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Original Article

Effect of *Ginkgo Biloba* and *Commiphora Opobalsamum* Extracts on Liver Fibrosis and Kidney Injury Induced By Carbon Tetra Chloride in Experimental Models

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ABSTRACT

Ginkgo biloba and *Commiphora opobalsamum* are herbal medicines ,which have important effects on health. The present study was designed to explore the ameliorative effect of Ginkgo *biloba* and *Commiphora opobalsamum* on liver and kidney damages induced by CCl_4 in experimental models. A total of 30 adult female albino rats were divided into five groups .Gp (1): Healthy, Gp (2): Positive control injected i.p by CCl_4, once. Gps from 3 to 5 were injected i.p. by CCl4, once. Then administered oraly by aquaes extract of either *G. biloba or C. opobalsamum* or a mixture of *G. biloba* and *C. opobalsamum*, respectively. The present results revealed significant elevation in liver enzymes activity and kidney functions in CCl_4 treated group as compared with control group. Treatment of rats with *G. biloba* decreased these levels significantly as compared to positive control and *C. opobalsamum* treated group. Albumin, total protein, increased significantly by *G. biloba* treatment as compared to positive control. Based on our results, it's clear that CCl_4 induce liver and kidney toxicity. Also, it could be concluded that *G.biloba*and *C. opobalsamum* extract showed significant ameliorative effect on liver fibrosis and kidney injury induced by CCl_4 in experimental models.

Key words: *Commiphora opobalsamum, Ginkgo biloba,* CCl_4, Hepatotoxicity, Renal toxicity

INTRODUCTION

Medicinal plants play a key role in the human health care system, about 80% of the world population rely on traditional medicine which is predominantly based on plant materials. The traditional medicine refers to a broad range of ancient natural health care practice. [1] Liver is one of the biggest organs in the body and maintains metabolism process and removal of toxic substances. Liver plays a vital role in maintaining the homeostasis of the body via the detoxification and elimination. Liver function can be reduced and hepatocytes damaged upon exposure to CCl_4, malnutrition, alcohol, drugs or infections. Kidneys handle several life-sustaining roles: They cleanse blood by removing waste and excess fluid, maintain the balance of salt, water and minerals in blood, and help regulate blood pressure by producing renin hormone. Also, it stimulates red blood cell production by erythropoietin secretion and produces an active form of vitamin D. When the kidneys become damaged, the extra salt, waste products and fluid buildup in the body and can cause swelling in ankles, vomiting, weakness, shortness of breath and poor sleep. If left untreated, diseased kidneys may stop functioning completely. Loss of kidney function is a serious and potentially fatal condition.

The *Ginkgo biloba* is an ancient tree in the earth, has been grown up in the forests for more than 150 million years and it is called "living fossil". The name of *ginkgo bioloba* comes from China and it means the silver fruit. The *Ginkgo biloba* tree is the present species of family (Ginkgoaceae) and of class (Ginkgoatae). The main active compounds in *Ginkgobiloba* extract are ginkgo flavone glycosides include (quercetin, isorhamnetin and kaempferol) and terpene lactones include (bilobalide and ginkgolides A & B). Active compounds in Ginkgo extract improve blood circulation, discourage clot formation, reinforce the walls of the capillaries, and protect nerve cells from harm when deprived of oxygen. The extracts are used to treat demential disorder, liver disease and memory impairment. The extract also possesses antioxidant, antiasthmatic, scavenge radicals, neuroprotective properties and wound healing effect, also it improves mental capacities in Alzheimer's patients

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[2]. *Ginkgo biloba* could have effect in patients with diabetic. Many interesting studies had shown that the electrical activity was increasing by *Ginkgo biloba* treatment in the beta cells of the pancreas. Which is an indicator of enhancing insulin secretion [3]. Also, *Ginkgo biloba* have evidence of potential estrogenic activity and a recent study has provided that *Ginkgo biloba* extract could be used as an alternative hormone replacement therapy "phaytoestrogen therapy"[4]**.** Furtheromre, *Ginkgo biloba* extract show significantly lower in the behavioral manifestations of tinnitus in the rat's clinical trial [5]. In addition, *Ginkgo biloba* treatment has been shown an improvement in cognitive performance in elderly people with dementia.

