

Hepatoprotective Activity of the Methanolic Extract of the Bark of *Khaya senegalensis* in Rats Against Carbon Tetrachloride (CCl₄) -Induced Hepatotoxicity in Adose of (800mg/kg I.P)

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Abstract: This study was carried out to investigate the hepatoprotective effect of the methanolic extract of the bark of sudanese plant *Khaya senegalensis*, which used in folk medicine for treatment of jaundice. The hepatoprotective effect was tested in rats against carbon tetrachloride (CCl₄) -induced hepatotoxicity. **Method:** Hepatoprotective extracts was given intraperitoneally one hour before injection of CCl₄ (800mg/kg I.P) and compared with silymarin, a standard hepatoprotective agent. **Result:** Methanolic extract of the bark of *Khaya senegalensis* showed a hepatoprotective effects against CCl₄- induced hepatotoxicity, which was evidenced by the significant decrease in ALT, AST and ALP. The methanolic extract of the bark of *Khaya senegalensis* possessed strong hepatoprotective effect, so we selected it for further biological activity -directed fractionation study. **Conclusion:** On the light of the present study it is concluded that the methanolic extract of the bark of *Khaya senegalensis* could protect liver against oxidative damages and could be used as an effective protector against CCl₄-induced hepatic damages

Keywords: Hepatoprotective, Methanolic, Hepatotoxicity, Carbon tetrachloride

1. Introduction

The predominant type of liver disease varies according to country and may be influenced by local factors. Causative factors of liver disorders include virus infection, exposure to certain chemicals e.g., the excessive inhalation of chlorinated hydrocarbons or overindulgence, medication with antibiotics, chemotherapeutic agents. In the recent years *in vivo* and *in vitro* test models have been developed for evaluation of plants for their anti-hepatotoxic activities (1). Medicinal and poisonous plants have always played an important role in African societies. However, among the more than 250.000 species of higher plants, only about 10% have been chemically investigated (2).

1.1 Hepatoprotective Plants

Medicinal plants represent one of essential sources in our country. Sudan is a large country and has excellent geographical localization and medicinal plants are widely spread in many areas. The use of plants and herbs for medicinal purposes spread overall the world. Therefore, a high consumption of medicinal plants is clearly observed in the developing Islamic and non-Islamic countries.

1.2 Plant Description

1.2.1 *Khaya senegalensis*

Khaya senegalensis (Desr.) A. Juss.: The plant belongs to the family *Meliaceae* and locally known as Mahogany tree (Senegal Mahogany). Other species: *K. grandifoliola*, *K. anthotheca* and *K. ivorensis*.

1.3 Hepatotoxic Agents

1.3.1 Carbon tetrachloride toxicity (CCl₄)

Carbon tetrachloride (CCl₄) is halogenated aliphatic hydrocarbon, used widely as industrial solvent, degreasing and cleaning agent. In laboratory animals causes central nervous system depression, liver injury, kidney injury and some degree of cardiotoxicity. Hepatotoxicity is also a common toxic effect that can occurs in humans after an acute or chronic exposure the severity of the lesion being dependent on the amount absorbed (3).

Carbon tetrachloride or tetrachloromethane, is formed by the action of chlorine or chloroform in sunlight, or in presence of iodine as a catalyst (4). There is a vasoconstriction of the sinusoids in the liver of rodents poisoned with chloroform and carbon tetrachloride (5) (6). These changes may indicate an impediment of the intralobular circulation leading to ischaemia of the hepatic cells. (7) reported that the peroxidative decomposition of rat liver microsomal lipids, as evidenced by the appearance of diene conjugation

absorption, is more than half maximal within 5 minutes after intra-gastric administration of CCl_4 in mineral oil. In the microsome fraction the degenerative process is completed within 15 minutes. Peroxidative decomposition of liver cell microsomal lipids is the most rapid pathological alteration yet noted for CCl_4 liver damage under comparable conditions of dosage and route of administration of this toxic haloalkane. As early as 1 to 5 hours after CCl_4 administration, there is fragmentation of the endoplasmic reticulum, depression of the ribosomal enzyme activity, inhibition of protein synthesis, accumulation of lipid and calcium in the hepatocytes and structural and functional damage to the mitochondria (8). (9) suggested that exposure to this solvent causes acute and chronic renal injuries.

