Antioxidant and hepatoprotective effects of *ginkgo biloba* leave extracts

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ABSTRACT

The present study was undertaken to investigate the in vitro and the in vivo antioxidant and hepatoprotective effects of Ginkgo biloba leave extracts. Qualitative analysis of different leaves extracts [ethanol 70% (Et), ethyl acetate (EA), and water (W)] revealed the presence of flavonoids, phenolic compounds, saponins, tannins, terpenes, and carbohydrates. The quantitative analysis of the leaves extracts revealed that the Et extract contains the highest amounts of tannins, saponins, flavonoids and phenolic compounds. The EA extract contains higher amounts of flavonoids and phenolic compounds and lower amounts of tannins and saponins compared to the W extract. The in vitro studies revealed that all the Ginkgo biloba leave extracts have a high antioxidant activity. The Et extract showed the highest activity followed by the EA extract then the W extract. Moreover, the in vivo studies revealed that all the Ginkgo biloba leave extracts have hepatoprotective activity, which evidenced by the effect of these extracts on the antioxidant enzymes activities [superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), glutathione–S–transferase (GST)], the liver marker enzymes [alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT)], the lipid peroxidation as well as inhibitory effects on nitric oxide (NO) release. The administration of these extracts significantly restored the elevated activities of the liver marker enzymes, and the antioxidant enzymes, which increase during hepatotoxicity. Also, they inhibit the release of NO. The treatment with the Et extract gave the best results for hepatoprotective effects. Moreover, the administration with this extract did not significantly affect the normal values of blood glucose (BG), total proteins (T.P), total lipids (T.L), total cholesterol (T.C), low and high density lipoprotein cholesterol (LDL-c and HDL-c). From this study it can conclude that the treatment with Ginkgo biloba leave extracts can inhibit the hepatotoxicity without toxic effect.

INTRODUCTION

Ginkgo biloba has been a popular remedy in traditional Chinese medicine for over 4000 years, and it has been a common herbal medicine in Europe since the 1730's[1]. The *Ginkgo* tree, the only existing tree in the family *Ginkgoaceae*, is the world's oldest living tree and is thus

sometimes referred to as a "living fossil"[2]. *Ginkgo* trees are now widely planted in China, Japan, Korea, France, Germany, and the United States for both ornamental and medicinal purposes.

Several human diseases have been associated with the overproduction of reactive oxygen species (ROS) and subsequently various antioxidants emerged as potential therapeutic agents that scavenge ROS. Free radical reactions are associated with a range of degenerative diseases and acute ischemic insults affecting liver and other tissues[3]. The free radical-mediated hepatotoxicity can be effectively managed by administration of such agents.

Kuo, et al.[4] demonstrated that *Ginkgo biloba* extract competitively inhibited rat hepatic microsomal CYP1A-mediated enzyme activity, as determined by experiments performed on extracts containing known amounts of terpene trilactones and ginkgo flavonol glycosides. The mechanism of action of *Ginkgo biloba* is not known. However, it appears to possess antioxidant activity[5].

The present study was undertaken to investigate chemical composition of various *Ginkgo biloba* extracts and to evaluate their hepatoprotective activity through scavenging peroxynitrite formation and antioxidant activity.

MATERIALS & METHODS

Plant material:

Leaves samples of *Ginkgo biloba* (*Ginkgoaceae*) were collected in May (2005) from tree cultivated in Orman garden, Giza, Egypt. All leaves were washed then air dried in shade at room temperature for 5–7 days, grinded to fine powder and kept for phytochemical analysis.

Plant extracts:

50 g of the air–dried powdered leaves of *Ginkgo biloba* were extracted separately by percolation in three different solvents. The first solvent was ethanol 70% (Et), the second solvent was ethyl acetate (EA) and the third one was water (W). All extracts were filtered, then freeze dried.

General phytochemical screening:

Preliminary phytochemical tests were carried out on the different *Ginkgo biloba* leaves extracts. Carbohydrates and/or glycosides, flavonoids, saponins, sterols and/or triterpens, and phenolic compounds were tested according the methods described by ***Lewis and Smith***[6], ***Geissman***[7], ***Shellard***[8], ***Balbaa***[9], ***Fransworth, et al.***[10], and **Swain and Hillis**[11], respectively.