Balessan is the local name of *Commiphora opobalsamum* (L.) Engl. family Burseraceae. This plant is one the most ancient plants, with a magnificent history of healing, and was a valuable medicinal agent in ancient Arabia. Active compounds in Balessan extract are The Triterpenes (Friedelin, Canophyllal and Oleanonic Acid) and The Flavonols (Syringic Acid, Mearnsetin and Quercetin) [6]**.**

The use of the ethanolic leaf extract of *Commiphora opobalsamum* in the therapy of microbial infections has been documented. This extract was found to inhibit Salmonella, typhi, *E.coli, Staphylococcus aureus, Streptococcus, Candida albicans, Bacillus subtilis and P. aeruginosa* [7].

Commiphora Opobalsamum extract containing ketonic steroid active substances such as guggulsterones have been shown to significantly reduce the serum level of very low density (VLDL) and low density lipoprotein (LDL). Moreover, it raise the levels of high density lipoprotein (HDL) cholesterol and decrease total cholesterol up to 30% in 12 weeks and a reduction of LDL by 35% and increase in HDL by 20% in 3 months. As the active ingredient obtained from *Commiphora mukul* acts as an antagonist to the farnesoid X receptor, which is involved in reducing cholesterol synthesis in the liver and increasing the body's fat-burning activity, and augmenting thermogenesis or heat production [8].

Commiphora mukul (c. mukul), is used for cardiac dysfunction disease. Several clinical investigations have proven that *C. mukul* as an effective medicine for myocardial infarction and atherosclerosis according to many chemical constituent such as esters, sterols, steroids and diterpenes have been known in C.mukul [9]. In this study we hypothesis and highlight the possible ameliorative effect of either *ginkgo biloba* or *Commiphora opobalsamum* or their combination on liver and kidney toxicity induced by CCl_4 in experimental model.

MATERIALS AND METHODS

Experimental Models: A 30 adult female Albino rats,"*Spargue – Dawley*" strains were used in this study. The weight of rats ranging from 100 –120. At the initiation of experimental treatments the animals were adapted to the laboratory conditions for 3 days. The rats caged in animal unit in a 12h light-dark cycle and an optimal temperature (23±3◦C). They have standard diet and free access to tap water; the rats were weighed once a week. Animal cared for accordance to the guidelines for animal experiments which were approved by the Ethical Committee of King Fahd Medical Research Centre (KFMR), Jeddah, KSA.

Preparation of Aqueous Extract of Plant: (50g dried powder) of plant were soaked in 0.5 liter of boiling distilled water, After 2 h it homogenized in the same distilled water, then filtered by filter paper until getting pure extraction [10].

Induction of Hepatotoxicity by CCl_4: Animals were Injected Intraperitoneally (i.p) with a solution of CCl_4 in olive oil1:1(v/v) 5 ml/Kg body weight, single dose according to He et al [11].

Experimental Design: Experimental animals were divided into 5 group's six rats each as follows:
Gp(1): Healthy control group : Rats were injected i.p. with a 5 ml/kg b.w of olive oil, once. Gp (2): CCl_4 injected group (Positive control): Rats were injected i.p. with 5 ml/kg b.w of CCl_4, once. Gp(3): CCl_4+ *Ginkgo Biloba* treated-group: Rats were injected i.p. with CCl_4 then treated orally by oral gavage with 5 ml/kg b.w of G.BE daily. Gp(4): CCl_4 + *Commiphora Opobalsamum* treated- group: Rats were injected i.p. with CCl_4 then treated orally by gavage with 5 ml/kg b.w of COE daily. Gp(5): CCl_4 + *Ginkgo Biloba* and *Commiphora Opobalsamum* treated- group: Rats were injected i.p. with CCl_4 then treated orally by gavage with 5 ml/kg b.w of GBE and COE daily.

Biochemical Analysis: At the end of experimental period, that last for four weeks. Blood samples were collected under anesthesia with diethyl ether from the orbital venous plexus of each rat, for estimation hepatic and renal biomarkers, include Total Protien (TP), Albumin, Total Bilirubin(TB), Urea, Creatinine, Alanine Aminotransferse (ALT), Aspartate Amino-transferase (AST) and Gammaglutamyl Transferase(GGT) Levels. Using commercial test kits (Crescent, Jeddah, Saudi Arabia).

