.001 compared to the control group, ^aP<0.05, ^bP<0.01, ^cP<0.001 compared to the BPA group. Data were analyzed by analysis of variance (ANOVA).

Table 3. Effect of glutamine on liver tissue biochemical markers of bisphenol A-treated rats

Dose (mg/kg)	Mean±SD				
	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	LDH(U/L)
Control	230.7±15.6	210.5±13.7	209.9±15.0	30.6±3.00	207.7±14.0
GTM 80	227.2±14.9	207.2±11.9	200.5±13.7	27.4±2.43	200.5±14.7
PBA 50	960.3±22.7 [#]	921.0±20.4 [#]	911.3±21.6 [#]	101.6±9.76 [#]	889.9±19.5 [#]
GTM 20+BPA 50	630.7±17.4 ^a	607.5±16.7 ^a	550.6±16.0 ^a	70.0±5.33 ^a	560.0±15.0 ^a
GTM 40+BPA 50	421.8±15.0 ^b	400.3±13.0 ^b	321.0±14.7 ^b	50.9±4.27 ^b	352.1±12.3 ^b
GTM 80+BPA 50	257.7±12.1 ^c	233.2±10.3 ^c	225.7±12.5 ^c	35.0±3.11 ^c	224.5±12.9 ^c

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Abbreviations: GM: Glutamine; BPA: Bisphenol A; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; LDH: Lactate dehydrogenase. [#]P<0.001 compared to the control group, ^aP<0.05, ^bP<0.01, ^cP<0.001 compared to the BPA group, n=5. Data are expressed as Mean±SEM. Data were analyzed by analysis of variance (ANOVA).

to the control group (Table 1). The BPA-administered group showed significantly (P<0.01) increased liver weight and significantly (P<0.01) decreased body weight when compared to the control group (Table 1). However, liver and body weight were significantly restored in the GM (20 mg/kg)+BPA (50 mg/kg), GM (40 mg/kg)+BPA (50 mg/kg), and GM (80 mg/kg) groups at P<0.05, P<0.01, and P<0.01, respectively when compared to the BPA group (Table 1).

Effect of glutamine on biochemical markers of bisphenol A-treated rats

The group administered with GM (80 mg/kg) showed normal (P>0.05) serum and liver CB, TB, AST, ALT,

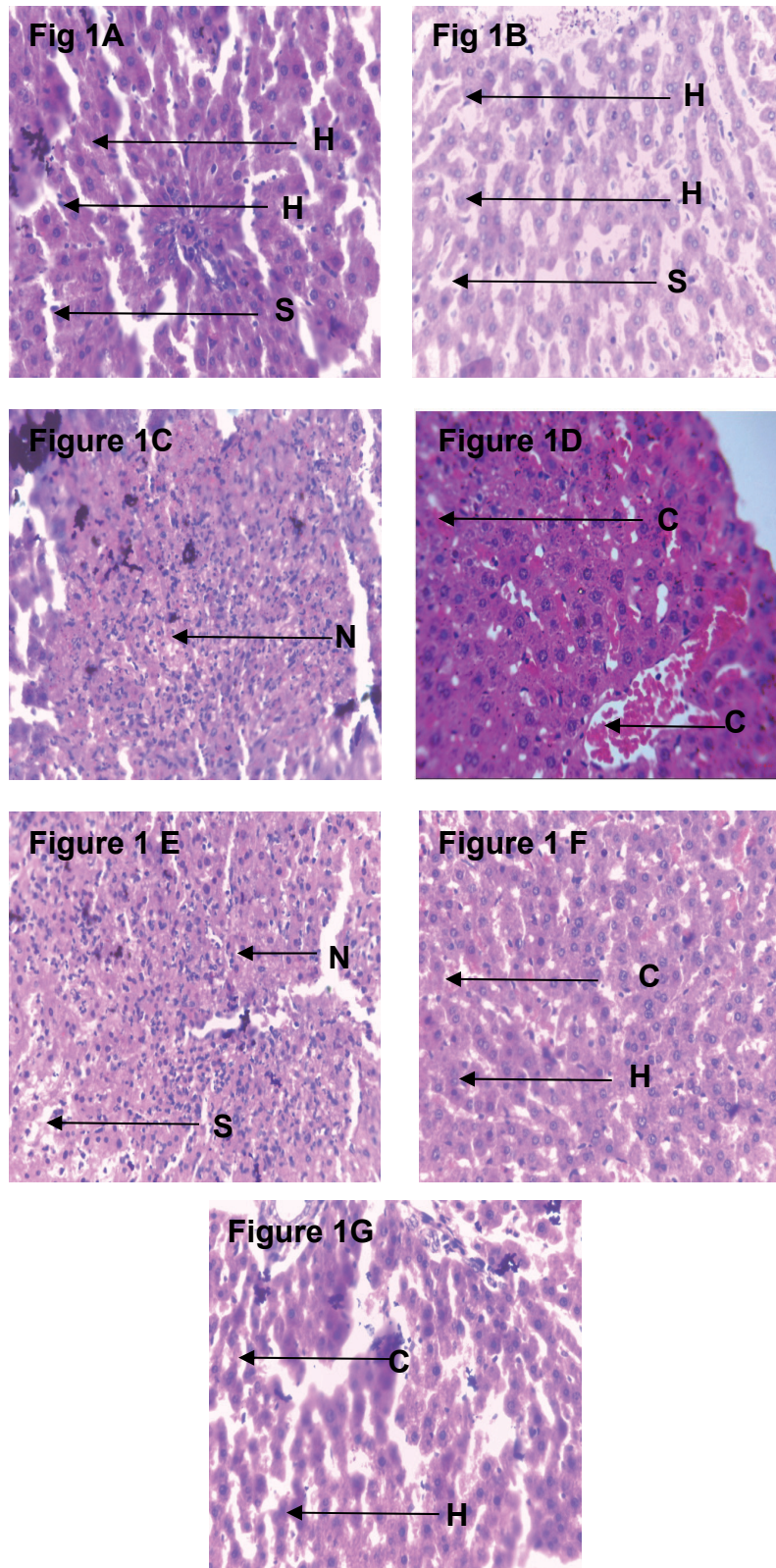
ALP, LDH, and GGT levels when compared to the control group (Tables 2 and 3). In contrast, the BPA-administered group showed significantly (p<0.001) increased serum and liver levels of the aforementioned markers when compared to the control group (Tables 2 and 3). However, in the groups treated with GM (20 mg/kg)+BPA (50 mg/kg), GM (40 mg/kg)+BPA (50 mg/kg), and GM (80 mg/kg)+BPA (50 mg/kg), the aforementioned markers significantly decreased in a dose-related fashion at P<0.05, P<0.01, and P<0.001, respectively when compared to the BPA-administered group (Tables 2 and 3).

