

Effect of *Tamarindus indica* L and *Ginkgo biloba* L., Leaves Extracts on Hepatorenal Functions of Male Rats intoxicated with CCl₄

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Abstract: The present study aimed to investigate the effect of oral administration of alcoholic leaves extract of *Tamarindus indica* L (TI), *Ginkgo biloba* L (GB) and their combination on serum levels of lipid profile, hepatorenal function as well as feed intake and body weight gain in hepatotoxic rats. The experiment was performed on 80 adult male rats distributed into 8 equal groups. Group (1) was kept as negative control (NC) (fed on basal diet), while the other 7 were subcutaneously administered a single dose of Carbon Tetrachloride (CCl₄) to induce experimental hepatic toxicity. Group (2) was left as a positive control (PN) group, hepatotoxic rats and groups (3), (4), (5) and (6) were orally administered TI extract 200 and 400 mg/kg, and GB extract at 200 and 400 mg/kg respectively, once daily for 4 weeks. Groups (7 and 8) were orally given combination of the small dose and the large dose of TI and GB extracts (200 and 400 mg/kg b.wt.) respectively. Feed intake, body weight gain were determined as well as the activity of Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, (TC) triglycerides (TG) lipoprotein fractions, blood urea nitrogen (BUN), uric acid and creatinine concentrations were also estimated. The results demonstrated that the high dose (400 mg/kg b.wt) of both extracts and their combinations significantly decreased the levels of serum AST and ALTenzymes, TC, TG, low density lipoprotein cholesterol (LDL-c), BUN, uric acid and creatinene in hepatotoxic rats. On the other hand the high dose of both extracts and their combinations increased high density lipoprotein cholesterol (HDL-c). Conclusively, TI and GB and their combinations are considered hepatoprotective herbs. This study recommended that consumption TI and GB are useful for patients who suffer formhepatorenal disorders.

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1-Introduction

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion[1]. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders[2]. Most of the hepatotoxic chemicals such as carbon tetrachloride (CCl₄) damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [3].

Nephrotoxicity represents a major health problem and accounts for high incidence among population all over the world [4]. The CCl₄ induced nephrotoxicity this is a multifaceted process that is observed by augmentation of plasma urea and creatinine levels along with necrosis of renal tubules that ultimately leads to renal failure [5]. One of the reasons behind this manifestation includes increased oxidative stress [6]. Numerous studies conducted in experimental animals model indicated that, reactive oxygen species (ROS) are potential mediators involved in CCl₄ [7].

Tamarindus indica L inn. (*Leguminosae*) seeds are reported to contain phenolic compoundspolymeric tannins and Fatty acids Andriamanantena [8], the leaves contain triterpenes Imam, Flavones, and flavonols[9] the pericarp contains (+)-catechin,

procyanidin B₂, (-) epicatechin, procyanidintrimer, procyanidin tetramer, procyanidinpentamer, procyanidinhexamer, taxifolin, apigenin, eriodictyol, luteolin, and naringenin. [10] Leaves have hepatoregenerative properties. Pulp of fruits have hypolipidemic, antioxidant effects [11].

Ginkgo biloba L. (Family: Ginkgoaceae), is an important herb medicine, achieving unprecedented popularity over the past decade, and the recognition of the important therapeutic effects shown by this plant [3]. Chemically, the active constituents of *Ginkgo biloba* leaf are mainly (kaempferol, quercetin and isorhamnetin), diterpene lactones namely Ginkgolides A, B, C, M and J and bilobalide, biflavones (ginkgetin, isoginkgetin, bilobetin) and organic acids such as 4-hydroxybenzoic acid, that have presented various pharmacological activities [12]. The extract of *G. biloba* leaves have been proved to be an effective antioxidant and found to possess cardioprotective, antiasthmatic, antidiabetic, and potent central nervous system activities, including enhancement of memory, concentration, mental alertness and decrease in mental fatigue [13]. Therefore, the purpose of this study was designed to investigate the effect of *Tamarindus indica* L and *Ginkgo biloba* L., Leaves Extracts on

Hepatorenal Functions of Male Rats intoxicated with CCl₄.