1.4 Parameters or Laboratory Tests used in Diagnosis

Alanine aminotransferase (ALT) which is very specific for hepatic tissue and is almost always absent in acute myocardial infarction. It is much more sensitive to hepatic damage, and levels rise faster and higher than those of AST in most types of hepatocellular damage (10). Aspartate aminotransferase (AST): Its levels rise in virtually all types of hepatic diseases. Its peak concentration and ratio to other enzymes reflect the type of hepatic damage. The highest concentrations of AST are located in cardiac and hepatic tissues. Other parameters used are alkaline phosphatase (ALP), Bilirubin, Serum proteins (Total protein & Albumin)

1.5 Standard Agents

1.5.1 *Silybum marianum*

This plant syn. *Carduus marianus* (*Compositae*) is one of the 'milk thistles'. In animal model 'milk thistle' limits hepatic injury associated with a variety of toxins, including galactosamine, carbon tetrachloride, acetaminophen, radiation, cold ischaemia, ethanol and *Amanita mushrooms* (parenteral silybin is marketed in Europe as antidote in *Amanita phalloides mushroom* poisoning). In animal model of cirrhosis, it reduced collagen accumulation and *in vitro* model it reduces expression of the profibrogenic cytokine TGF- β . In acute viral hepatitis studies have generally involved sample sizes and have shown mixed outcomes of improved liver function e.g. (aminotransferase values, bilirubin, prothrombin time) or no effect. Studies in chronic viral hepatitis and toxin induced injury have also been of small size but have reported mostly favourable result (3).

1.6. Objectives:

- 1) To investigate and confirm the hepatoprotective activity of the plant extract against carbon tetrachloride-induced hepatic damage.
- 2) To study the fractions of the hepatoprotective plant extract.
- 3) To select the most potent fraction of the plant extract.

2. Material

2.1 Animals

Male and female albino rat weighing 100 to 210 gm were obtained from animal house of Faculty of Pharmacy,

University of Khartoum & National Centre for research, Khartoum, Sudan. Food and water made freely accessible.

2.2 Drugs

Drugs and agents used in this study are as follows: Carboxy methyl cellulose, Carbon tetrachloride, Silymarin, Sodium chloride, Chloroform,, Methanol,, Petroleum ether, Dichloromethane, Ethyl acetate, and Corn oil

3. Methods

3.1 Preparation of Drugs

The drug solutions were freshly prepared daily, 1% carboxy methyl cellulose in normal saline and Carbon tetrachloride dissolved in corn oil. The drugs were administered either Intraperitoneal (I.P) or orally (P.O)

3.2 Preparations of Plants Material

3.2.1 *Khaya senegalensis*

Plant was obtained from Elobied, North Kordofan, and Western Sudan.

3.2.2 Preparation of Extracts

3.2.2.1 Methanolic extract

100gm of the dried small pieces of plant homogenized with one liter of 80% methanol. The mixture was filtered with Whatman No.1 filter paper and the filtrate was dried to a solid mass under air at room temperature.

3.2.2.2 Fractionation of *Khaya senegalensis* bark

500 gms of powdered bark of *Khaya senegalensis* were divided equally into 4 flasks containing 500 ml of petroleum ether. They were macerated for 48 hours with occasional shakers, then filtered and evaporated under reduced pressure using the Rota vapor. The residue of bark powder of *Khaya senegalensis* was divided into 4 flasks each containing 500ml of 80% methanol, shook with flask shaker for 24 hrs. The mixture was filtered with Whatman No. 1 filter paper and the filtrate was evaporated using Rota vapor under reduced pressure and was dried to a solid mass at room temperature.

3.2.2.2.1 Solvent-solvent extraction

10 gms of methanolic extract of bark of *Khaya senegalensis* dissolved in 200 ml of distilled water and mixed with 3 portions of 250 ml of dichloromethane in a separating funnel, the mixture separated in aqueous layer and dichloromethane layer, which was evaporated using Rota vapor under reduced pressure and was dried to a solid mass at room temperature. The aqueous layer mixed with 3 portions of 250 ml of chloroform in a separating funnel. The mixture separated into aqueous layer and chloroform layer which was evaporated using Rota vapor under reduced pressure and was dried to a solid mass at room temperature.

The aqueous layer extracted with 6 portions of 250 ml of ethyl acetate in a separating funnel, the mixture separated into aqueous layer and ethyl acetate layer, which was evaporated using Rota vapor under reduced pressure and was dried to a solid mass at room temperature. The rest of

aqueous layer freeze dried to a solid mass and weighed which contained the aqueous methanolic extract.

