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Ameliorative Roles of Guarana (*Paullinia cupana*) Against Carbon Tetrachloride-Induced Acute Hepatotoxicity in Rats

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Abstract: Several biological effects of Guarana (*Paullinia cupana*) have been demonstrated, but little information is available on its effects on the liver. This study aimed to investigate the ameliorative effects of Guarana seed powder on carbon tetrachloride (CCl_4)- induced hepatotoxicity in rats. Thirty adult male rats were sorted equally into five groups (G1-G5), 6 rats each. The first group (G1) received olive oil intraperitoneally (IP) for four weeks (control group). G2 group received IP injections of CCl_4 (0.5 ml/kg) dissolved in olive oil (1:1 v/v) three times weekly for four weeks (toxicant group). G3 group received a daily oral dose of Guarana (30 mg/kg) for three weeks (Guarana group). G4 received IP CCl_4 for four weeks then received Guarana orally for three weeks (toxicant/Guarana group). G5 group received CCl_4 IP for four weeks then administered daily IP injection of metformin (250 mg/kg) for three weeks (toxicant/metformin group). During the experimental period, food and water intakes and body weight were recorded and weight gains were calculated. At the end of the experiments blood samples were obtain for measurement of liver functions tests. Liver tissue homogenate was prepared and oxidative stress markers in it were measured. Liver was examined histologically. Results showed that treatment with CCl_4 significantly increased serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) enzymes and bilirubin and malondialdehyde (MDA) in liver tissue homogenate, while decreased activity of the antioxidant enzyme glutathione S-transferase (GSH) in liver tissue homogenate and damage liver cells. Compared to CCl_4-treated rats, treatment with Guarana effectively suppressed lipid accumulation, liver injury and significantly decreased the serum levels of ALT, AST, ALP, GGT and bilirubin compared. Our finding demonstrates that treatment with Guarana counteracts CCl_4-induced hepatotoxicity in rats by decreasing oxidative stress and increasing biological antioxidant power and improved liver cells.

Key words: Carbon tetrachloride · Hepatotoxicity · Metformin · Oxidative stress · *Paullinia cupana* · Rats

INTRODUCTION

The liver, as a main organ of metabolism and excretion of waste, is constantly endowed with the task of detoxification. Hepatotoxicity is a prevalent health problem that represents 38% of all hepatic problems worldwide [1]. Injury of liver can be caused by toxic chemicals, environmental pollutants, chemotherapeutic agents, minerals, fungal products, bacterial metabolites and infiltration of virus from ingestion or infection [2].

Carbon tetrachloride (CCl_4) is a transparent, odorless and non- flammable material. CCl_4 causes acute liver toxicity in human and experimental animals [3]. Metabolic activation of CCl4 by cytochrome P450 to the free radicals (trichloromethyl and trichloromethyl peroxy radicals) is reported to enhance lipid peroxidation and protein oxidation in the liver leading to membrane damage and liver injury [4]. Hepatotoxicity using CCl_4 is a common model used to measure the efficiency of several anti-hepatotoxic drugs [5, 6].

The lack of effective modern medications to treat acute and chronic liver injury has led to the development of researches using various experimental models to detect the hepato-protective activity of various medicinal plants. A candidate medication includes the seed extract of *Paullinia cupana* Mart. var. sorbilis (Sapindaceae) that is a plant popularly known as Guarana that is native to the central Amazon basin and exists in Brazil, Colombia,

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Equador, Peru, Venezuela and the Republic of Guyana [7]. Chemically, Guarana seeds mainly contain methyl-xanthine derivatives and rich in caffeine, theophylline and theobromine, which have beneficial effects on the central nervous system. It also presents high concentration of polyphenols, such as tannins, flavonoids and catechins substances with antioxidant action [8]. The broad spectrum of medicinal activities of Guarana ranging from stimulation of the central nervous system, in cases of physical or mental stress, to appetite suppression and increased body metabolism. It can also be used as an aphrodisiac for impotence, antipyretic and acts as anti-platelets aggregation, antioxidant and antibacterial [9 - 12]. *Paullinia cupana* had protective effect against diethyl nitrosamine-induced DNA damage in mouse liver [13] and gastric lesions induced by ethanol and indomethacin [13].