2. Material and Methods

Material and Methods

Chemicals and Drugs

All chemicals with high analytical grade and CCl₄ were purchased from Sigma, USA. The tested kits for determinations of total cholesterol, triglycerides, lipoprotein fractions, liver enzymes as well as kidneys function were purchased from Biosystems (Barcelona, Spain).

Plant

Tamarindus indica (TI), *Ginkgo biloba* L (GB) were obtained from iHerb.com, HERB PHARM, Saudi Arabia.

Experimental animals

A total of 80 adult Male Wister albino rats, weighing 175 ± 5 grams, were purchased from King Fahd Medical Research Center. Basal diet constituents were purchased from Baghafar Company for Pharmaceutical and Chemical, Jeddah, KSA.

Basal diet preparation, hepatotoxicity induction

Diet was set as described in Reeves et al. [14]. Hepatotoxicity was induced by a subcutaneous administration of a single dose of CCl₄ (30%V/V) in paraffin oil (1ml/kg) for 2 days from start of the experimental period, to induce acute hepatic damage according to the method described by Nadeem et al., (1996).

Preparation of herbt extract

The leaves powder of the two herbs *Tamarindus indica* (TI), *Ginkgo biloba* L (GB) had been mixed with 90% ethylic alcohol by 1part in 3. Container closed with par film and covered with aluminum foil to avoid potentially light effect on extract components. Before use, extracts kept in refrigerator.

Experiment and grouping of rats

The 80 male Wister rats weighing about 180 ± 5 g was used in the study. The animals were housed in cages and was fed on standard chow and water *ad libitum* in a steady environment (room temperature $22 \pm$ three °C, room humidity $50 \pm$ five%) with a 14 h mild and 10 h darkish cycle. The animals were kept beneath observation for one week previous to the beginning of the experiment. After acclimatization period, rats were divided into two main groups. Group (I) (n=10) (NC) fed on normal diet. Group (II) (n=50) was subcutaneous administration of a single dose of CCl₄ (30%V/V) in paraffin oil (1ml/kg) for 2 days from start of the experimental period to induce hepatotoxicity, after that blood samples were taken from ratsto analyze the activity of liver enzymes. Hepatotoxic rats were distributed into7 sub- groups (n=10 rats each group) and all rats were fed on rat pallets. The groups were kept as follows:

Group (1): Control negative group (NC)

Group (2): Control positive group (PC)

Group (3): Treated with *Tamarindus indica* (200 mg/kg) (TI)

Group (4): Treated with *Tamarindus indica* (400 mg/kg) (TI)

Group (5): Treated with *Ginkgo biloba* (200 mg/kg) (GB)

Group (6): Treated with *Ginkgo biloba* (400 mg/kg) (GB)

Group (7): Treated with Both TI + GB (200 mg/kg)

Group (8): Treated with TI + GB (400 mg/kg)

At day twenty eight blood samples were collected for biochemical analyses.

Determination of biological evaluation

Each day feed intake (FI) for all groups were recorded throw out the experimental time (8 weeks). Body weight gain percentage (BWG %) and feed efficiency ratio (FER) were calculated consistent with the approach of [15].

Determination of hepatic enzymes activity

Aspartate and alanine aminotransferase (AST and ALT) were chemically determined according to Bergmeyer et al., [16].

Determination of serum blood lipid

Total cholesterol, triglycerides, lipoprotein fractions level were assessed by commercial chemical kits *protocol* [17].

Determination of kidney function

Blood urea nitrogen was determined using Bio Mérieux kits according to [18], Serum uric acid was determined using the enzymatic colorimetric method as described by [19]. Serum creatinine concentrations were colorimetrically determined by Jaffe reaction [20].

Statistical analysis

All results were presented as the mean \pm SD. Data were evaluated using SPSS 22 for windows. An analysis of variance, L.S.D. test will be performed to test the differences in the treatment [21].

