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Physiological Studies of the Protective and Therapeutic Effects of Guarana (*Paullinia cupana)* Seeds against Thioacetamide-Induced Hepatotoxicity in Male Rats

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Abstract: Current study was designed to evaluate hepatoprotective Guarana *(Paullinia Cupana)* powder seeds effects on Thioacetamide (TAA)-induced hepatotoxicity in rats. Seventy male rats were divided into 7 equal groups. First group (G1, control) received saline, second group (G2) received TAA (200 mg/kg) intraperitoneally (i.p.) three times weekly for four weeks, third group (G3) received orally Guarana (30 mg/kg) daily for 21 days, (G4-a) group received orally Guarana then TAA as G2 and G3, (G4-b) received orally silymarin (100 mg/kg) daily for 21 days then TAA as G2, (G5-a) group received TAA then Guarana as G2 and G3. (G5-b) group received TAA then silymarin as G2 and G4-b. At experimental end, liver function tests and oxidative stress markers were measured. Liver was examined microscopically. Treatment of rats with TAA led to significant decrease in body weight gain but increased in liver weight and index. TAA caused significant increased in liver enzymes; while total protein, albumin, globulin, catalase, superoxide dismutase, GST were decreased versus control. Liver sections after TAA showed fibrosis and hepatocytic destruction. Treating with Guarana attenuated changes in liver functions, oxidative stress and histopathological changes. In conclusion, Guarana supplementation counteracts TAA-induced hepatotoxicity by reducing oxidative stress and improved liver cells due to antioxidant properties.

Key words: Hepatotoxicity · Thioacetamide · Guarana (*Paullinia cupana)* · Oxidative Stress

INTRODUCTION

The liver is the most complex and largest organ in human body [1]. It is accountable for most of endocrine and exocrine functions as tissue growth, destroying foreign organisms, supply nutrients and energy as well as reproductive functions and hormonal synthesis. It also plays important roles in carbohydrate, proteins and fat metabolisms as well as bile formation and vitamins storage [2]. Liver diseases are worldwide problem and associated with high morbidity and mortality rates. It is due to a variety of causes as inflammations, exposure to environmental pollutants, chemicals, drugs, viral infections, alcohol abuse and metabolic disorders [3-5]. Most chronic liver injuries led to hepatic fibrosis and cirrhosis [6].

Liver fibrosis is a serious and dangerous threat to human health especially when advanced to cirrhosis or hepatocellular carcinoma [7]. Thioacetamide (TAA) is a thio-sulfur-carrying compound that usually incorporated into motor fuel, textile, leather and papers formation [8]. TAA is commonly used to make acute liver injury in experimental animal models [9]. A single TAA dose led to centrilobular hepatic necrosis, while chronic TAA intake led to fibrosis or cirrhosis [6, 10]. TAA causes a major oxidative stress by formation of a toxic metabolite (Thioacetamide-s-oxide), which causes cell membrane injury by interfering with RNA movement from nucleus to cytoplasm [11]. Meanwhile, others reported that TAA enhanced hepatic DNA formation and mitosis for liver regeneration [11].

The lack of potent modern medications to treat liver injury led to research about hepatoprotective activity of many plants using many experimental animal models. Guarana is a hiking plant, belongs to Sapindaceae family that is native to central Amazon basin and cultivated exclusively in Brazil. Guarana seeds contained large amounts of methylxanthines (Theophylline, caffeine and

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theobromin), saponins and polyphenols, especially tannins [1] Guarana is largely used in traditional medications as well as in high-energy drinks and dietary supplements, mostly due to stimulant effects on central nervous system and memory maintenance [12]. The pharmacological effects reported of Guarana include weight loss [13] lowering platelet thromboxane formation, protecting against gastric lesions, antioxidant activity [14] and anti-inflammatory actions [15]. Although there are studies documented many biological actions of Guarana, little knowledge is known on its effects on diseased liver especially in TAA hepatotoxicity model.

Therefore, this experimental study aimed to evaluate the protective and therapeutic actions of Guarana *(Paullinia Cupana)* on hepatotoxicity induced by TAA on male rats as well as possible mechanism of its action.

MATERIALS AND METHODS

Chemicals: Thioacetamide was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). TAA was mixed in distilled water based on manufacture protocol. Guarana was purchase from I Herb (CA, USA) and was conserved dry and protected from light at -20°C until administration. It was dissolved in water and mixed well until powder was dissolved. The solution was prepared. Silymarin was purchased from local pharmacy in Jeddah, Saudi Arabia. It was mixed well with water till complete dissolving.

Animals and Experimental Design

Animals: Seventy adult male Wister albino rats with body weight of 180- 250 g were used. The rats were getting from animal house of King Fahd Medical Research Center, King Abdulaziz University (KAU), Jeddah, Saudi Arabia. The principles of laboratory animal care were followed throughout the duration of experiment and instruction given by King Abdulaziz University research ethics committee (Ethical approval # 434-19). The animals were housed in standard cages (5 per cage) at an ambient temperature of (21±1°C) with 12 h light-dark cycle. The animals had free access to water and eat *ad libitum* on normal commercial chow diet.