Table 4. Effect of glutamine on liver oxidative stress markers of bisphenol A-treated rats

Dose (mg/kg)	Mean±SD				
	SOD (u/mg protein)	CAT (u/mg protein)	GSH (µg/mg protein)	GPx (u/mg protein)	MDA (nmol/mg protein)
Control	40.97±3.43	43.96±5.00	22.44±2.11	26.90±2.71	0.18±0.05
GM 80	42.04±3.67	44.07±4.51	21.64±2.32	28.35±2.15	0.14±0.03
BPA 50	15.73±1.68 [#]	17.32±1.67 [#]	7.54±0.11 [#]	9.36±0.37 [#]	0.79±0.01 [#]
GM 20+BPA 50	20.82±2.64 ^a	22.81±2.00 ^a	11.67±0.12 ^a	12.48±0.67 ^a	0.57±0.07 ^a
GM 40+BPA 50	27.35±3.66 ^b	29.56±4.63 ^b	14.72±1.56 ^b	17.79±1.74 ^b	0.33±0.04 ^b
GM 80+BPA 50	36.47±3.57 ^c	39.73±5.71 ^c	20.83±2.60 ^c	24.88±2.47 ^c	0.20±0.06 ^c

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Abbreviations: SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; MDA: Malondialdehyde; GPx: Glutathione peroxidase; GM: Glutamine; BPA: Bisphenol A. n=5, Data are expressed as Mean±SEM, [#]P<0.001 compared to the control group, ^aP<0.05, ^bP<0.01, ^cP<0.001 compared to the BPA group. Data were analyzed by analysis of variance (ANOVA).



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Figure 1. A: Liver of the control rats. Figure 1B: Liver of GM (80 mg/kg)-administered rats. Figure 1C: Liver of BPA (50mg/kg)-administered rats. Figure 1D: Liver of the BPA (50 mg/kg)-treated rats. Figure 1E: Liver of the GM (20 mg/kg)+BPA (50 mg/kg)- administered rats. Figure 1F: Liver of the GM (40 mg/kg)+BPA (50 mg/kg)-administered rats. Figure G: Liver of the GM (80 mg/kg)+BPA (50 mg/kg)-administered rats.

H: Normal hepatocytes, CV: Central vein congestion, N: Hepatocyte necrosis, C: Sinusoids congestion Hand E x400.

Effect of glutamine on liver oxidative stress markers of bisphenol A-treated rats

The group treated with GM (80 mg/kg) showed normal liver antioxidants (GSH, CAT, GPx, and SOD) and MDA levels when compared to the control group (Table 4). In contrast, the BPA-administered group showed significantly ($P < 0.001$) decreased liver antioxidant levels and significantly ($P < 0.001$) increased liver MDA levels when compared to the control group (Table 4). But liver antioxidant levels significantly increased and MDA levels decreased in a dose-related fashion in groups treated with GM (20 mg/kg)+BPA (50 mg/kg), GM (40 mg/kg)+BPA (50 mg/kg), and GM (80 mg/kg)+BPA (50 mg/kg) at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively when compared to the BPA-administered group (Table 4).