3. Results and Discussion

From data in Table (1) non-significant difference in initial body weight between all experimental groups. There was significant decrease in final body weight and weight gain in Cont+ve group, as well as FI noticeable decrease in Cont+ve group compared with Cont-ve group. Treatment with TI induced significant increase in final weight and weight gain % compared with Cont+ve rats. The high dose of GB improved biological evaluation in treated rats, there was non-significant difference in final weight compared with Cont-ve group. Combination of TI and GB improved biological evaluation in treated rats, their values were non-significant as compared with

Cont-ve group, at the same time there was significant increase in final weight and weight gain %, as well as FI compared with Cont+ve rats. In hepatotoxic rats the reduction in biological evaluation was agreed with [22 - 24]. The GB and TI improved weight gain % and FI. These results confirmed with the study done by Guo *et al.* [25] who found that GB significantly suppressed

the hepatotoxic effect of CCl₄. The same results regarding TI reported by Shammi *et al.* [26]. In addition the combination of GB and TI normalized the biological values, thus indicated the potent hepatotoxic effects of the treatment with leaves extract mixture.

Table 1: Effect of *Tamarindus indica* and *Ginkgo biloba* leaves extract and their mixture on body weight gain % and feed intake in hepatotoxic rats

Experimental groups	Biological evaluation			
	Initial weight g	Final weight g	Weight gain %	FI g
Group (1) Cont -ve	177.43 ± 6.43 ^a	254.63 ± 11.64 ^a	45.70 ± 1.07 ^a	22.43
Group (2) Cont+ve	175.56 ± 3.14 ^a	205.33 ± 9.02 ^d	17.43 ± 1.08 ^d	13.59
Group (3) TI extract (200 mg/kg b.wt)	174.35 ± 5.02 ^a	224.29 ± 6.12 ^c	28.22 ± 1.34 ^c	15.27
Group (4) TI extract (400 mg/kg b.wt)	173.41 ± 5.13 ^a	228.12 ± 6.16 ^{bc}	31.72 ± 1.13 ^c	16.09
Group (5) GB extract (200 mg/kg b.wt)	173.80 ± 3.43 ^a	239.33 ± 6.11 ^b	35.34 ± 0.91 ^{bc}	18.99
Group (6) GB extract (400 mg/kg b.wt)	175.43 ± 4.29 ^a	248.43 ± 7.21 ^a	39.44 ± 1.21 ^b	19.29
Group (7) Mixture (200 mg/kg b.wt)	174.87 ± 3.62 ^a	253.92 ± 8.42 ^a	45.20 ± 1.62 ^a	21.09
Group (8) Mixture (400 mg/kg b.wt)	175.46 ± 2.62 ^a	252.32 ± 6.46 ^a	43.80 ± 3.54 ^a	23.76

Means (n=10 rat) with different letters in the same column considered significant (P < 0.05)

Liver enzymes activities are illustrated in Table (2). There was significant increase in AST and ALT in Cont+ve group compared with Cont-ve group. Treatment with TI at 200 and 400 mg induced significant decrease in liver enzymes compared with Cont+ve rats. The low and high doses of GB improved AST and ALT in treated rats, there was significant difference compared with Cont+ve group. Combination of TI and GB improved liver functions in treated rats, the values of high dose of TI and GB treated rats revealed non-significant difference as compared with Cont-ve group, at the same time there was significant decrease compared with Cont+ve rats.

Subcutaneously administered of CCl₄ induced dramatically significant increase in liver enzymes activities compared with normal rats. The obtained results were confirmed by Tirkey *et al.* [27] and Anand *et al.* [28]. Thus indicated that there was cellular and mitochondrial damage and loss of functional integrity of liver cells membrane [29-30]. Treatment with TI and GB induced significant restorations of liver enzymes activities. Thus indicated that the mixture of TI and GB induced healing of hepatic parenchyma and regenerated of hepatocytes. These results confirmed by Pimple *et al.* [31]. These effect could be explained by the presence of many

active compounds in TI as flavonoids, polyphenols, beta-carotene and ascorbic acid, which have antioxidant properties [32-33]. In addition, flavonoids, ginkgo-flavone glycosides, terpenoidas antioxidant compounds in the GB [34-36].