Experimental Design: The rats were equally classified into seven groups (10 rats each). Group 1 (Negative Control Group): The animals were injected intraperitoneal (i.p.) with saline solution (0.9% NaCl, vehicle) in a dose of 1 ml/kg B.W. for 4 weeks. Group 2 (Positive Control Group): The animals were injected i.p. with TAA (200 mg/kg) 3 times/ week for 4 weeks to induce liver fibrosis [4, 7]. Group 3 (Safety Experimental Group): The animals were orally supplemented with Guarana at dose of 30mg/kg/day daily for 21 days [16]. Group 4 (Protective Experimental Group 4a&b) (G4-a): The animals were received via oral gavage Guarana daily for 21 days then injected i.p. with TAA for 4 weeks and (G4-b) group: the animals received via oral gavage Silymarin for 21 days then injected i.p. with TAA for four weeks. Group 5 a&b (Therapeutic Experimental Groups), (G5-a) group: The animals injected i.p. with TAA for four weeks then received oral gavage of Guarana for 21 days. (G5-b) group: The animals injected i.p. with TAA for 4 weeks then received Silymarin for 21 days.

During the experimental period, animals were observed for any abnormalities. Total body weights were estimated at start and end of study duration by means of digital balance. The weight gain was calculated (Final body weight - initial body weight) / initial body weight x 100) [10]. Twenty four hours after experimental end, blood sampling was taken then rats were scarified by cervical dislocation after given diethyl ether anesthesia. The abdomen was opened and livers were excised, washed with saline, dried and weighted. Liver index was calculated as liver weight/ final body weight X 100 [17]. The liver was cut into two pieces, one piece was homogenized and supernatant was used for determination of oxidative stress markers and the other piece was utilized for histological examination.

Liver Functions and Enzyme Assessment: Blood samples were taken from retro-orbital veins into plain tube at end of the study. Five ml of blood was centrifuged at 11,000 g for 15 min and the blood sera were kept at -20°C till utilized. Sera were utilized to estimate levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (TBIL), total proteins (TP), albumin (ALB), globulin, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) using commercially available kits.

Preparation of Hepatic Homogenate: Equal weights of liver tissue from different groups were utilized for preparing liver homogenate. The weighed frozen liver was homogenized in a glass-Teflon homogenizer with 50 mM phosphate buffer (pH 7.4) to get 1:9 (w/v) whole homogenate. The homogenates were centrifuged at 11,000 g for 15 min at 4°C and supernatant was utilized for measurement of MDA, SOD, CAT and GST using

commercial available kits. Total protein contents were determined by method of Lowry *et al*. [18] using bovine serum albumin as a standard.

Histological Studies: Part of liver lobe was cut and fixed in 10% formalin solution for about 24 hours and embedded in paraffin. The remaining liver parts were frozen in dry ice and kept at -80°C for biochemical analysis. The sections were cut at (5µm thick) and stained by hematoxylin -eosins stain, then examined by light microscope for histopathological investigation [8].

Statistical Analysis: The data were expressed as mean +/- standard error (SE) and were analyzed using the IBM SPSS Statistics for Windows, version 23 (IBM SPSS, IBM Corp., Armonk, N.Y., USA). Statistical comparisons were performed by a One-Way analysis of variance (ANOVA). The results were considered statistically significant if *P*-values were < 0.05.

RESULTS

Table (1) showed that percentage change of the body weight gain in TAA group was significantly decreased (P =0.005), while, was significantly increased in groups (G. + TAA), (TAA + G.) and (TAA + S.) (P = 0.001for all) versus control group. TAA administration to rats for 4 weeks resulted in significant decreased in the body weight gain versus G., G. + TAA, S. + TAA. TAA + G. and TAA + S. groups (P = 0.001for all). Liver weight and index in TAA group were significantly increased compared with control (P =0.018 and P <0.001). Meanwhile, treatment of the rats with G. alone, (G. + TAA), (S. + TAA), (TAA + G.) and (TAA + S.) led to significant decrease in percentage changes of liver index versus control and TAA groups.

As shown in Table (2), in TAA group serum levels of total bilirubin, AST, ALT, GGT, ALP and LDH were significantly increased, while, total protein, albumin, globulin were significantly decreased versus control, G., G. + TAA, S. + TAA, TAA + G. and TAA + S. groups. Compared with control group, significant elevation of serum levels of total bilirubin in G. + TAA, S. + TAA, TAA + G. (P =0.001, P =0.004, P =0.002); GGT in G. + TAA, S. + TAA, TAA + S. (P =0.001, P= 0.031, P =0.034); ALP in TAA + S. (P =0.002); LDH in S. + TAA (P =0.003).

Serum levels of MDA was significantly increased while of SOD, catalase and GST were significantly decreased in TAA group versus control, G., G. + TAA, S. + TAA, TAA + G. and TAA + S. groups. Meanwhile, compared to control, significant decrease of catalase serum levels were observed in G. + TAA and TAA+G, groups (P =0.004 and P =0.003) and of GST serum levels in S.+TAA, TAA+G. and TAA+S. groups (P =0.001, P =0.011 and P =0.005) (Table 3).

In liver tissue homogenate, MDA was significantly increased in TAA versus control and G. groups (P =0.001 for both). Meanwhile, tissue homogenate MDA levels in G.+TAA, S.+TAA, TAA+G. and TAA+S. were significantly increased versus control (P =0.001 for all) but significantly decreased versus TAA group (P =0.001, P =0.017, P =0.001 and P =0.001, respectively). SOD liver homogenate levels were significantly decreased in TAA group (P =0.001), but significantly increased in

Table 1: Effect of Guarana *(Paullinia cupana)* against Thioacetamide (TAA)-induced hepatotoxicity on total body weight gain (%), liver weight (Grams) and liver index (%) in adult male rats.