The present study aims to evaluate the ameliorative effects of Guarana against CCl_4-induced acute hepatotoxicity as compared to metformin in rats to improve the quality of care in the treatment of liver disorders.

MATERIALS AND METHODS

Chemicals: Glucophage XR (750 mg) (metformin hydrochloride) were purchase from local pharmacy (Merck Serono, Darmstadt, Germany) Carbon tetrachloride CCl4 was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Commercial kits for the measurement of activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), bilirubin, malondialdehyde (MDA), reduced glutathione (GSH) were obtained from Siemens Healthcare Diagnostics Inc. (Newyork, DE 19714, USA).

Paullinia Cupana Powder: A *Paullinia cupana* Mart. var. sorbilis powder seed was supplied by I Herb (USA) and was conserved dry and protected from light at -20ºC until administration. The solution administered to the animals was prepared on the day of use by diluting the Guarana powder seeds in water [14].

Animals: Thirty male adult Wister albino rats with an average weight of 200-250 g were provided by Animal House of King Fahd Medical Research Center, King Abdulaziz University (KAU), Jeddah, Saudi Arabia. Animals were left for one week in the lab for acclimatization with fixed humidity (65%), temperature (20 ± 1°C) and light/dark (12hrs/12hrs) period. Rats had free access to standard rodent chow and water ad libitum. It was housed one rat per cage. All the experimental procedures involving the animals and their care were done following ethical guidelines of the Animal Care and Research Ethical Committee on animal use at KAU, Jeddah, Saudi Arabia, which were in compliance with the Guidelines for Care and Use of Laboratory Animals in Biomedical Research as adopted and promulgated by the World Health Organization and the United States National Institutes of Health, Bethesda, Maryland, 1985, no. 85-23.

Experimental Protocol: Animals were randomized sorted into 5 equal groups; 6 rats in each group. Group 1 (GI) represents the control group, which received single intraperitoneally (IP) (0.50 ml/kg) of olive oil for 4 weeks. G2 (toxicant group) received CCl_4 in olive oil (1:1 v/v) IP at dose of 0.50 ml/kg, 3 times weekly for 4 weeks to induced liver toxicity [15]. G3 (guarana group) received a daily oral dose of Guarana (30 mg/kg) for 3 weeks as described [16]. G4 and G5 groups received CCl_4 IP for 4 weeks then received a daily oral dose of Guarana (30 mg/kg) or IP injection of metformin (250 mg/kg) for 3 weeks, respectively. Metformin was used as the standard hepatotoxicity protective agent to compare its therapeutic effects with that of Guarana.

Body Weight Determination: The total body weights, food intake and water intake of all rats were recorded on a weekly basis throughout the experimental period. The animals were observed for any behavioral or clinical abnormalities. Weight gain (%) was calculated according to the following formula

Final body weight (grams) - initial body weight (grams)/ initial body weight (grams) X 100

At the end of the experiment, rats were sacrificed by cervical dislocation and abdomen was opened and liver was carefully excised and weighted then organ indexes (organ weight (grams)/ final body weight (grams) X 100) were determined. Tissues from liver were cut into thin slices (2 x 2 cm) and immersed in 10% isotonic buffered formalin fixative for histological study.

Biochemical Analysis: At the end of the experiment, from all the animals 4 ml of blood was withdrawn from retro-orbital plexus under mild ether anesthesia into plain tube. The serum was separated by centrifuging at 4000 rpm for 15 min and sorted and kept at -20°C for analysis of various parameters. Liver function tests as

serum levels of ALT, AST, GGT, ALP and bilirubin were measured using standard technique using automated analyzer (Dimension Vista, USA).

Preparation of Hepatic Homogenate: Equal weights of liver tissue from all groups were used for preparing hepatic homogenate. The weighed frozen liver tissue was homogenized in a glass-Teflon homogenizer with 50 mM phosphate buffer (pH 7.4) to obtain 1:9 (w/v) whole homogenate. The homogenates were centrifuged at 11, 000 g for 15 min at 4°C and the supernatant was used for the measurement of MDA and GSH using commercial available kits. Total protein contents were determined by the method of Lowry et al. [17], using bovine serum albumin as a standard.