Quantitative determination of the phytochemicals in the different extracts of *Ginkgo biloba***:**

Total tannins, saponins, flavonoids, and phenolic compounds were determined according to the methods described by ***Balbaa***[9], ***Ebrahimzadeh and Niknam***[12], ***Zhuang et al.***[13], and **Swain and *Hillis***[11], respectively.

Free radical scavenging assay:

The antioxidant activity of the different *Ginkgo biloba* leaves extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydryzyl ($DPPH^{\bullet}$) according to ***Brand–***

***Williams et al.*[14]** and the inhibition percentage of the DPPH$^{\bullet}$ by the samples was calculated according to the formula described by ***Yen and Duh*[15]** as follows:

% Inhibition = [(Ac (0) – Ac (t)) /Ac (0)] × 100

Where: Ac (0) was the absorbance of the control at t = 0 min and Ac (t) was the absorbance of the antioxidant, which varied with the different content.

Superoxide radical scavenging activity by various *Ginkgo biloba* extracts was determined according to **Chung, *et al*.[16]**.

Determination of thiobarbituric acid (MDA):

Lipid peroxidation was measured in the liver according to the method of **Burits and Bucar[17].**

Experimental animals:

Forty male Sprague - Dawley rats (175-200g) were housed in the animal house of Research Institute of Ophthalmology. The rats were kept under normal laboratory conditions for two weeks before commencing the experiments.

Route of administration:

Animals were divided into five groups (each group eight rats). Group one represents the negative control, which administered 10% di-methyle sulphoxide (DMSO). Group two represents the positive control, which administered a single oral dose of carbon tetrachloride (CCl_4) (1:1 in liquid paraffin + 10 % DMSO) to induce liver damage. The single dose was equal 2.0 ml/kg body weight. Groups three, four and five were given with ethanolic, ethyl acetate, and water extracts (100mg/kg/body weight) respectively for 14 days. After six hrs from the last dose of extracts, all groups except group one were administered with a single oral dose of CCl_4 to induce liver damage. Rats were decapitated after 72 hrs from the CCl_4 administration, then blood and liver were taken from various groups and samples were prepared according to each method.

Preparation of Liver tissues for biochemical analyses:-

Liver tissues were washed twice with cold saline solution, placed into glass bottles, labeled, and stored at −80 ˚C until processing. Liver tissues were homogenized in Tris–HCl buffer 0.1 M (pH 7.4). The homogenates were then centrifuged at 5000×g for 60 min. and the clear supernatant fluid was taken for assaying the enzymes activity. Tissue malondialdehyde (MDA) and nitric oxide levels were determined as describe later.

Biochemical analysis:

Fasting blood glucose (FBG) was estimated by colorimetric method of **Trinder[18].** Total proteins (T.P) were determined according to the method of **Lowry, et al.[19].**

The glutathione peroxidase (GSH-PX) activity was estimated using 1–chloro-2,4-dinitrobene (CDNB) as substrate according to the method described by ***Habig, et al.*[20].** Superoxide dismutase activity (SOD) was measured by the method of ***Santa-mará, et al.,* [21]**. The activity of alkaline phosphatase (ALP) was determined according to ***Empfehlungen*[22].** Activities of aspartate transaminase (AST) and alanine transaminase (ALT) were estimated according to ***Reitman and Frankel*[23].** Hepatic glutathione-S–transferase (GST) activity was

assayed in the liver tissue according to the method described by ***Ahmed, et al.***[24].

Serum total lipids (T.L), cholesterol (T.C), Low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) were measured by using BioMerrie Kits according to the methods describe by ***Zollner and Kirsch***[27], ***Tietz***[26], ***Bergmenyer***[27], and ***Stein***[28], respectively.

Serum and liver nitric oxide was estimated using Greiss reagent according to the method of ***Green, et al.***[29].

Statistical analysis:

Statistical analyses were done using *SPSS (version 10) program*. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

RESULTS

Preliminary phytochemical screening of the different *Ginkgo Biloba* **leaves extracts:-**

As shown in table (1), the phytochemical study of the different extracts of *Ginkgo biloba* leaves [ethanolic extract 70% (Et), ethyl acetate (EA), and water (W) extracts] revealed the presence of flavonoids, tannins, saponins, sterols and/or triterpens and phenolic compounds. Carbohydrates and/or glycosides were found only in the ethanol and water extracts.