Effect of glutamine on liver histology of bisphenol A-treated rats

The control (Figure 1A) and GM-administered (80 mg/kg) groups (Figure 1B) showed normal liver histology, but the BPA-administered group showed hepatocyte necrosis, sinusoid, and central vein congestions (Figure 1C and D). GM (20 mg/kg)+BPA (50 mg/kg)-administered group showed hepatocyte necrosis (Figure 1E). The GM (40 mg/kg)+BPA (50 mg/kg)-administered group (Figures 1G) and (80 mg/kg)+BPA (50 mg/kg)-administered group (Figures 1G) showed vascular congestions.

Discussion

The present study examined whether BPA-induced hepatotoxicity in rats can be surmounted by GM supplementation. In the current study, the BPA-administered group showed decreased body weight and increased liver weight. Similarly, Hassan et al. [23] reported decreased body weight and increased liver weight in the PBA (0.1-50 mg/kg)-treated rats for 28 days. Also, Ahmed et al. [31] showed decreased body weight and increased liver weight in the PBA (150 mg/kg)-treated rats for 70 days. This could be attributed to the induction of inflammation in the liver and the suppression of appetite in rats by BPA [32, 33]. In the BPA-administered group, serum biochemical markers (CB, TB, AST, ALT, ALP, LDH, and GGT) were elevated. This observation is a sign of hepatotoxicity supported by the elevated levels of the aforementioned markers in rats treated with BPA (10 mg/kg) reported by Ola-Davies et al. [34]. It is also supported by elevated levels of liver markers in BPA (50 mg/kg)-treated rats for 28 days reported by Elhamalawy et al. [35]. A high dose of BPA (50 mg/kg) was reported to increase liver biochemical markers as observed in this

study [23]. This may be due to increased hepatic syntheses of the aforementioned biochemical markers and the disruption of the hepatic membrane causing the leakage of the biochemical markers into the blood [36]. In the current study, BPA caused depletion of liver antioxidants and elevated liver MDA levels, which are signs of oxidative stress characterized by lipid peroxidation. Similarly, Abdel-Wahab [37] reported liver oxidative stress characterized by lipid oxidation in BPA (10 mg/kg) treated rats. The finding in this study is also supported by oxidative liver damage in rats treated with BPA (30 mg/kg) for six weeks reported by Eweda et al. [38]. BPA might have caused hepatic oxidative stress by reacting with oxygen radicals, transforming them into reactive metabolites that are potent oxidants that can cause cell damage [39]. BPA also undergoes an oxidation-reduction reaction forming peroxide anions and superoxide anions in cells, which can cause oxidative stress and cellular damage [40]. In this study, the BPA-administered group showed hepatocyte necrosis, sinusoids, and central vein congestions. Abdel-Rahaman et al. [41] reported similar findings in the BPA (10 mg/kg)-treated rats for 30 days. Kamel et al. [6] also observed similar liver morphologic changes in the BPA (20-100 mg/kg)-treated rats. This may be due to damage to liver biomolecules (lipids, proteins, and DNA) caused by BPA.

In the current study, GM supplementation protects against BPA-induced changes in liver biochemical markers and histology. These were characterized by the restored body and liver weights and decreased serum biochemical markers (CB, TB, AST, ALT, ALP, LDH, and GGT). Also, liver antioxidant levels were up-regulated, and MDA levels decreased while liver histology was restored. The observation in this study correlates with the protective effect of GM in rats with severe acute liver failure [24]. It also correlates with the restored liver activity in deltamethrin-treated rats supplemented with GM reported by Gunduz et al. [42]. GM supplementation might have restored body and liver weight by increasing appetite and inhibiting liver inflammation. The restored serum and liver biochemical markers by GM may be attributed to its stabilizing effect on the hepatic cell membrane, which might have prevented the leakage of biochemical markers into the blood. GM might have surmounted BPA-induced hepatic oxidative stress through its antioxidant activity. GM is important for the synthesis of GSH. It supplies glutamate to the liver, thereby preserving GSH levels in the liver [43]. GSH is the most important and non-enzymatic antioxidant, which directly reacts with ROS, generating oxidized GSH and donating electrons for peroxide reduction, catalyzed by GPx. The activity of GSH can help overcome the menace of

ROS, leading to reducing oxidative stress [44]. Furthermore, GM might have restored liver histology through hepatocyte regeneration, growth, and repairs.

Conclusion

GM supplementation attenuates BPA-induced hepatotoxicity in a dose-related fashion in Wistar rats. This shows that GM may be used effectively against BPA-related hepatotoxicity.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of the Department of Pharmacology, Faculty of Pharmacy, Madonna University, Rivers State, Nigeria (Code: REC/PHARM/023/2022).

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Authors' contributions

Design, literature review, manuscript drafting, and final approval: All authors; Experimentation: Elias Adikwu and Ben Enoluomen Ehigiator; Data interpretation: Elias Adikwu; Data analysis: Ben Enoluomen Ehigiator and Theodore Mmamsichukwu Ajaekwe.

Conflict of interest

The authors declared that there was no conflict of interest.

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