3.3. General phytochemical tests for the major components of the tested plant

The following phytochemical screening tests of the chemical constituent were conducted according to the methods established by (11).

3.4 Assessment of hepatoprotective activity against CCl₄-induced hepatotoxicity

Hepatotoxicity was induced by carbon tetrachloride (800 mg/kg in 10% corn oil intraperitoneally) (12). Hepatotoxins were injected 1 hour after the administration of the extract or the standard. Blood samples were drawn by cardiac puncture or from orbital plexuses under light ether anesthesia into heparinized capillary tubes 24 hour later. Serum was separated by centrifugation for 5 minutes at 4000 rpm. The serum samples and livers were kept refrigerated at -20°C until used for serum ALT determination. The most hepatoprotective constituents will be subjected to further biochemical serum and liver assessments.

3.5 Estimation of ALT, AST, ALP, total bilirubin, total protein, albumin, urea, creatinine and cholesterol in serum

The above parameters were determined using commercially diagnostic kits (Dialab, Australia) by standard automated technique using of Hitachi analyzer, model 911.

3.6 Statistical analysis

The observations in each group were compiled and tabulated for the assessment of mean and standard error of mean (Mean \pm SEM). Statistical comparison between different groups was done using one-way analysis of variance (ANOVA) followed by post HOC test where appropriate. Significance was accepted at $P < 0.05$.

4. Result

General phytochemical tests for major components of the tested plant revealed presence of flavonoids, tannins, triterpenoids and saponins. The extract is devoid of coumarins, unsaturated sterols, and alkaloids.

4.1. Effect of silymarin against carbon tetrachloride induced hepatotoxicity in rats

Silymarin, a standard hepatoprotective drug, caused significant ($P < 0.05$) dose dependent hepatoprotective activity at the doses (12.5 and 25 mg/kg) against carbon tetrachloride-induced hepatotoxicity by decreasing the level of AST, ALT and ALP. The percentage of inhibition of ALT was being (85.3% and 90.4%) respectively as shown in (Table 1). Silymarin itself didn't produce any significant changes in total bilirubin, total protein, albumin and

cholesterol against carbon tetrachloride-induced hepatotoxicity (Table1).

4.2 Effect of methanolic extract of the bark of *Khaya senegalensis* against carbon tetrachloride - induced hepatotoxicity in rats

Methanolic extract caused significant ($P < 0.05$) hepatoprotective activity by reducing the level of AST, ALT and ALP against carbon tetrachloride induced-hepatotoxicity at the doses (12.5, 25 and 50 mg/kg), but not in a dose dependent manner. The percentage of inhibition was being (95.9%, 92.6% and 96.2%) respectively as shown in (Table 1). For methanolic extract of the bark of *Khaya senegalensis*, no significant changes in total protein, total bilirubin, albumin and cholesterol at all doses against carbon tetrachloride-induced hepatotoxicity (Table 1).

4.3. Effect of intraperitoneal administration of tested fractions of methanolic extract of the bark of *Khaya senegalensis*

4.3.1. Effect of petroleum ether and dichloromethane extracts against carbon tetrachloride- induced hepatotoxicity

Intraperitoneal administration of petroleum ether and dichloromethane extracts in a dose of 25 and 50 mg/kg against carbon tetrachloride induced-hepatotoxicity didn't possess hepatoprotective activity ($P > 0.05$). It failed to reduce the elevated level of ALT & AST (Table.1)

4.3.1.1 Effect of chloroform extract against carbon tetrachloride -induced hepatotoxicity

Oral administration of chloroform extract in a dose of 25 and 50 mg/kg against carbon tetrachloride-induced hepatotoxicity caused significant ($P < 0.05$) hepatoprotective activity by decreasing the elevated level of ALT, but no significant decrease of the level of AST activity at a dose of 25 and 50 mg/kg. The percentage of inhibition of ALT was 48.9% and 49.1% respectively as shown in (Table 1).

4.3.1.2 Effect of ethyl acetate extract against carbon tetrachloride-induced hepatotoxicity

Intraperitoneal administration of ethyl acetate extract in the dose of 25 mg/kg against carbon tetrachloride-induced hepatotoxicity caused significant ($P < 0.05$) hepatoprotective activity by decreasing the elevated level of ALT, but no significant decrease at the dose of 50 mg/kg. At the dose of 25 and 50 mg/kg significantly ($P < 0.05$) decreased the elevated level of AST. The percentage of inhibition of ALT was 52.6% and 39.28% respectively as shown in (Table.2).