Groups	Body weight gain (%)	Liver weight (grams)	Liver index (%)
G 1 (control)	11.81±3.19	7.86±0.59	3.38±0.22
G2 (TAA)	-1.69±1.45	9.39±0.22	4.27±0.11
Significance	^{1}P=0.005	^{1}P=0.018	^{1}P=0.001
G3 (G.)	20.15±1.75	8.24±0.53	2.83±0.09
Significance	^{1}P=0.072; ^{2}P=0.001	^{1}P=0.546; ^{2}P=0.069	^{1}P=0.010; ^{2}P=0.001
G4a (G.+TAA)	27.77±4.01	8.21±0.45	2.76±0.18
Significance	^{1}P=0.001; ^{2}P=0.001	^{1}P=0.574; ^{2}P=0.063	^{1}P=0.004; ^{2}P=0.001
G4b (S.+TAA)	20.10±2.27	8.16±0.26	2.94±0.10
Significance	^{1}P=0.073; ^{2}P=0.001	^{1}P=0.615; ^{2}P=0.056	^{1}P=0.037; ^{2}P=0.001
G5a (TAA+G.)	31.62±3.29	8.17±0.44	2.74±0.13
Significance	^{1}P=0.001; ^{2}P=0.001	^{1}P=0.615; ^{2}P=0.056	^{1}P=0.003; ^{2}P=0.001
G5b (TAA+ S.)	30.12±4.81	8.18±0.41	2.80±0.09
Significance	^{1}P=0.001; ^{2}P=0.001	^{1}P=0.608; ^{2}P=0.057	^{1}P=0.007; ^{2}P=0.001

Data are expressed as mean +/- standard error (SE). Pairwise comparison between each 2 groups was done using Post Hoc Test (least significant test). ^{1}P: significance versus G1 (control) group; ^{2}P: comparison versus G2 (TAA) group.

Table 2: Effect of Guarana *(Paullinia cupana)* against Thioacetamide-induced hepatotoxicity on liver functions in adult male rats.

Groups	Total bilirubin (umol/L)	Total protein (g/L)	Albumin (g/L)	Globulin (g/dL)	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	LDH (U/L)
G1 (control)	6.68±2.74	74.50±1.65	11.33±0.49	63.17±1.90	93.83±4.18	69.67±3.40	3.96±0.30	123.67±9.91	346.17±44.11
G2 (TAA)	24.72±2.74	50.17±3.23	8.17±0.40	42.00±3.34	242.17±19.63	171.50±17.38	38.93±1.31	343.00±18.92	751.50±61.84
Significance	[1]P=0.001	[1]P=0.001	[1]P=0.001	[1]P=0.001	[1]P=0.001	[1]P=0.001	[1]P=0.001	[1]P=0.001	[1]P=0.001
G3 (G.)	6.64±0.71	72.67±3.72	11.83±0.60	60.83±3.62	108.17±5.10	67.33±4.47	4.27±0.30	101.00±8.20	373.67±30.54
Significance	[1]P=0.988; [2]P=0.001	[1]P=0.580; [2]P=0.001	[1]P=0.442; [2]P=0.001	[1]P=0.494; [2]P=0.001	[1]P=0.320; [2]P=0.001	[1]P=0.823; [2]P=0.001	[1]P=0.793; [2]P=0.001	[1]P=0.186; [2]P=0.001	[1]P=0.699; [2]P=0.001
G4a (G.+TAA)	17.25±1.30	75.33±2.22	12.50±0.34	62.83±2.02	107.50±8.95	71.00±1.24	12.20±1.09	151.17±13.15	367.17±44.08
Significance	[1]P=0.001; [2]P=0.005	[1]P=0.801; [2]P=0.001	[1]P=0.078; [2]P=0.001	[1]P=0.922; [2]P=0.001	[1]P=0.343; [2]P=0.001	[1]P=0.898; [2]P=0.001	[1]P=0.001; [2]P=0.001	[1]P=0.111; [2]P=0.001	[1]P=0.768; [2]P=0.001
G4b (S.+TAA)	14.36±1.87	75.17±1.78	11.50±0.43	63.67±1.93	107.83±6.17	70.00±3.43	6.62±0.88	153.67±8.41	574.83±52.23
Significance	[1]P=0.004; [2]P=0.001	[1]P=0.840; [2]P=0.001	[1]P=0.797; [2]P=0.001	[1]P=0.883; [2]P=0.001	[1]P=0.331; [2]P=0.001	[1]P=0.974; [2]P=0.001	[1]P=0.031; [2]P=0.001	[1]P=0.083; [2]P=0.001	[1]P=0.003; [2]P=0.017
G5a (TAA+G.)	15.18±2.27	73.83±1.47	11.33±0.33	62.50±1.75	115.66±7.36	60.00±4.34	5.22±0.56	111.33±8.27	405.50±41.94
Significance	[1]P=0.002; [2]P=0.001	[1]P=0.840; [2]P=0.001	[1]P=1.000; [2]P=0.001	[1]P=0.845; [2]P=0.001	[1]P=0.133; [2]P=0.001	[1]P=0.356; [2]P=0.001	[1]P=0.294; [2]P=0.001	[1]P=0.468; [2]P=0.001	[1]P=0.406; [2]P=0.001
G5b (TAA+ S.)	9.72±1.51	72.67±0.71	12.00±0.50	60.67±1.09	111.00±10.24	68.33±2.87	6.57±0.87	181.00±12.36	433.83±65.62
Significance	[1]P=0.223; [2]P=0.001	[1]P=0.580; [2]P=0.001	[1]P=0.307; [2]P=0.001	[1]P=0.464; [2]P=0.001	[1]P=0.235; [2]P=0.001	[1]P=0.898; [2]P=0.001	[1]P=0.034; [2]P=0.001	[1]P=0.002; [2]P=0.001	[1]P=0.223; [2]P=0.001