Table (1): Preliminary Phytochemical Screening of the Different *Ginkgo Biloba* Leaves Extracts.

Extracts / Phytochemical	Et1	EA	W
Carbohydrates and or glycosides	+++	-	++
Flavonoids	+++	+++	++
Saponins	++	+	++
Tannins	+++	+	++
Sterols and or triterpens	++	+	+
Phenolic compounds	+++	++	++

(+++) High amount (++) moderate amount (+) Low amount (-) Absent.

Chemical analysis of the different *Ginkgo biloba* **leaves extracts:**

The obtained data in table (2) showed that the percentage of tannins and saponins compounds were (2.19%, 0.59%, 1.48%) and (1.05%, 0.29%, 0.89%) based on the dry weight, respectively for the ethanolic, ethyl acetate, and water extracts. While the percentage of flavonoids and phenolic compounds were (1.55%, 1.49%, 0.98%) and (2.59%, 1.98%, 1.58 %) based on the dry weight, respectively for the ethanol 70%, ethyl acetate, and water extracts. The highest concentration of tannins, saponins, flavonoids and phenolic compounds were found in the ethanolic extract.

Table (2): Total Tannins, Saponins, Flavonoids and Phenolic Compounds Contents in the Different *Ginkgo Biloba* Leaves Extracts.

Compounds Extracts	Total tannins %	Total saponins %	Total flavonoids %	Total Phenolic compounds %
Ethanol 70% (Et)	2.19	1.05	1.55	2.95
Ethyl acetate (EA)	0.59	0.29	1.49	1.98
Water (W)	1.48	0.89	0.98	1.58

All determinations based on the dry weight.

Antioxidant activity of the different *Ginkgo biloba* extracts according to the DPPH radical scavenging method:-

Table (3) shows that rutin (standard) is superior inhibitor of DPPH• compared to *Ginkgo biloba* leaf extracts and gave high percentage inhibition 82.40 % and 91.27 % for 50, 100 µg/ml. The results in table (3) show that the decrease in absorbance of DPPH• radical concerning to all extracts compared with rutin standard. While water extracts showed more decrease in the inhibition effect for all concentrations than other extracts.

Table (3): Comparison of Free Radical Scavenging Activity of different Ginkgo biloba leaves extracts with rutin standard expressed as % inhibition.

Samples	DPPH• radical decolouration 100%		
	*10 (µg/ml)	*50 (µg/ml)	*100 (µg/ml)
Rutin (Standard)	58.1	82.40	91.27
Ethanol 70% (Et)	59.47	73.80	89.91
Ethyl acetate (EA)	47.59	55.47	68.49
Water (W)	29.57	45.68	61.09

**Different concentrations of extract. All values mean of 3 replicates.*

Antioxidant activity of different extracts from *Ginkgo biloba* leaves according to the Superoxide scavenging activity method:-

Table (4) shows that ethanolic and ethyl acetate extracts of *Ginkgo biloba* leaves showed a good free radical scavenging activity. The activities were increased by increasing the concentration of the extract. The highest superoxide scavenging activity was observed with Et followed by EA extract, and finally the water extract.

Table (4): **Superoxide Radical Scavenging Activity of Rutin Standard expressed as % inhibition and Different Ginkgo Biloba Extracts.**

Samples	Superoxide scavenging activity 100 %		
	*10 **(µg/ml)**	*50 **(µg/ml)**	*100 **(µg/ml)**
Rutin (Standard)	69.87	91.24	95.34
Ethanol 70% (Et)	72.59	80.12	92.57
Ethyl acetate (EA)	64.9	69.57	87.59
Water (W)	35.68	55.49	78.17

** Different concentrations of extracts. All values mean of 3 replicates*

Fasting blood glucose, total proteins and MDA contents:-

Table (5) shows the levels of fasting blood glucose (FBG), total proteins (T.P) and malondialdehyde (MDA) in the negative control, the positive control and in the groups treated with the different *Ginkgo biloba* extracts. The obtained data from this table revealed that the highest decrease in the FBG was observed in the rats administrated with the ethanolic extracts compared with the positive control. On the other hand there was no significant change in the levels of total proteins in the different groups. The level of MDA content in positive control group was significantly increased under oxidative stress. But in the various groups treated with *Ginkgo biloba* extracts there was significant decrease in the levels of MDA content, the lowest content was observed with ethanolic extract.