Statistical Analysis: All data express as mean ± standard error (SE), and analyzed by software package SPSS. A probability statistical was considered a significant of less than 5% (P<0.05).

RESULTS

The present results revealed significant elevation in liver enzymes activity (ALT, AST, Total bilirubin and GGT) as well as kidney functions (urea and creatnine) in CCL_4 treated group compared with healthy control group. Treatment of rats with Ginkgo biloba decreased these levels significantly as compared to positive control and *Commiphora opobalsamum treated* group. On the other hand, (Albumin and total protein), increased significantly by *Ginkgo biloba* treatment as compared with positive control (Table 1,2,3).

DISCUSSION

The present study showed that CCl_4 induced liver fibrosis through elevation of plasma liver enzyme ALT, AST, GGT, total bilirubin and reduction of total Protein and albumin levels. Our results are in agreement with Aleynik et al. [12], they suggested that, Carbon tetrachloride (CCl_4) is utilized to experimentally induce liver damage. Formation of trichloromethyl ($CCl_{31/2}$and /or $CCl_3OO_{1/2}$) as result of Peroxidation of membrane lipids secondary. The toxic effect of CCl_4 produce free radicals. These free radicals are susceptive to starting a chain of lipid peroxidation reactions by removing hydrogen from polyunsaturated fatty acids (PUFA). Peroxidation of lipids, in particular the consist of PUFA lead to produce severe cell damage and dramatic changes in the characteristic of biological membranes which play role in the diseases pathogenesis. Lipid peroxidation produce cross-links on proteins of cell membrane that generate malondialdehyde (MDA) during PUFA degeneration. The proteins break down produce free amine units that binding to the two-aldehyde units of MDA covalently and that lead to an irreversible the polymerization process. However, the oxidation of protein resulting in oxidative damage that produce reversible disulfide bonded polymers [11].

Moreover, it's observed that CCI_4 is bio-transformation by the cytochrome *p*450 to the tri-chloromethyl which is a type of free radical. The interact of tri-chloromethyl with oxygen form a tri-chloromethylperoxyl radical, which could break down the lipids on the membrane of endoplasmic reticulum. The tri-chloromethylperoxyl free radical leads to lipid peroxidation and the disturbance of Ca^{++} regulation causing cell damage. In addition, leakage of high amount of enzymes into the blood circulation are related with huge necrosis in the liver. Administration of CCI_4 results in an increasing of serum AST and ALT level [6].These observations supports our finding.

It is clear from our results that *Ginkgo biloba* extract has a strong ameliorative effect on liver fibrosis than on kidney injury induced by CCl_4 as it cause significant regression of liver enzyme. Moreover, Total protein and albumin content increased significant in group treated with *Ginkgo biloba*, this may be due to the ability of two groups of main*Ginkgo biloba* components: terpenoids and flavonoid glycosides [13]. It has been reported that flavonoids origin nuclear can reduce hydroxyl function group, which could capture oxygen-derived free radicals, such as superoxide anion, hydroxyl and peroxyl radicals, and nitric oxide. Act as a donator of hydrogen atom to terminate pathological aggravation of free radical chain reaction and lipid peroxidation, and relieve the injury caused by oxygen-derived free radicals and lipid peroxidation. Consequently, it reduces lipid peroxidation. Thus *Ginkgo biloba* extract has wide antioxidant effects and can be used in diseases treatment [6,14].

The present study revealed that *Commiphora opobalsamum* extract have ameliorative effect on liver and kidney injury induced by CCl_4 as it cause significant reduction of liver and kidney functions. Also, total protein and albumin increased slightly in the group treated with *Commiphora opobalsamum*, this may be due to the mechanism hepatoprotection of *Commiphora opobalsamum* extract on CCI_4 that induced liver injury. The extract of *Commiphora opobalsamum*s up press the increasing in AST and ALT activity as well as reduction of total bilirubin level. The possibilities suggests of the extract may have efficiency in preserving the normal function of the liver. These observations in the present study may be explained as follows:(i) the inhibiting of cytochrome *p*450 activity (ii) the preventing of lipid peroxidation (iii) the stabilizing of the hepatocellular membrane (iv)the synthesis of protein increased. Furthermore, an ethanolic extract of *Commiphora opobalsamum* offered a significant replenishing of the non-protein sulfhdryl level. Thus, sulfhydryl seems to have a role hepatoprotection through its antioxidant potential.