Histopathological Examination: A portion of the median lobe of the liver was dissected and fixed in 10% neutral buffered formalin solution for 24 h. The remaining livers were frozen quickly in dry ice and stored at -80ºC for biochemical analysis. Fixed slices of liver was washed thoroughly with water and processed for paraffin embedding in automatic machine. Five-micron paraffin sections were stained by hematoxylin and eosin using standard techniques, then examined, photographed and labeled to show the histological changes due to the different treatments.

Statistical Analysis: Statistical analysis was performed using SPSS version 20 (SPSS, Chicago, IL). All the experimental data was expressed as means and standard deviation (SD). For significance verification by groups, one-way ANOVA was performed, followed by least significant difference (LSD) test. *P* values of <0.05 were considered statistically significant.

RESULTS

Influence of guarana seed in alleviating the CCl_4-induced acute hepatotoxicity in rats was compared to metformin, the standard protective agent used at commercial scale. Comparison of food intake (g) and water intake (ml) across time in different treated groups showed that animals of group G2 showed significant decreased in food and water intake compared to G1 and animals in this group were sacrificed at the 4th week to demonstrate the effect of CCl_4 on the liver (Tables 1 & 2). Interestingly, comparison of guarana group (G3) versus the control (G1) indicated that the first showed significantly higher food intake at the 6th and 7th weeks. Guarana and metformin in G4 and G5 groups, respectively, alleviated the influence of CCl_4 as no significant differences were scored at the 6th and 7th weeks for either treatment as compared with the control except that rats of G4 group that showed significantly higher ability to take up food at the 6th week (Tables 1 & 2).

Body weights of the rats significantly decreased in the CCl_4-treated group as compared with the control (G1) or rats of the guarana (G3), guarana/CCl_4 (G4) or metformin/CCl_4 (G5) groups. Guarana and metformin in G4 and G5 groups did not completely alleviate the influence of CCl_4 as body weight was slightly decreased in G4 group, while slightly increased in G5 group due to the effect of metformin (Table 3). Results for liver index indicated no significant differences among the five groups (Table 3).

Liver function parameters in terms of enzyme (ALT, AST, ALP and GGT) activities as well as the levels of serum bilirubin are shown in Table 4. Significant increases were scored for these parameters due to CCl_4 treatment in G2 group as compared to the control as well as the other three groups (Table 4). There are no significant differences scored among rats in the control as compared to those in G3, G4 and G5 groups in terms of ALT and ALP enzyme activities as well as the bilirubin level. Interestingly, AST activity levels of rats in guarana group (G3) were significantly lower than the control group. Treatments of guarana and metformin did not completely alleviate the influence of CCl_4 in terms of GGT enzyme activity as rats in groups G4 and G5 showed significant increase in enzyme activity (Table 4).

Although, there are significant differences in rats of groups G3, G4 and G5 as compared with those of group G2 in terms of the two oxidative stress markers, neither guarana nor metformin were able to significantly alleviate the influence of CCl_4 (Table 5).

Histopathological Results: Liver of control rat showed normal structure as previously described in literature, central vein (CV) demarcated the center of liver lobules. Hepatocytes were polyhedral in shape and had one or two active vesicular nuclei and homogenously stained cytoplasm. They were arranged in regular cords radiating from the central vein and separated by thin wall blood sinusoids. Portal area (PA) was found at the periphery and contains branches of portal vein, bile duct and hepatic artery (Fig. 1 G1). In CCl4 group, histological changes in the form of hepatocyte cell necrosis, fibroblast proliferation with fine collagen deposition mainly in portal areas and bridging the adjacent lobules with inflammatory

Table 1: Comparison of food intake (g) across time in different studied groups (G3-G5) versus control (G1) and CCl_4 (G2) groups