Data are expressed as mean +/- standard error (SE). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase; ALP: alkaline phosphatase, LDH: lactate dehydrogenase. Pairwise comparison between each 2 groups was done using Post Hoc Test (Least significant test). [1]P: significance versus G1 (Control) group; [2]P: comparison versus G2 (TAA) group.

Table 3: Effect of Guarana *(Paullinia cupana)* against Thioacetamide-induced hepatotoxicity on serum levels of oxidative stress markers in adult male rats

Groups	MDA (nmol/ml)	SOD (ng/ml)	Catalase (nmol/ml)	GST (ng/ml)
G 1 (control)	2.52±0.17	5.77±0.13	68.08±0.62	56.05±1.26
G2 (TAA)	9.65±0.80	3.36±0.44	34.27±3.70	22.99±2.82
Significance	[1]P=0.001	[1]P=0.001	[1]P=0.001	[1]P=0.001
G3 (G.)	2.53±0.17	5.63±0.56	65.38±0.65	57.15±3.41
Significance	[1]P=0.983; [2]P=0.001	[1]P=0.738; [2]P=0.001	[1]P=0.441; [2]P=0.001	[1]P=0.732; [2]P=0.001
G4a (G.+TAA)	2.65±0.08	5.93±0.18	57.27±2.63	51.33±1.64
Significance	[1]P=0.786; [2]P=0.001	[1]P=0.720; [2]P=0.001	[1]P=0.004; [2]P=0.001	[1]P=0.148; [2]P=0.001
G4b (S.+TAA)	2.68±0.12	5.82±0.13	67.09±1.06	40.46±2.47
Significance	[1]P=0.786; [2]P=0.017	[1]P=0.902; [2]P=0.017	[1]P=0.777; [2]P=0.017	[1]P=0.001; [2]P=0.017
G5a (TAA+G.)	2.75±0.12	5.77±0.24	57.13±4.33	47.52±2.26
Significance	[1]P=0.618; [2]P=0.001	[1]P=1.000; [2]P=0.001	[1]P=0.003; [2]P=0.001	[1]P=0.011; [2]P=0.001
G5b (TAA+ S.)	2.72±0.06	6.03±0.10	69.82±0.84	46.60±0.75
Significance	[1]P=0.667; [2]P=0.001	[1]P=0.546; [2]P=0.001	[1]P=0.617; [2]P=0.001	[1]P=0.005; [2]P=0.001

Data are expressed as mean +/- standard error (SE). MDA: malondildehyde; SOD: superoxide dismutase; CAT: catalase; GST: glutathione S-transferase. Pairwise comparison between each 2 groups was done using Post Hoc Test (Least significant test). [1]P: significance versus G1 (Control) group; [2]P: comparison versus G2 (TAA) group.

G., G.+TAA, S.+TAA and TAA+S. versus control (P =0.007, P =0.001, P =0.001, P =0.020, respectively). Meanwhile, SOD in liver tissue homogenate were significantly increased in G., G. + TAA, S. + TAA, TAA+G. and TAA+S. versus TAA group (P=0.001for all). Liver tissue homogenate levels of catalase and GST in TAA group were significantly decreased versus control (P =0.001 for both) but were significantly increased in G., G.+TAA, S.+TAA and TAA+G. and TAA+S. groups versus control and TAA groups (Table 4).

H &E -stained sections in rat liver from both control and Guarana groups demonstrated normal hepatic architecture with hepatic cords radiating from clear central veins and were separated by sinusoids, without inflammation or necrosis. The liver sections of these groups showed normal hepatocytes with preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein. After exposure to TAA, liver sections showed an abnormal morphology characterized by noticeable fibrosis with extracellular matrix

Table 4: Effect of Guarana *(Paullinia cupana)* against Thioacetamide-induced hepatotoxicity on liver tissue homogenate levels of oxidative stress markers in adult male rats