There was significant hyperlipidemic in Cont+ve group compared with Cont-ve group. Treatment with TI at 200 and 400 mg induced significant improved in lipid tested parameters compared with Cont+ve rats. The low and high doses of GB induced hypolipidemic effects in treated rats, there was significant difference compared with Cont+ve group. Treatment with TI and GB revealed noticeable hypolipidemic effects in treated rats, their values were significant difference as compared with Cont+ve group, at the same time there was significant decrease compared with Cont-ve rats Tables (3 and 4). Thus indicated that the combination of leaves of TI and GB have potent hypolipidemic and hypocholesterolemic effects.

Hyperlipidemic effect of subcutaneously administered of CCl₄ in hepatotoxic rats was in agreed with El-Habib *et al.* [37] and Al-Dosari [38]. This effect could be explained by increase the fatty acids synthesis and TG from acetate, which influenced by CCl₄. In addition, CCl₄ inhibit the bile acids synthesis thus increase cholesterol level [39]. In addition, CCl₄

increases the availability of esterification of fatty acids [40]. The improved in lipid parameters by treatment with either TI or GB extract, revealed normalize in their values as compared with normal range in contr-

ve group, thus could be explained by antioxidant compounds in leaves extract as confirmed by Olatunde *et al.* [32], Ross [33], Oteiza *et al.* [41] and Bhendric [42].

Table 2: Effect of *Tamarindus indica* and *Ginkgo biloba* leaves extract and their mixture on levels of serum liver enzymes (AST and ALT) in hepatotoxic rats

Experimental groups	Parameters	
	ALT U/L	AST U/L
Group (1) Cont -ve	33.246± 2.23 ^a	67.245± 2.07 ^a
Group (2) Cont+ve	56.231± 2.621 ^a	78.472± 1.66 ^a
Group (3) TI extract (200 mg/kg b.wt)	48.821± 1.69 ^b	72.381± 2.42 ^{ab}
Group (4) TI extract (400 mg/kg b.wt)	34.001± 0.98 ^c	69.176± 1.76 ^b
Group (5) GB extract (200 mg/kg b.wt)	46.764± 1.75 ^b	74.653± 2.81 ^{ab}
Group (6) GB extract (400 mg/kg b.wt)	39.357± 2.33 ^c	71.874± 0.89 ^b
Group (7) Mixture (200 mg/kg b.wt)	45.541± 1.34 ^c	72.458± 1.98 ^b
Group (8) Mixture (400 mg/kg b.wt)	34.867± 1.56 ^a	68.382± 0.78 ^a

Means (n=10 rat) with different letters in the same column considered significant (P < 0.05)

Table 3: Effect of *Tamarindus indica* and *Ginkgo biloba* leaves extract and their mixture on total cholesterol (TC) and triglycerides (TG) in hepatotoxic rats

Experimental groups	Parameters	
	TC mg/dl	TG mg/dl
Group (1) Cont -ve	94.47± 1.87 ^a	52.33± 1.41 ^c
Group (2) Cont+ve	114.365± 2.05 ^c	133.958± 1.09 ^d
Group (3) TI extract (200 mg/kg b.wt)	95.64± 1.11 ^{ab}	122.073± 2.13 ^c
Group (4) TI extract (400 mg/kg b.wt)	74.415± 2.73 ^b	104.26± 1.97 ^{ab}
Group (5) GB extract (200 mg/kg b.wt)	94.91± 3.51 ^{ab}	118.356± 1.17 ^c
Group (6) GB extract (400 mg/kg b.wt)	73.82± 1.91 ^b	100.798± 2.18 ^{ab}
Group (7) Mixture (200 mg/kg b.wt)	78.105± 1.23 ^b	109.048± 2.19 ^b
Group (8) Mixture (400 mg/kg b.wt)	62.825± 2.42 ^{cb}	98.26± 1.94 ^a

Means (n=10 rat) with different letters in the same column considered significant (P < 0.05)

Table 4: Effect of *Tamarindus indica* and *Ginkgo biloba* leaves extract and their mixture on levels of serum lipoprotein fractions (HDL-c, LDL-c and VLDL-c) in hepatotoxic rats