	Groups				
Weeks	G1 (control)	G2 (CCl_4)	G3 (guarana)	G4 (CCl_4/guarana)	G5 (CCl_4/metformin)
1st week	23.60±0.85	7.77±1.96	21.23±0.51	9.87±2.30	7.60±1.04
Significance		$^1P = 0.0001$	$^1P = 0.082$; $^2P = 0.0001$	$^1P = 0.0001$; $^2P = 0.117$	$^1P = 0.0001$; $^2P = 0.892$
2nd week	22.83±1.72	9.67±2.06	22.47±1.62	14.55±1.95	17.10±1.06
Significance		$^1P = 0.0001$	$^1P = 0.799$; $^2P = 0.0001$	$^1P = 0.0001$; $^2P = 0.006$	$^1P = 0.002$; $^2P = 0.0001$
3rd week	24.77±2.57	12.30±1.02	24.10±1.55	21.60±0.56	32.10±16.82
Significance		$^1P = 0.074$	$^1P = 0.917$; $^2P = 0.088$	$^1P = 0.623$; $^2P = 0.167$	$^1P = 0.268$; $^2P = 0.010$
4th week	15.10±11.92	6.43±1.23	21.37±1.34	23.90±0.36	21.73±0.41
Significance		$^1P = 0.078$	$^1P = 0.185$; $^2P = 0.007$	$^1P = 0.074$; $^2P = 0.003$	$^1P = 0.163$; $^2P = 0.006$
5th week	20.53±1.96	Animals scarified	23.60±0.27	22.70±1.56	21.40±2.95
Significance			$^1P = 0.088$	$^1P = 0.208$	$^1P = 0.599$
6th week	22.47±0.47	Animals scarified	24.60±0.61	23.63±0.74	21.57±0.50
Significance			$^1P = 0.002$	$^1P = 0.042$	$^1P = 0.098$
7th week	21.67±1.63	Animals scarified	23.77±0.64	23.37±0.95	21.03±0.95
Significance			$^1P = 0.048$	$^1P = 0.095$	$^1P = 0.501$

Data are expressed as mean+/- standard deviation. ^1P: significance versus control group; ^2P: significance versus CCL_4 group using One-way ANOVA (LSD) test

Table 2: Comparison of water intake (ml) across time in different studied groups (G3-G5) versus control (G1) and CCl_4 (G2) groups

	Groups				
Weeks	G1 (control)	G2 (CCl_4)	G3 (guarana)	G4 (CCl_4/guarana)	G5 (CCl_4/metformin)
1st week	34.43±4.31	15.00±1.32	25.60±1.74	14.23±4.18	10.77±0.95
Significance		$^1P = 0.0001$	$^1P = 0.004$; $^2P = 0.001$	$^1P = 0.0001$; $^2P = 0.752$	$^1P = 0.0001$; $^2P = 0.103$
2nd week	31.67±4.56	16.10±6.46	24.77±7.48	26.47±0.58	18.20±1.40
Significance		$^1P = 0.003$	$^1P = 0.116$; $^2P = 0.056$	$^1P = 0.224$; $^2P = 0.027$	$^1P = 0.007$; $^2P = 0.612$
3rd week	30.33±2.85	28.63±15.36	20.60±4.00	32.20±5.46	29.77±11.95
Significance		$^1P = 0.827$	$^1P = 0.229$; $^2P = 0.315$	$^1P = 0.811$; $^2P = 0.649$	$^1P = 0.942$; $^2P = 0.884$
4th week	32.07±1.07	36.73±2.82	23.80±0.46	38.27±1.55	31.30±2.98
Significance		$^1P = 0.018$	$^1P = 0.001$; $^2P = 0.0001$	$^1P = 0.004$; $^2P = 0.377$	$^1P = 0.654$; $^2P = 0.008$
5th week	31.33±2.40	Animals scarified	27.43±2.41	34.83±1.05	25.53±2.87
Significance			$^1P = 0.070$	$^1P = 0.098$	$^1P = 0.014$
6th week	31.37±0.49	Animals scarified	24.97±1.50	32.83±2.47	29.73±2.67
Significance			$^1P = 0.004$	$^1P = 0.391$	$^1P = 0.343$
7th week	32.07±1.69	Animals scarified	25.60±2.05	31.47±1.78	29.87±1.15
Significance			$^1P = 0.002$	$^1P = 0.677$	$^1P = 0.152$

Data are expressed as mean+/- standard deviation. ^1P: significance versus control group; ^2P: significance versus CCL_4 group using One-way ANOVA (LSD) test.