Groups	MDA (nmol/mg protein)	SOD (ng/ mg protein)	Catalase (nmol/ mg protein)	GST (ng/ mg protein)
G 1 (control)	0.52±0.03	2.93±0.04	31.62±0.50	18.74±0.40
G2 (TAA)	1.74±0.06	1.24±0.13	22.79±0.73	9.31±0.33
Significance	[1]P=0.001	[1]P=0.001	[1]P=0.001	[1]P=0.001
G3 (G.)	0.56±0.02	3.32±0.18	44.72±0.24	42.57±0.58
Significance	[1]P=0.451; 2P=0.001	[1]P=0.007; [2]P=0.001	[1]P=0.001; [2]P=0.001	[1]P=0.001; [2]P=0.001
G4a (G.+TAA)	0.83±0.02	3.26±0.06	48.77±0.53	37.07±0.50
Significance	[1]P=0.001; [2]P=0.001	[1]P=0.001; [2]P=0.001	[1]P=0.001; [2]P=0.001	[1]P=0.001; [2]P=0.001
G4b (S.+TAA)	1.46±0.03	4.23±0.07	39.96±0.50	29.90±0.70
Significance	[1]P=0.001; [2]P=0.017	[1]P=0.001; [2]P=0.001	[1]P=0.001; [2]P=0.001	[1]P=0.001; [2]P=0.017
G5a (TAA+G.)	1.12±0.05	3.14±0.03	44.46±0.38	31.90±0.48
Significance	[1]P=0.001; [2]P=0.001	[1]P=0.130; [2]P=0.001	[1]P=0.001; [2]P=0.001	[1]P=0.001; [2]P=0.001
G5b (TAA+ S.)	1.22±0.02	3.26±0.06	52.04±0.19	34.61±0.40
Significance	P=0.001; [2]P=0.001	[1]P=0.020; [2]P=0.001	[1]P=0.001; [2]P=0.001	[1]P=0.001; [2]P=0.001

Data are expressed as mean +/- standard error (SE). MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GST: glutathione S-transferase. Pairwise comparison between each 2 groups was done using Post Hoc Test (Least significant test). [1]P: significance versus G1 (Control) group; [2]P: comparison versus G2 (TAA) group.

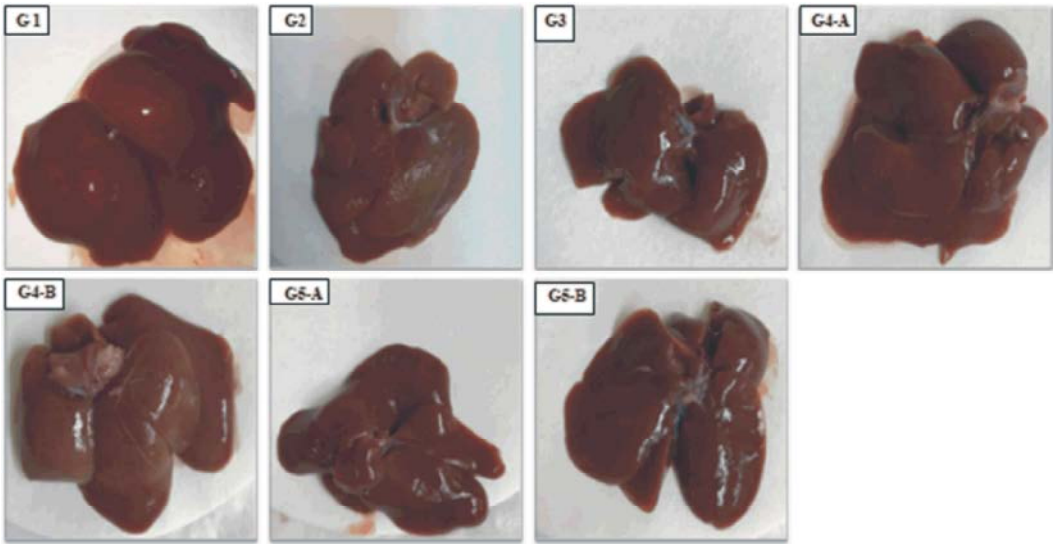

Fig. 1: Photographs of gross morphology of rat liver in different studied groups.

collagen contents and damage of hepatocytes structure. There were bundles of collagen surrounding lobules that resulted in fibrous septa and distorted tissue structure. Rats treated with Guarana plus TAA liver sections showed that fibrosis degree was substantially less than TAA alone treated rats, as well as reduced hepatocytes inflammation and necrosis. Liver tissues from Silymarin-treated rats showed mild collagen deposition and livers from rats treated with Guarana showed moderate deposition of collagen fibers and moderate congestion around central vein, this supports Guarana protective effects against TAA-induced hepatic toxicity. However, treatment with Guarana and Silymarin (30 and 100 mg/kg) showed improved morphology and reduced in fibrosis processes compared with liver sections of rats treated with only TAA (Figs. 1, 2, 3).