The ameliorative effect of *Commiphora opobalsamum* may be also due to a number of phytochemical constituents, including flavonoids, saponin, volatile oils, sterol and/or triterpenes. All of these constituents are known to exhibit antioxidant activity, offer protection against cell damage and possess free radical scavenging effects. Interestingly, some *Commiphora opobalsamum*s pieces have been shown to possess diversified activities through various mechanisms. These include *Commiphoramolmol* and

Commiphoramukul, which showed pharmacological effects that included anti-inflammatory, antihepatotoxic, anticholesterolemic, antiulcer and cytotoxic actions [6].

CONCLUSION

Based on our result it's clear that CCl_4 induce hepatic and renal toxicity through increasing AST, ALT, urea and creatinine and decreasing albumin and total protein biomarkers. Also, it could be concluded that *Ginkgo biloba* extract has a significant strong ameliorative effect on liver fibrosis than on kidney injury. *Ginkgo biloba* extract has a better effect than *Commiphora opobalsamum* extract and treatment of both plants *Ginkgo biloba* and *Commiphora opobalsamum* did not give the same effect, further studies are needed to clarify this issue.

Table 1: Effect of *Ginkgo biloba* and *Commiphora opobalsamum extract* on liver toxicity (mean ±SE)

Groups	AST (U/L)	ALT (U/L)	GGT (U/L)	Total Bilirubin (mg/dl)
Control (Healthy Control)	72.28 ± 3.65	58.14 ± 1.67	19.30 ± 1.88	1.13 ± 0.075
CCl_4 (Positive Control)	106.24 ± 4.99[a]	86.23 ± 1.93[a]	30.21 ± 1.31[a]	1.93 ± 0.058[a]
CCl_4 + GBE	73.01 ± 1.38[b]	62.43 ± 3.67[b]	20.79 ± 1.48[b]	1.24 ± 0.273[b]
CCl_4 + COE	79.50 ±1.75[b]	68.36 ± 3.06[b]	23.70 ± 1.69[b]	1.32 ± 0.175[b]
CCl_4+COE+GBE	81.03 ± 1.70[b]	77.98 ± 2.98[b]	28.46 ± 1.68[b]	1.34 ± 0.057[b]

a Significant change at $p<0.05$ in comparison with control group.
b Significant change at $p<0.05$ in comparison with CCl_4 treated group

Table (2) Effect of *Ginkgo biloba* and *Commiphora opobalsamum* extract on protein content (mean ± SE).

Groups	Albumin (g/dl)	Total Protein (g/dl)
Control (Healthy Control)	4.80 ± 0.212	7.89 ± 0.096
CCl_4 (Positive Control)	2.33 ± 0. 322[a]	4.69 ± 0.258[a]
CCl_4 + GB.E	4.70 ± 0.379[b]	7.65 ± 0.166[b]
CCl_4 + CO.E	4.10 ± 0.641[b]	7.30 ± 0.281[b]
CCl_4+CO.E+GB.E	3.83 ± 1.208[b]	6.57 ± 0.104[b]

a Significant change at $p<0.05$ in comparison with control group.
b Significant change at $p<0.05$ in comparison with CCl_4 treated group

Table (3) Effect of *Ginkgo biloba* and *Commiphora opobalsamum* extract on kidney functions (Mean ± SE)

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Control (Healthy Control)	0.66 ± 0.016	35.98 ± 1.72
CCl_4 (Positive Control)	0.90 ± 0.092[a]	53.58 ± 8.35[a]
CCl_4 + GB.E	0.71 ± 0.017[b]	39.80 ± 2.98[b]
CCl_4 + CO.E	0.75 ± 0.148[b]	47.48 ± 3.36[b]
CCl_4+CO.E+GB.E	0.83 ± 0.049[b]	48.98 ± 1.55[b]

a Significant change at $p<0.05$ in comparison with control group.
b Significant change at $p<0.05$ in comparison with CCl_4 treated group

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