Table (5): Fasting Blood Glucose (FBG), Total Proteins (T.P) and Malondialdehyde (MDA) in Control and Different *Ginkgo Biloba* Extract Groups.

Parameters / Groups	FBG (mg/dl) mean±S.E.	T.P (g/dl) mean±S.E.	MDA (nmol/ml) mean±S.E.
Control (-)	117.34 ± 15.20 c	6.92 ± 0.41 a	1.60±0.53 c
Control (+)	221.61 ± 29.71 a	5.59 ± 0.31 b	2.70±1.61 a
Ethanol 70% (Et)	128.70 ± 21.01 c	6.12 ± 0.32 b	1.63±0.79 c
Ethel acetate (EA)	172.00 ± 23.60 b	6.21 ± 0.19 b	1.70±0.90 c
Water (W)	200.11 ± 27.10 ab	5.98 ± 0.41 b	1.98±1.19 b

Means in the same column with the same letter are not significant at P< 0.05.

Anti-Hyperlipidemic Activity of the different *Ginkgo biloba* Extracts:-

Data in table (6) show a high anti-hyperlipidemic activity of various *Ginkgo biloba* extracts, the highest effect was observed in the group administrated with the Et extract, followed by EA extract and finally by the water extract. On the other hand, the lowest decrease in the lipid profile was observed in the group administered with the Et extract compared with the other two extracts.

Table (6): Total Lipids (T.L), Total Cholesterol (T.C), Low Density Lipoprotein (LDL-c) and High Density Lipoprotein (HDL-c) in Controls and Different Extracts of *Ginkgo Biloba* Leaves.

Samples	T. L (mg/dl) mean±S.E.	T.C (mg/dl) mean±S.E	HDL-c (mg/dl) mean±S.E.	LDL-(mg/dl) mean±S.E.
Control (-)	560.54±42.25e	108.23±8.57 e	36.78±1.24d	70.70±2.14 e
Control (+)	890.24±57.15a	230.58±12.24a	25.80±1.98c	188.70±11.14a
Ethanol 70%(Et)	580.7±32.65 d	135.54±7.89 d	56.35±3.14a	98.96±4.57 d
Ethyl acetate (EA)	620.89±28.65c	162.67±11.24c	49.62±2.31b	106.37±6.78 c
Water (W)	780.98±43.25b	219.38±13.57b	36.46±2.14d	169.6±12.47 b

Means in the same column with the same letter are not significantly at P< 0.05**.**

The Protective Activity of *Ginkgo biloba* Extracts Against Hepatotoxicity by its Modulating Effect on Defense Enzymes and Nitric Oxide:-

The obtained data in table (7) show that the *Ginkgo biloba* extracts caused a significant decrease in the AST and ALT activity compared to the positive control, the same trend was observed with the ALP activity . In addition, the activity of antioxidant enzymes (GSH-PX and SOD)) and also nitric oxide were decreased after administration of different *Ginkgo biloba* extracts.

Table (7): Blood Glutathione Peroxidase (GSH-PX), Superoxide Dismutase (SOD), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Nitric Oxide (NO) in Control and Rats Treated with Different *Ginkgo Biloba* Extracts.

Parameters / Groups	AST µ/l mean±S.E	ALT µ /l mean±S.E	ALP µ /l mean±S.E	SOD µ /ml mean±S.E	GSH-PX µm/min/mg mean±S.E	NO µmol/ml mean±S.E
Control (-)	217.34 ±15.20 c	41.34 ±2.97 a	145.86 ± 6.05 d	30.14 ±0.99e	0.99 ±0.04 c	53.25 ±2.54e
Control (+)	321.61 ±29.71 a	96.78 ± 8.21 b	301.75 ±23.27 a	79.42 ±03.71a	1.59 ±0.03 a	102.21 ±4.58a
Ethanol (70)	228.70 ±21.01 c	66.19 ±5.21 d	169.86 ± 9.31d	41.05 ±02.13d	1.34 ±0.05 ab	76.59 ±4.21d
Ethel acetate	272.40 ±23.60 b	71.98 ±5.21c	214.73 ±8.99 c	51.35 ±1.47 c	1.37 ±0.02 ab	85.89 ±3.58 c
Water (W)	300.11 ±27.10ab	87.32 ±7.81eb	257.95 ±14.01b	63.25 ±4.05 b	1.28 ±0.03 b	89.49 ±5.28 b