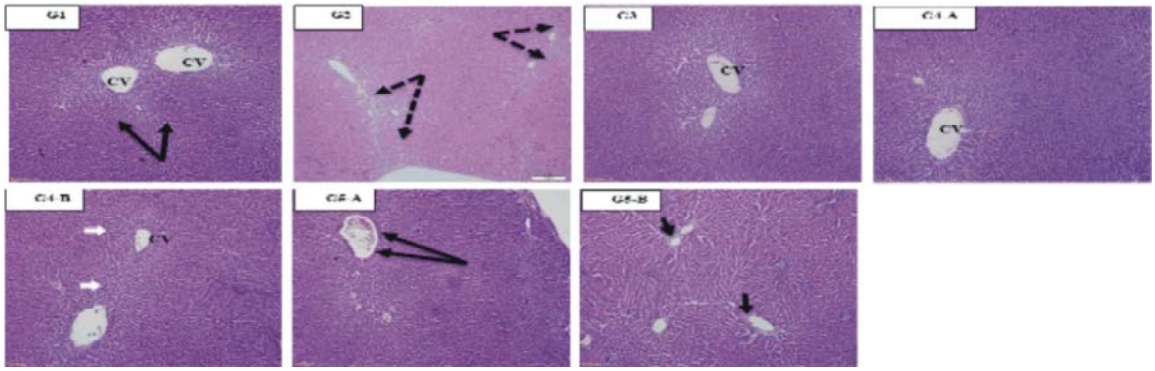

Fig. 2: A photomicrograph of H&E stained sections in rat liver of all studied groups. (All are H&E 100x).
G1: control showed normal hepatocytes (Thin black arrows) arranged in radial pattern around central vein (CV).
G2: Liver displays fibrous septa extends from portal area (Arrow) forming porto-portal bridging fibrosis (Grade III fibrosis).
G3: Liver display normal hepatocytes arranged in radial pattern around central vein.
G4-A: Liver display normal hepatocytes arranged in radial pattern around central vein.
G4-B: Liver display normal hepatocytes arranged in radial pattern around central vein with dilation of hepatic sinusoids (White arrow).
G5-A: Liver display mild fibrocollagenous tissue proliferation arises from portal area (Arrow).
G5-B: Liver display fibrocollagenous tissue proliferation arises from portal area (Arrow).

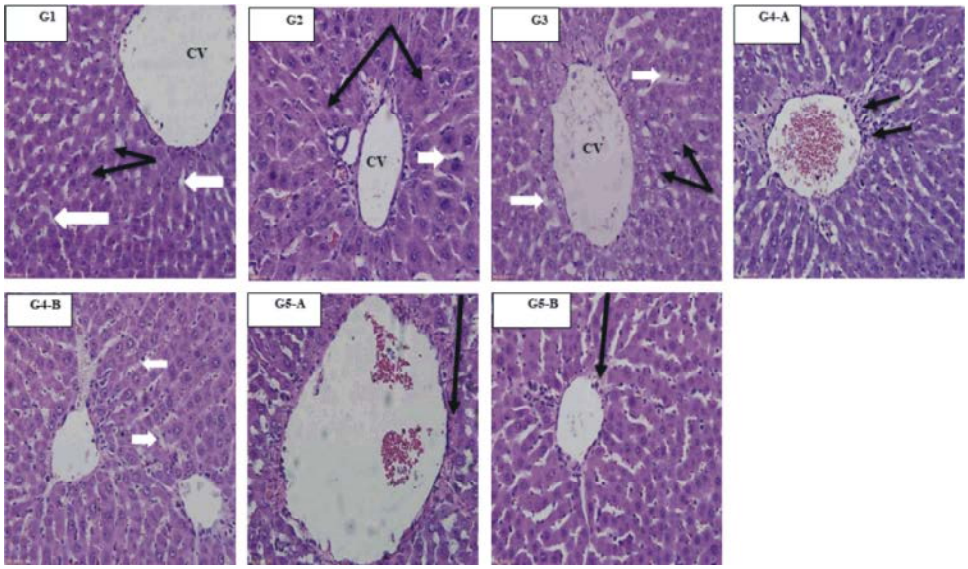

Fig. 3: A photomicrograph of H&E stained sections in rat liver of all studied groups (All are H&E 400x).
G1: Liver display normal hepatocytes (Black arrows) arranged in radial pattern around central vein (CV) and normal hepatic sinusoids (White arrows).
G2: Liver displays necrosis of hepatocytes (Black arrows) and congestion in hepatic sinusoids (White arrow).
G3: Liver display normal hepatocytes (Black arrows) arranged in radial pattern around central vein and normal hepatic sinusoids (White arrows).
G4-A: Liver display fibrous tissue proliferation from portal area (Black arrow) forming intralobular radiating pattern (Grade I fibrosis) extending from portal area.
G4-B: Liver display congested hepatic sinusoids (White arrow).
G5-A: Liver display mild fibrocollagenous tissue proliferation arises from portal area (Black arrow).
G5-B: Liver display lymphocytic infiltration in hepatic parenchyma and fibrocollagenous tissue proliferation arises from portal area (Black arrow)..

DISCUSSION

Hepatic damage has become a serious health problem due to utilization of several prescription drugs and exposure to various toxins [9]. TAA proves highly useful as in experimental liver injury model. It has been used for years as the lesions caused by this hepatotoxic drug are similar to most cases of liver diseases in human, which makes it a good model to study the mechanism *in vivo* [4]. Natural products, especially herbs with immense medicinal history of exhibiting beneficial effects against liver diseases are considered an alternative therapeutic approach. In the present study, the Guarana seed was examined as a promising therapy for treating hepatofibrosis in experimental rat model [19].

The present study demonstrated that TAA at a dose of 200 mg/kg body weight 3 times weekly for a period of 4 weeks induced decreased body weight gain in male Wistar rats. Similar observations were noted in experimental animals treated with TAA [20-22]. The relative decrease in body weight gain in TAA treated rats may be due to malnutrition resulting from reduced nutrients absorption from intestine of treated rats, energy utilization and metabolic inefficiency caused by TAA [23]. TAA increased protein catabolism and hampered utilization of food consumed during intoxication period, thereby causing a decrease in body weight [17]. Meanwhile, the weight gain was significantly increased when rats treated with Guarana or Silymarin compared to TAA group. Guarana could help in the better utilization of nutrients in diet thereby increasing body weight gain of rats [24].