Experimental groups	Parameters		
	VLDL-c mg/dl	LDL-c mg/dl	HDL-c mg/dl
Group (1) Cont -ve	10.466± 0.32 ^c	14.762± 1.89 ^d	66.243± 2.44 ^a
Group (2) Cont+ve	22.874± 0.22 ^a	68.523± 2.22 ^a	42.562± 1.98 ^d
Group (3) TI extract (200 mg/kg b.wt)	19.128± 0.36 ^a	56.432± 2.11 ^b	46.522± 1.23 ^d
Group (4) TI extract (400 mg/kg b.wt)	14.443± 0.64 ^b	33.753± 0.98 ^c	55.624± 1.59 ^c
Group (5) GB extract (200 mg/kg b.wt)	18.982± 0.45 ^a	53.241± 1.67 ^b	46.133± 2.34 ^d
Group (6) GB extract (400 mg/kg b.wt)	14.764± 0.536 ^b	31.542± 1.12 ^c	54.492± 1.26 ^c
Group (7) Mixture (200 mg/kg b.wt)	15.621± 0.25 ^b	39.655± 1.97 ^c	53.762± 1.99 ^c
Group (8) Mixture (400 mg/kg b.wt)	12.365± 0.23 ^c	22.26± 1.87 ^{cd}	63.634± 1.37 ^a

Means (n=10 rat) with different letters in the same column considered significant (P < 0.05)

Table (5) showed the kidney functions in treated hepatotoxic rats. There was significant increase in urea, uric acid and creatinine levels in Cont+ve group compared with Cont-ve group. Oral administration of TI and GB at low and high doses induced significant improved in kidney functions compared with Cont+ve rats. Treatment with mixture of TI and GB to

hepatotoxic rats ameliorated urea, uric acid and creatinine levels, their values was nearly with the normal level, there was non-significant difference as compared with Cont-ve group. At the same time, their values were significant difference as compared with Cont+ve group.

Table (5): Effect of *Tamarindus indica* and *Ginkgo biloba* leaves extract and their mixture on kidney functions in hepatotoxic rats

Experimental groups	Parameters		
	Urea nitrogen mg/dl	Uric acid mg/dl	Creatinine mg /dl
Group (1) Cont -ve	29.43 ± 1.67 ^d	2.54 ± 1.12 ^d	0.86 ± 0.54 ^d
Group (2) Cont+ve	67.45 ± 1.32 ^a	6.12 ± 0.06 ^a	4.1 ± 2.54 ^a
Group (3) TI extract (200 mg/kg b.wt)	59.56 ± 3.32 ^b	4.96 ± 0.23 ^b	3.4 ± 1.34 ^b
Group (4) TI extract (400 mg/kg b.wt)	40.43 ± 2.49 ^c	3.75 ± 0.73 ^c	1.5 ± 0.98 ^{cd}
Group (5) GB extract (200 mg/kg b.wt)	56.85 ± 2.11 ^b	4.25 ± 0.67 ^b	2.8 ± 2.21 ^c
Group (6) GB extract (400 mg/kg b.wt)	35.25 ± 1.85 ^{cd}	3.22 ± 0.72 ^c	1.2 ± 0.75 ^d
Group (7) Mixture (200 mg/kg b.wt)	31.33 ± 2.43 ^d	2.92 ± 0.21 ^d	0.9 ± 0.43 ^d
Group (8) Mixture (400 mg/kg b.wt)	29.76 ± 1.53 ^d	2.63 ± 0.02 ^d	0.83 ± 0.34 ^d

Means (n=10 rat) with different letters in the same column considered significant (P < 0.05)

Several studies revealed that CCl₄ has potentially toxicity to kidney [43-44]. Thus impairment in its functions and decreased its clearance rate induced elevated in urea, creatinine and uric acid [45-46]. The reduced kidney function levels after treatment with TI and GB proven that they have potent capacity to treated, prevent and ameliorate oxidative stress and its damage on liver and kidney [47-48].

In conclusion, the results suggested that either TI and GB leaves extracts alleviated hepatorenal toxicity induced by CCl₄. Thus could be attributed to their active biochemical compounds that have potent antioxidant activity. On the other hand, although the mixture of TI and GB was the most effective but its induced noticeable hypolipidemic effect thus could attributed to their synergistic effects, therefore it should be care when used mixture of herbal medicine.

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