Table 3: Comparison of total body, liver and kidney weights in different studied groups (G3-G5) versus control (G1) and CCl_4 (G2) groups.

	Groups				
Parameter	G1 (control)	G2 (CCl_4)	G3 (guarana)	G4 (CCl_4/guarana)	G5 (Ccl_4/metformin)
Initial body weight (g)	237.67±13.08	257.83±17.16	226.00±10.94	268.50±12.57	232.50±15.06
Significance		$^1P = 0.019$	$^1P = 0.159$; $^2P = 0.001$	$^1P = 0.001$; $^2P = 0.197$	$^1P = 0.526$; $^2P = 0.004$
Final body weight (g)	277.17±13.23	223.50±13.18	250.17±11.23	261.20±12.76	235.80±25.20
Significance		$^1P = 0.0001$	$^1P = 0.007$; $^2P = 0.016$	$^1P = 0.109$; $^2P = 0.002$	$^1P = 0.0001$; $^2P = 0.258$
Body weight gain (g)	39.50±17.87	-37.00±8.98	24.17±7.14	-8.00±11.90	4.60±16.99
Significance		$^1P = 0.0001$	$^1P = 0.062$; $^2P = 0.0001$	$^1P = 0.0001$; $^2P = 0.004$	$1P = 0.0001$; $^2P = 0.0001$
Liver index (%)	2.94±0.48	3.54±1.16	3.33±0.41	2.73±0.09	2.77±0.58
Significance		$^1P = 0.134$	$^1P = 0.264$; $^2P = 0.600$	$^1P = 0.558$; $^2P = 0.054$	$^1P = 0.645$; $^2P = 0.068$

Data are expressed as mean+/- standard deviation. ^1P: significance versus control group; ^2P: significance versus CCL_4 group using One-way ANOVA (LSD) test.

Table 4: Comparison of liver function parameters in different studied groups (G3-G5) versus control (G1) and CCl_4 (G2) groups.

	Groups				
Parameter	G1 (control)	G2 (CCl_4)	G3 (guarana)	G4 (CCl_4/guarana)	G5 (Ccl_4/metformin)
ALT (IU/L)	75.02±4.88	415.42±32.51	82.01±11.34	93.60±21.09	96.48±16.71
Significance		$^1P = 0.0001$	$^1P = 0.544$; $^2P = 0.0001$	$^1P = 0.114$; $^2P = 0.0001$	$^1P = 0.070$; $^2P = 0.0001$
AST (IU/L)	113.42±14.26	365.00±29.85	93.32±9.25	117.17±8.64	101.58±8.40
Significance		$^1P = 0.0001$	$^1P = 0.042$; $^2P = 0.0001$	$^1P = 0.693$; $^2P = 0.0001$	$^1P = 0.219$; $^2P = 0.0001$
ALP (IU/L)	166.50±38.44	462.42±64.30	188.13±28.33	161.38±26.64	185.08±14.23
Significance		$^1P = 0.0001$	$^1P = 0.337$; $^2P = 0.0001$	$^1P = 0.819$; $^2P = 0.0001$	$^1P = 0.408$; $^2P = 0.0001$
GGT (IU/L)	2.20±0.40	16.78±1.25	2.18±0.39	12.60±0.85	7.47±0.83
Significance		$^1P = 0.0001$	$^1P = 0.972$; $^2P = 0.0001$	$^1P = 0.0001$; $^2P = 0.0001$	$^1P = 0.0001$; $^2P = 0.0001$
Bilirubin (umol/L)	2.53±0.56	17.02±3.55	2.87±0.40	4.03±0.63	3.66±0.50
Significance		$^1P = 0.0001$	$^1P = 0.724$; $^2P = 0.0001$	$^1P = 0.131$; $^2P = 0.0001$	$^1P = 0.250$; $^2P = 0.0001$

Data are expressed as mean+/- standard deviation. ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma glutamyl transferase. ^1P: significance versus control group; ^2P: significance versus CCL_4 group using One-way ANOVA (LSD) test.