*Means in the same column with the same letter are not significantly at P< 0.05***.**

Effect of Different *Ginkgo biloba* Extracts against Hepatotoxicity of Carbon Tetrachloride in Liver Rats:-

The protective effects of various extracts of *Ginkgo biloba* leaves against liver damage induced by carbon tetrachloride (CCl_4) were evaluated in rats. The results in Table (8) show that CCl_4 administration significantly damages the liver which is evident from the very high activities of liver antioxidant enzymes. Acute CCl_4 administration significantly increased the level of liver injury marker enzymes such as GSH-PX and SOD. It also increased the activity of glutathione-S-transferase, but treatment with different extracts 14 days before CCl_4 treatment offered considerable protection to liver as evidenced from the levels of most biochemical parameters. Administration of Et decreased the activities of GSH-PX, SOD and GST in the liver compared to the positive control.

The data of table (8) revealed that the nitric oxide content was decreased in response to the administration of various *Ginkgo biloba* extracts and the lowest decrease in the nitric oxide levels was observed with Et, then the E.A. and finally with water extract.

Table (8): Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-PX), Glutathione-S-Transferase (GST), Nitric Oxide (NO), and Malondialdehyde (MDA) Content of the Homogenized Liver Tissues in Controls and Rats Treated with Different *Ginkgo biloba* Extracts.

Parameters Groups	SOD µ/mg protein mean±S.E.	GSH-PX µ/mg protein mean±S.E.	GST µm/mg pt mean±S.E.	NO µmol/mg pt mean±S.E.	MDA nmol/mg mean±S.E.
Control (-)	91.07 ±6.90e	1611.9 ±99.87c	0.99 ±0.04 a	120.89 ±4.58 d	1.64 ±0.78 c
Control (+)	277.2 ±19.01a	2781.9 ±159.1a	1.59 ±0.03b	230.17 ±8.24 a	2.70 ±1.61a
Ethanol (70%)	121.3 ±5.2 d	1756.9 ±91.01c	1.40 ±0.03bc	158.95 ±7.54 c	1.602 ±0.53c
Ethyl acetate (EA)	179.38 ±9.02 c	2054.7 ±113.27b	1.37 ±0.02d	188.95 ±6.57 b	1.70 ±0.91 c
Water (W)	205.85 ±12.73b	2243.16 ±107.91b	1.38 ±0.05e	191.54 ±11.24 b	1.98 ±0.12 b

Means in the same column with the same letter are not significantly at P< 0.05.

DISCUSSION

Ginkgo biloba leaves were used in traditional Chinese medicine, and now grows throughout the world. This popular herbal medicine is extracted from the fan-shaped leaves of the ancient *Ginkgo biloba* tree. The mechanism of action is not yet well understood. Some studies suggest that *Ginkgo biloba* may be beneficial for

treating the symptoms of cancer, Alzheimer's disease, learning, memory, attention, or concentration in elderly.[30,31]. It may have anti-inflammatory, anti-oxidant, anti-coagulant properties and may also increase blood flow to the brain.

***Wang, et al.*[32]** found that the contents of flavonoids in the leaves of *Ginkgo biloba* gathered from different producing areas are different. For this reason the current was directed to study the chemical composition of *Ginkgo biloba* in Egypt. The results of phytochemical screening of different *Ginkgo biloba* extracts revealed the presence of carbohydrates and or glycosides, tannins, saponins, flavonoids, sterols and or triterpens and phenolic compounds. Also after extraction with different solvents, the chemical constituents were determined and the results are shown in table (2). Preliminary photochemical screening and chemical analysis of Et (tables 1and 2) showed the presence of flavonoids which are natural products having significant biological activities including antiradical and antioxidant properties.