Rats exposed to TAA showed a significant increase of liver weight compared with control group. Our data is in agreement with previous studies [3, 6]. Meanwhile, liver weight was decreased in G., G.+TAA, S.+TAA, TAA+G., TAA+S. groups compared to TAA group. These results indicate that 30 mg/kg Guarana was as effective as Silymarin in protecting rat liver against fibrosis. Previous studies indicated that an increase in liver weight was typical symptoms of TAA-induced hepatic toxicity [22, 25]. Measurement of liver index is a more accurate approach to determine the changes in liver size compared to liver weight alone as liver weight largely depends on rat size [17]. This study revealed that liver index was significantly increased in TAA treated group compared to control. Liver index was significantly decreased in rats administered Guarana or Silymarin compared to control and TAA groups. Al-Attar and Shawush [20] reported significant increase of liver index in adult male Wistar rats after TAA administration for 12 weeks. Similar observations were also detected in many experimental studies using TAA to induce hepatotoxicity [26-29]. Increased liver weight resulting from TAA injection seems to be due to accumulation of extracellular matrix (ECM) proteins such as collagen [30]. Guarana is a well-known and widespread plant used popularly and new reports assessing other biological activities are of interest. Guarana is rich in methylxanthines such as caffeine, theobromine and theophylline and contains tannins, saponins, cathechins, epicatechins, proanthocyanidols as well as trace concentrations of many other compounds [31].

In this study, TAA significantly increased serum total bilirubin content while, significantly decreased serum total protein, albumin and globulin levels versus control group. These results were in accordance with many studies on TAA-induced liver fibrosis and cirrhosis in experimental animals [3, 7, 25, 32]. Elevated total bilirubin levels due to blockage of bile ductile caused by inflammation and fibrosis in portal triads and/or regurgitation of conjugated bilirubin from necrotic hepatocytes to sinusoids [33]. Additionally, Al-Attar [34] reported that chronic TAA administration for a period of 10 weeks increased serum total bilirubin levels in Wistar male rats. El-Baz *et al.* [35] reported that TAA treatment at a dose of 200mg/kg for 6 weeks in male Wister rats led to significant elevation of bilirubin serum levels. Jain and Singhai [36] showed that the level of total protein was significantly decreased in rat treated with TAA at a dose (100 mg/kg) for 6 days. Kadir *et al.* [26] and Hessin *et al.* [37] reported decrease in serum albumin levels in TAA hepatotoxic animals. Abdou *et al.* [38] reported significant decrease in serum globulin levels in TAA treated rats in a dose of 250 mg/kg body weight three time a week for 8 weeks. The total protein levels are depressed in hepatotoxic conditions due to disturbances in the carbohydrate, protein, lipid metabolisms or perturbed protein biosynthesis in cirrhotic liver [32]. TAA acts as an electrophilic agent and leads to formation of s-oxide that can covalently bind to the lysine residues forming adducts with sulfhydryl groups hence lowering the protein levels and causing significant liver damage [9]. In the current study, the serum levels of total bilirubin were significantly decreased, while total protein, albumin and globulin were significantly increased in animals receiving Guarana or Silymarin before or after TAA administration compared to TAA group. However, Guarana was able to restore the low albumin level to normal comparable to Silymarin effects. Wang *et al.* [39] showed that total bilirubin was improved after flavonoid treatment, suggesting that flavonoids can function as a

replenishment method in ameliorating liver damage and preventing liver fibrosis. Abdel-Hamid *et al.* [40] showed that the level of total protein was elevated in rats treated with individual catechin. Alkiyumi *et al.* [41] showed that the treatment with Silymarin at a dose (50 mg/kg) by oral administration three times a week for two months led to significant restore of total bilirubin levels. Bardi *et al.* [29] showed that treatment the rats with Silymarin at a dose (50mg/ kg) daily led to increase of serum albumin levels.

In the present study, TAA administration caused significant elevation in serum levels of AST, ALT, GGT, ALP and LDH indicating that liver is susceptible to TAA-induced toxicity. Similar observations were noted in experimental animals treated with TAA [7, 27, 29, 41, 42]. TAA causes lipid destruction and protein denaturation on the cell membrane of rat liver cells leading to intracellular enzyme leakage and increased serum AST level [43]. Elevation of the activity of liver enzymes after TAA administration indicates cellular leakage, loss of structural and functional integrity of the liver [8]. Ghosh *et al.* [44] reported that treatment with TAA at a dose of 100 mg/Kg for 56 days caused an elevation in LDH serum level in male mice. An elevated serum ALP level could be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells in presence of increasing biliary pressure [45]. In contrast, the increased level of these enzymes was significantly inhibited in all experimental groups that were treated with Guarana or Silymarin versus TAA group. Kober *et al.* [46] reported that a single dose intraperitoneally of CCl4 caused an elevation in serum level AST activity that was significantly inhibited by the pretreatment orally daily with Guarana in all different concentrations (100, 300 and 600 mg/kg) for a period of 14 days. Abboud *et al.* [47] indicated that consumption of Guarana by male Wistar rats with alloxan induced diabetes without treatment had a beneficial effect on hepatic (AST, ALT). Caffeine is well known as the major constituent of Guarana [12]. Rezaie *et al.* [48] indicated that caffeine tended to prevent liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes through membranes, exhibiting hepatoprotective activity. Shin *et al.* [49] showed that oral administration of proanthocyanidins (20 mg•kg-1 daily for four weeks) remarkably prevented the elevation in levels of ALP induced by dimethylnitrosamine in rats. Yang *et al.* [50] showed that treatment the rats with Silymarin at a dose (50 mg/kg/day) led to significant reduction in ALP enzyme induced by TAA. Silymarin is anti-cirrhotic effects was recorded in experimental animals [27, 32].