Table 5: Comparison of oxidative stress markers in different studied groups (G3-G5) versus control (G1) and CCl_4 (G2) groups in liver tissue homogenate.

	Groups				
Parameter	G1 (control)	G2 (CCl_4)	G3 (guarana)	G4 (CCl_4/guarana)	G5 (Ccl_4/metformin)
MDA (nmol/mg protein)	8.89±1.27	90.02±9.65	6.80±0.60	53.54±9.22	42.09±10.82
Significance		$^1P = 0.0001$	$^1P = 0.642$; $^2P = 0.0001$	$^1P = 0.0001$; $^2P = 0.0001$	$^1P = 0.0001$; $^2P = 0.0001$
GSH (umol/ mg protein)	52.93±1.49	24.86±2.86	74.88±7.96	28.20±3.16	35.83±4.97
Significance		$^1P = 0.0001$	$^1P = 0.0001$; $^2P = 0.0001$	$^1P = 0.0001$; $^2P = 0.226$	$^1P = 0.0001$; $^2P = 0.0001$

Data are expressed as mean+/- standard deviation. MDA: malondialdehyde; GSH: (reduced glutathione. ^1P: significance versus control group; ^2P: significance versus CCL_4 group using One-way ANOVA (LSD) test

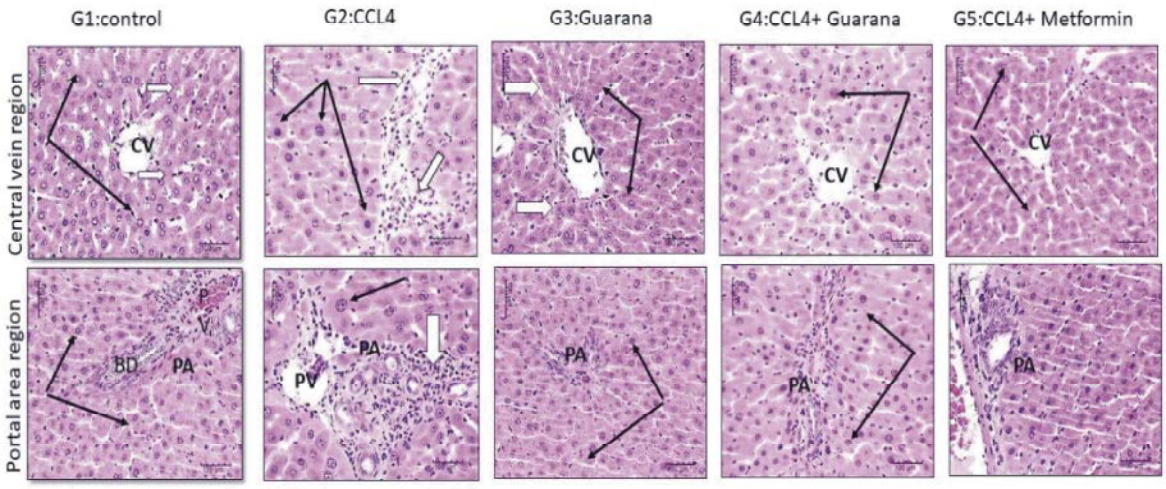

Fig. 1: Sections in rat liver stained by H&E stain to show both central vein (CV) and portal area(PA) regains of:Group 1(GI): Control group showing normal hepatocytes with rounded central active vesicular nuclei (black arrows) separated by thin wall blood sinusoids (white arrows). Group2 (G2): Toxicant group Showing, histological changes in the form of hepatocyte cell necrosis(black arrows), fibroblast proliferation with fine collagen deposition mainly in portal areas and bridging the adjacent lobules with inflammatory cell infiltrate (white arrows). Group 3 (G3): Guarana group Showing no apparent histological changes were observed in liver tissue and hepatocytes looked normal similar or more healthy than control (black arrows) either in central vein region (CV) or portal area (PA) (white arrows). Group 4 (G4) (CCL4+ Guarana) marked protection was observed and liver tissue around central vein (CV) looked normal with absence of any signs of cell necrosis or fibroblasts proliferation. Portal area (PA) also showed normal contents (black arrows). Group 5 (G5): (CCL4 + metformin) marked protection from CCl4 induced degenerative and fibrotic changes were observed (black arrows).