The results of Table (3) show that the Et extract showed high percentage inhibition of $DPPH^{\bullet}$. These results suggested that the decrease in the absorbance of $DPPH^{\bullet}$ radical was due to its reduction by different antioxidants. From these data, the Et1 extract of *Ginkgo biloba* showed good free radical- scavenging activity depending on the concentration used and it contain high significant amount of natural flavonoids and phenolic compounds. These results are agreement with those obtained by **Zhang, *et al.*[33]** who reported that natural mixtures of phenolic compounds have high antioxidant activity. Based on these experimental results, it is suggested that *Ginkgo biloba* leaf extracts comprise effective potential source of natural antioxidants.

Superoxide radical scavenging activity of the different *Ginkgo biloba* extracts is shown in table 4. Rutin is an important anti–lipoperoxidant agent and also a strong scavenger of hydroxyl and superoxide radicals as reported by ***Lachman, et al.*[34],** so it was used as a standard in this study. The data of the current study suggested that all *Ginkgo biloba* extracts have superoxide scavenging activity. The most scavenging activity was observed in the Et extract followed by EA extract and lastly the W extract. This may be depends on the flavonoids content of these extracts. The higher flavonoids content the higher scavenging activity. This data was in agreement with ***Sudheesh and Vijayalakshmi*[35]**, who showed that fractions rich in flavonoids obtained from *Punica granatum* extracts have potential anti-peroxidative effect.

The obtained data of table (5) revealed that the highest decrease in the FBG compared with the positive control was observed in the animal administrated with the ethanolic extract. This may be due to the effect of Ginkgo biloba on the stimulation of glucose uptake or the activation of insulin receptor as reported by ***Pinakini, et al.,*[36]**. There were no significant changes in the total protein concentrations in the groups administrated with the different

extracts. The lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl_4. The obtained data of table 5 revealed that the MDA content was significantly increased in the positive control, but the various *Ginkgo biloba* extracts treatment caused significant decrease in the levels of MDA content. The lowest content was observed with ethanolic extracts, which may be due to the high antioxidant activity in Et extract compared with other extracts. Also**,** *Ginkgo biloba* extract was also found to have a protective effect against lipid peroxidation in hepatic microsomes by inhibiting this lipid peroxidation[37].

Oxidative stress, which involves excess accumulation of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide (H_2O_2) and hydroxyl radical, can damage cells by lipid peroxidation and alteration of protein and nucleic acid structure. Data of table (6) showed a high anti-hyperlipidemic activity of *Ginkgo biloba* extracts. Treated treatment with different extracts indicating the potentiality of Et as antioxidant by preventing the peroxidative damage caused by CCl_4 more than other extracts. This is may be due to high contents of antioxidant compounds and flavonoids resulted in the inhibition of lipid peroxidation. Flavonoids have inhibitory effect on microsomal Cyt P450 enzyme or on lipid peroxidation. It may be interfering with the Cyt P450 and ultimately hindering the biotransformation of CCl_4. At the same time Et may also possess antioxidant activity, which inhibited the deleterious effect of free radicals generated by CCl_4 influencing the membrane rigidity by preventing inhibiting the membrane peroxidation[38]. Also the antihyperlipidemic activity may be due to the protective activity of Et extract against induced hepatotoxicity that modulates lipid metabolism and lipid profile to more or less normal levels.

It is thought that the natural antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by preventing free radical formation[39]. The free radical-mediated hepatotoxicity can be effectively managed by administration of such agents possessing antioxidants free radical scavengers. Protective effects of Ginkgo biloba extracts against hepatotoxicity by GSH-PX and SOD defense enzymes modulation are shown in tables (7) in the blood and table (8) in the liver. Damage of the liver cells by CCl_4 cause leakage of cellular enzymes to the blood. CCl_4 is one of the most commonly used hepatotoxins in the experimental study of liver diseases[40]. Administration of CCl_4 causes oxidative damage through free-radical generation. In the present study, the rats were administered with a single oral dose of CCl_4 to induce liver damage and the rats were killed after 72 hours from the administration of CCl_4. This time is enough to generate a high amount of free radicals and as a result, the activity of antioxidant enzymes increased to overcome these high amounts of free radicals. The liver damage in the positive control was evidenced by an elevation in the serum maker enzymes