Histologically, in contrast to control group, TAA administration resulted in extensive hepatocellular damage, including inflammatory cells and hepatic necrosis. However, the rats treated with Guarana or Silymarin showed obvious improvement indicated by mild to moderate infiltration of lymphocytes and less hepatocyte necrosis in liver morphology. Our findings were confirmed by disturbed microscopic images showing features of degeneration, necrosis, inflammatory infiltration and elements of fibrosis.

TAA is a toxic agent that causes hepatocytes necrosis and it contributes to cirrhosis development through multiple action mechanisms, such as oxidative stress, decrease of the antioxidant system response and lipid peroxidation [51]. MDA is a major oxidation product of per oxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation [52, 53]. SOD plays crucial role in defense mechanisms governing the anti-oxidant activities and hence in prevention of diseases linked to oxidative stress [27]. Catalase is a major intracellular antioxidant enzyme. Its function is to protect cells from H_2O_2 accumulation by catalyzing its decomposition into water and oxygen [54]. The current study demonstrated that in TAA alone treated group, serum and tissue homogenate levels of MDA were significantly increased, while, SOD, catalase and GST were significantly decreased versus control. These results are consistent with many previous studies [3, 8, 55, 56]. The increased MDA content might have resulted from an increase of ROS as a result of stress condition in the rats with TAA intoxication. Wong *et al.* [28] indicated that the serum levels of MDA were significantly increased in rats administered with TAA at a dose (200 mg/kg). It was suggested that hepatotoxins including TAA induced liver damage by forming free radicals, which then react with cellular lipids to promote lipid peroxidation [57]. Low level of SOD and GST by TAA was previously reported [58]. Hepatotoxicity by TAA requires metabolic activation with the formation of the reactive metabolites, S-oxide (TASO) and S, S-dioxide (TASO2), which binds to microsomal lipids, leading to is peroxidation, as well as ROS production, such as hydroxyl, peroxide and superoxide radicals. ROS affect antioxidant defense mechanisms and they decrease SOD activity, leading to liver damage, cirrhosis and hepatocellular carcinoma [51].

In the present study, the serum and liver tissue homogenate levels of MDA were reduced; while SOD, catalase and GST were significantly increased versus TAA in Guarana or Silymarin treated rats suggesting its cytoprotective and curative activities against TAA.

Natural antioxidants eliminate oxidative stress caused by CCl4 and other hepatotoxicant [59]. In a study conducted by Bittencourt *et al.* [60], Guarana was also able to modulate the activity of MDA. In addition to antioxidant activity, Guarana presented effects on NO modulation in fibroblast NIH-3T3 cells exposed to sodium nitroprusside. The antioxidant activity of plant-derived phenolic compounds is exhibited through various mechanisms including free radicals scavenging, metal ions chelation and anti-lipid peroxidation [61]. Yang *et al.* [50] revealed that pre-administered procyanidin B2 significantly inhibited the increase of MDA in the liver of the CCl4-treated mice. Bittencourt *et al.* [60] reported that Guarana modulate SOD enzyme activity. Yang *et al.* [22] reported that TAA administration at a dose (150 mg/kg, twice per week) for 6 weeks to male rats significantly reduced SOD activity liver tissue homogenate but Silymarin oral administration at a dose (50 mg/kg) daily for 6 weeks significantly increased SOD activity. Catechin was reported to have superior antioxidant abilities compared to antioxidant nutrients vitamin C, β-carotene and vitamin E [62] and scavenge free radicals through decreasing MDA content and enhancing SOD activity [63]. GST is a soluble enzyme located in cytosol, which plays a significant function in xenobiotics detoxification [58]. It increases the solubility of hydrophobic substances and metabolizes toxic compounds to non-toxic ones, which means they have an increasing liver protective activity [64]. Samarghandian *et al.* [65] showed that injection the rats with Catechin at a dose (20mg/kg, 40mg/kg) caused increase serum GST level. Flavonoids were proven to decrease oxidative stress, inflammation and lipid peroxidation in mouse model with alcohol-induced hepatic damage [66]. Ghosh *et al.* [44] reported that liver GST level was decreased in TAA treated mice at a dose 100 mg/kg for 56 days; meanwhile, treatment with Silymarin at a dose 150 mg/kg for 8 weeks led to restore this enzyme activity to normal.

CONCLUSIONS

The results of the present study indicated that Guarana *(Paullinia cupana)* had hepatoprotective effect against TAA induced biochemical and histological changes in rat liver. The oral administration of Guarana seeds powder at dose of 30 mg/kg for three weeks significantly decreased the serum levels of liver enzymes of AST, ALT, ALP, GGT, LDH and bilirubin. It can be concluded that the supplementation of Guarana counteracts TAA -induced hepatotoxicity in rats by reducing oxidative stress and improved liver cells due to their antioxidant properties.

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