cell infiltrate (Fig. 1 G2). In G3 where guarana was given for 3 weeks, no apparent histological changes were observed in liver tissue either in central vein region (CV) or portal area (PA) and hepatocytes looked normal similar or more healthy than control (Fig. 1, G3). In G4 (guarana/CCl_4), marked protection was observed and liver tissue around central vein (CV) looked normal with absence of any signs of cell necrosis or fibroblasts proliferation. Portal area (PA) also showed normal contents (Fig. 1. G4). In G5 (metformin/CCl_4) marked protection fromCCl4 induced degenerative and fibrotic changes was observed (Fig. 1.G5).

DISCUSSION

Guarana is a plant originated in Brazil seeds are widely used in pharmaceuticals and foods as a tonic, a stimulant of the central nervous system [18], augmentation of learning and memory [19] and had *in vitro* antioxidant properties [20]. The results of the study revealed that body weights gain of the rats significantly decreased in the CCl4-treated group but increased in the treated groups with Guarana and Metformin compared with control, however, there was no significant change in the body weight of group that supplemented by Guarana alone compared with normal control. The reduction in body weight in CCl4 treated group observed in this study explained by impaired the activation and utilization of nutrients due to mal-digestion and/ or malabsorption caused by gastrointestinal disturbances of CCI4 administration [21]. Guarana could help in the better utilization of nutrients in the diet thereby increasing body weight of rats. Also, the results of this study revealed that liver weights index was insignificantly changed among rats of the CCl4-treated group compared with control. This result may be due to the low dose and short duration of the exposure to CCl4. Meanwhile, Eidi *et al*. [15] reported that liver weight significantly increased among the CCl4-treated rats that were reversed with the administration of Guarana. The accumulation of lipids [22] and collagen [23] could contribute to a rise in liver weight/body weight ratio in CCl4-treated rats as compared to control in their study.

In this study, we used an experimental model of CCl4-induced acute hepatotoxicity in rats because this chemical is a potent hepato-toxin and a single exposure can rapidly lead to severe hepatic necrosis and steatosis [24]. The results obtained in this study indicated that the treatment with CCl4 caused a significant increase in the serum levels of liver enzymes as AST, ALT, GGT, ALP and bilirubin compared with control group [25]. CCl4 produces hepatic injury that leads to large increases in both ALT and AST activities [21]. These enzymes activities reflect acute liver damage and hepatocellular disorders. Since these enzymes are cytoplasmic in nature, upon liver injury, these enzymes enter into the circulatory system due to altered permeability of the cell membrane [26]. The histologic alterations probably occurred as a result of the lipid peroxidation and breakdown of the membrane structure. While, the treatment with Guarana in this study was effective in decreasing the ALT, AST, GGT, ALP and bilirubin when compared with the CCl4 treated group. Therefore, treatment with Guarana showed a marked tendency towards normalization of all measured biochemical parameters in CCl4 treated rats. These phenomena were also confirmed by histological observations. Histologically, in contrast to the control group, CCl4 administration resulted in extensive hepatocellular damage, including inflammatory cell inflammation and hepatocyte necrosis. However, the rats treated with Guarana or Metformin showed obvious improvement indicated by mild to moderate infiltration of lymphocytes and less hepatocyte necrosis in liver morphology. Kober and his coworkers [27] reported that a single dose intraperitoneally of CCl4 caused an elevation in serum levels of ALT and AST activities that were 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. The AST activity in the groups pre-treated with Guarana was 4–7 times lower than in CCl4 group, whereas the ALT activity was 4–6 times lower. They suggest that the Guarana may stabilize the hepatic cellular membrane and protect the hepatocytes against toxic effects of CCl4, which may decrease the rate of leakage of the enzymes into the bloodstream.