(table 7), namely AST, ALT, and ALP as reported by ***Johnson and Kroening***[40] .Also, administrations of CCl_4 cased a significant increase in the activities of the antioxidant enzymes such as GSH-PX, SOD and GST in the blood (table 7) as well as in the liver. In the present study the crude ethanol, ethyl acetate and water extracts were evaluated for the protective effect against liver damage induced by CCl_4 in rats. The data revealed that *Ginkgo biloba* extracts has decreased the levels of the previous enzymes in the blood but the levels did not reach the normal values, which indicate hepatoprotective activity of these extracts. The most significant decrease in these enzymes was observed in the rats administrated with the Et extract. In addition to the previous results, administration with the Et extract decreased the activities of SOD and GSHPX enzymes in the blood as well as in the liver more than the other extracts. Generally, the administration of the different extracts showed an improvement in the activities of different antioxidant enzymes. Based on these results, it is clear that the inhibitory effect of Et extract of *Ginkgo biloba* leaves showed the highest inhibitory effect on these enzymes. This may be partly due to its action as free radical scavenger and its ability to chelate ions responsible for the promotion of lipid peroxidation. Moreover, treatment with Et of *Ginkgo biloba* leaves before CCl_4 treatment offered considerable protection to liver as evidenced from the levels of biochemical parameters.

In another explanation, the inhibitory effects of *Ginkgo biloba* extracts on rat hepatic enzyme activity may be due to the inhibition by the flavonoids (kaempferol and quercetin) that detect chemically in *Ginkgo biloba*. ***Kuo, et al.***[41] found that isorhamnetin is a potent inhibitor of rat hepatic microsomal CYP1A. Kaempferol, quercetin, and isorhamnetin all have multiple hydroxyl groups. This result is consistent with the idea that flavonoids possessing hydroxyl groups tend to be inhibitors of these enzymes[42]**.** Kaempferol has a hydrogen atom and isorhamnetin has a methoxy group, whereas quercetin has a hydroxyl group. Thus, it appears that a hydroxyl renders the flavonol molecule less potent with respect to the inhibition of rat hepatic enzymes. In addition to the flavonoids, *Ginkgo biloba* extracts are known to contain many other chemicals, including carbohydrates and/or glycosides as shown in table (1). The results in the current study suggested that glycoside may be decrease the inhibitory effect on ALP, GHS PX, GST, and SOD as reported in tables (7,8). Based on these results of the phytochemical analysis of *Ginkgo biloba* leaves, it was observed that the highest concentration of glycosides and flavonoids found in the ethanolic extract, which recorded the best results for the *in vivo* inhibition of rat hepatic enzyme activity.

Nitric oxide (NO) is a free radical with many well-known physiological functions; one these are its abilities to act as either a cytoprotective or a cytotoxic agent[43]. It is accepted that the difference between the protective and the deleterious effects of this free radical is determined both by

concentration and time of exposure and by cell type[44]. The effect of *Ginkgo biloba* extract a on the NO production was studies by ***Varga, et al.***[45]. They found that *Ginkgo biloba* directly acts as a NO scavenger and concomitantly inhibits the expression of iNOS mRNA. Thus, *Ginkgo biloba* may act as a potent inhibitor of NO production under the condition of ischemia/reperfusion, improving the recovery of post-ischemic cardiac function. These results showed the lowest decrease in nitric oxide level had been observed in rats administrated with Et extract, then the E.A extract and finally with the water extract. This may be due to the inhibitory effect of Ginkgo flavonoids on the inducible nitric oxide synthetase and scavenge peroxynitrite formation.

In conclusion: the Et extract of *Ginkgo biloba* has the highest activity against lipid peroxidation and free radical scavenging effect compared to that of ethyl acetate and the water extracts. In addition, it has the most protective activity against liver injury caused by CCl_4 administration. Moreover, it was the most effective extract on the NO. This may be due to its high contents of antioxidant compounds and flavonoids compared to the other two extracts. Finally, the present study can say that *Ginkgo biloba* has a very wide range of pharmacological actions.