The used dosed of Guarana in the present doses was 30 mg/kg orally for 3 weeks showed no changes in the liver functions of the rats. Meanwhile, Otobone *et al*. [19] and Antonelli-Ushirobira *et al*. [16] reported that after 90 days of treatment of guarana at doses of 150 mg/kg in rats and 300 mg/kg in male Swiss mice, respectively, the animals showed biochemical changes indicating that the liver is the target organ in case of possible toxicity of Guarana.

Similar to the liver's enzymes activities, the liver tissue homogenate level of MDA was significantly increased but GSH was significantly decreased in the CCl4 compared with control group, which were significantly improved by treatment with Guarana or

Metformin. Previous studies have reported that oxidative stress plays an essential role in the hepatic injury mediated by CCl4 [21, 28, 29]. Szymonok-Lesiuk *et al*. [30] had found that CCl4 intoxication can lead to alteration in gene expression and depletion of superoxide dismutase (SOD) and catalase (CAT) activities in kidney and heart. Significantly decreased liver GSH content was reported by Ohta *et al*. [31] in CCl4-injected rats. The antioxidant activity and/or the inhibition of free radical generation are important in terms of protecting the liver from CCl4-induced damage [28].

Several studies have reported that Guarana shows a number of beneficial effects probably because this plant is a source of bioactive substances with multifaceted activity [32] the same was documented by the present studies. *In vitro* studies revealed the antioxidant effects of Guarana probably due to a high concentration of polyphenols, such as tannins [20]. We infer that the hepato-protective effects of Guarana observed in this study may be attributed to the action of several bioactive compounds present in Guarana [33- 35]. Kober and colleagues {27] reported that pretreated of male Wistar rats with Guarana powder (100, 300 and 600 mg/kg) daily for 14 days before treatment with a single dose of CCl4 (1 ml/kg, intraperitoneally) results in decrease in the ALT and AST activities and DNA damage index of the liver when compared with the CCl4-treated group. *In vitro* studies reported the Guarana seeds antioxidant effects in rat brain homogenates [36], NIH-3T3 fibroblasts [14], 3T3-L1 adipocytes [37] and adipocytes from human lipoaspirates [38]. Despite the guarana has recognized antioxidant activities *in vitro*, some results regarding its protective activity seem to be contradictory. Non-enzymatic antioxidant potential, decreased the basal levels of free radical generation and reduced both SOD and CAT activities in human neuronal SH-SY5Y cells. However, Guarana-treated cells developed signs of neurite degeneration and the neurotoxicologically effects were exerted in part by disruption of redox homeostasis. In a study conducted by Bittencourt *et al.* [14], Guarana was also able to modulate the activity of SOD and CAT. In addition to antioxidant activity, Guarana presented effects on NO modulation in fibroblast NIH-3T3 cells exposed to sodium nitroprusside. On the other hand, Costa Krewer *et al*. [39] carried out a study on 637 elderly individuals classified as either those who habitually drank Guarana or those who never drank Guarana and there were no significant differences in nitric oxide (NO) metabolites between the two groups. For this reason, despite the Guarana present antioxidant activity and seems to modulate NO *in vitro*, some results of *in vivo* studies are different and appear to be contradictory. For this reason, further investigations are required to elucidate other mechanisms that may be involved in these pathways.

In conclusion, carbon tetrachloride through the influencing on liver cells and destruction of cell membrane significantly increased the serum levels of liver enzymes of AST, ALT, ALP and GGT and bilirubin. The oral administration of Guarana extract at dose of 30 mg/kg for three weeks significantly decreased the amounts of these enzymes. Due to these changes it can be concluded that the Guarana extract moderated the toxic effect of carbon tetrachloride on the activity of liver cells. These relative improvements or recovery are likely due to the presence of antioxidant compounds such as flavonoids in guarana extract.

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