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تأثير مستخلصات ورق نبات جينكو *بيلوبا* فحماية الخلايا الكبدية ومضادات للأكسدة

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تعتبر أوراق نبات الجينكو بيلوبا من المصادر الهامة للحصول على المركبات الثانوية كالفينولات والفلافونيدات ولها عدة استخدامات في الطب الشعبي في عديد من البلدان فى جميع أنحاء العالم حيث يستخدم المستخلص المائي للأوراق في أمراض عديدة مثل مرض السكر و الجلطات وامراض القلب وذلك للمحتوى

العالي من الفلافونيدات ولهذا صمم هذا البحث لدراسة تاثير نشاط مستخلصات نبات الجينكو بيلوبا فى حماية الكبد من الشوارد الحرة التى تصدر نتيجة لمعاملة الكبد برابع كلوريد الكربون .ودراسة أسباب و طرق هذه الحماية و ذلك بإستخدام التجارب *In vitro* and *in vivo* أولاً : لتجربة الـ *In vitro* تم إجراء عمليات استخلاص نبات الجينكو بيلوبا باستخدام مذيبات مختلفة مثل الأيثانول 70٪ (المستخلص الأول) خلات الأيثايل (المستخلص الثانى) والماء (المستخلص الثالث) وقد تم إجراء تحليل وصفى وكمي للمركبات الموجودة في كل مستخلص على حدى .أوضحت النتائج احتواء المستخلص الكحولي وخلات الأيثايل والمائي على كل من التنينات والفينولات والفلافونيدات والصابونين .و اوضحت النتائج أن مستخلص الكحول الإيثيلى يحتوى على أعلى من المكونات التنينات والفينولات والفلافونيدات والصابونين .أوضحت نتائج تجربة نشاط المستخلصات الثلاث كمضادات الأكسدة وذلك بإستعمال مادة ال , ($DPPH^{\bullet}$)أوضحت النتائج أن المستخلصات الثلاث تحتوى على أعلى نسبة من مضادت الأكسدة خاصةَ مستخلص الكحول الإيثيلى الذى أظهر أعلى المحتويات فى إحتوائه على مضادات الأكسدة .أيضاَ أظهر نشاط واضح فى تثبيط اكسيد النيتريك و عدم ترسيب انيونات السوبر اكسيد .

ثانياَ: لتجربة الـ *In vivo* لدراسة تأثير المستخلصات السابقة على نشاط الإنزيمات المضادة للأكسدة فى الدم و ايضاَ الكبد ,تم إستخدام عدد اربعون فأر من فئران التجارب قسمت إلى خمس مجموعات ,المجموعة الأولى تناولت (10% Dimethyl sulphoxide-DMSO) وهى تمثل مجموعة الكونترول الموجب ,أما المجموعة الثانية تناولت (CCl_4 + 10% DMSO) وهى تمثل الكونترول السالب ,أما المجموعات الثانية و الثالثة و الرابعة تناولت كمية 100مج/كجم من وزن الجسم من مستخلصات الكحول الإيثيلى ,وخلات الإيثيل و المستخلص المائى على التوالى ,أيضاَ تم إجراء عملية سمية للكبد باستخدام رابع كلوريد الكربون بالنسبة للمجموعات الثلاث الأخيرة.

تم تقدير نشاط أنزيمات السوبر أكسيد ديسميوتيز , ,الجلوتاثيون اس ترانسفيريز ,الجلوتاثيون بيرأوكسيديز,أكسيد النيترك ,المالوندالدهيد ,البروتينات الكلية و نسبة السكر فى الدم ,وكذلك تم تقدير الدهون مثل نسبة الكوليستيرول الكلى ,الدهون العالية و المنخفضة فى الكثافة ,الجلسريدات الثلاثيه. فوجد أن المستخلص الكحولي له تأثير واضح على تقليل الآثار السامة لرابع كلوريد الكربون بالمقارنة بالمستخلصات الأخرى وذلك بأنه قلل نشاط الإنزيمات المضادة للأكسدة التى تم تقديرها فى هذه التجربة .وكذلك كان له القدرة العالية على تقليل نيبة المالوندالديهيد.

ونستخلص من هذه الدراسة أن المستخلص الكحولي بمفرده له تأثير مضاد للأكسدة عالي مقارنة بباقي المستخلصات وأن مستخلصات نبات الجينكو بيلوبا ليس له اى تأثير سام .