4.3.2 Effect of aqueous methanolic extract against carbon tetrachloride - induced hepatotoxicity

Intraperitoneal administration of aqueous methanolic extract in a dose of 50 mg/kg didn't protect the liver against toxicity induced by carbon tetrachloride as evidence by non-significant decrease in serum ALT and AST activity (Table.2).

Table 1: Effect of intraperitoneal administration of methanolic extract of the bark of *Khaya senegalensis* and silymarin on liver function test (ALT, AST, ALP, total bilirubin, total protein, albumin and cholesterol) after treatment of carbon tetrachloride

Treatment	ALT (IU/ml)	AST (IU/ml)	ALP (IU/ml)	Total bilirubin(mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	% of inhibit. of ALT
Control	70 ± 1.93	98.4 ± 22.8	118 ± 4.6	0.46 ± 0.04	7.42 ± 0.18	3.2 ± 0.09	61.0 ± 5.9	-
Carbon tetrachloride 800mg/kg	470 ± 8.1**	571.8 ± 35.3**	456 ± 81.9**	0.38 ± 0.03	6.78 ± 0.12**	3.0 ± 0.07	53 ± 6.8	-
Silymarin 12.5 mg/kg	68.8 ± 6.2*	149.6 ± 6.9*	238 ± 39.3*	0.20 ± .04	6.16 ± 0.42	2.82 ± 0.18	54.60 ± 6.57	85..3%
Silymarin 25 mg/kg	45.2 ± 3.9*	115.2 ± 4.9*	257 ± 8.2*	1.0 ± 0.45	6.92 ± 0.28	3.24 ± 0.172	57.80 ± 8.33	90.40%
<i>K. senegalensis</i> 12.5 mg/kg	18 ± 1.22*	20.4 ± 1.6*	300 ± 27.1*	0.38 ± 0.07	5.5 ± 0.37	2.6 ± 0.24	50.0 ± 6.0	95..9%
<i>K. senegalensis</i> 25 mg/kg	34.6 ± 7.82*	13.4 ± 1.8*	194.6 ± 15.9*	0.34 ± 0.04	6.5 ± 0.48	3.1 ± 0.23	54.2 ± 2.4	92.60%
<i>K. senegalensis</i> 50 mg/kg	17.8 ± 2.47*	11.4 ± 1.5*	246.2 ± 14.4*	0.46 ± 0.08	6.2 ± 0.23	2.8 ± 0.17	57.0 ± 4.3	96..2%

Values were expressed as mean ± SEM (n = 5), P < 0.05* significant difference from carbon tetrachloride.

P < 0.05** significant difference from control

Table 2: Effect of intraperitoneal administration of tested fractions of methanolic extract of the bark of *Khaya senegalensis* and silymarin on liver enzymes after treatment of carbon tetrachloride

Treatment	ALT (IU/ml)	AST (IU/ml)	% of inhibition of ALT
Control	65.6 ± 4.83	164 ± 13.9	-
Carbon tetrachloride 800mg/kg	462.8 ± 64.8**	812.6 ± 129.6**	-
Silymarin 12.5 mg/kg	68.8 ± 6.2*	149.6 ± 6.9*	85.2%
Silymarin 25 mg/kg	45.2 ± 3.9*	115.2 ± 14.9*	90.3%
Petroleum ether 25mg/kg	532.4 ± 120.3	878.2 ± 19.8	-
Petroleum ether 50mg/kg	572.8 ± 22.5	1196.6 ± 25.9	-
Dichloromethane 25mg/kg	926.8 ± 237.2	783.6 ± 190.7	-
Dichloromethane 50mg/kg	691.6 ± 52.2	1361 ± 61.8	-
Chloroform 25 mg/kg (P.O)	236.4 ± 20.6*	706.6 ± 99.3	48.9%
Chloroform 50 mg/kg (P.O)	235.2 ± 71.09*	597.4 ± 60.8	49.1%
Ethyl acetate 25mg/kg	219 ± 86.8*	387 ± 79.4*	52.6%
Ethyl acetate 50mg/kg	281 ± 78.47	422.6 ± 93.9*	39.28%
Aqueous methanolic 50mg/kg	440.2 ± 6.0	767.2 ± 31.3	4.88%

Values were expressed as mean ± SEM (n = 5), P < 0.05* significant difference from carbon tetrachloride.

P < 0.05** significant difference from control

5. Discussion

In Sudan in folk medicine watery maceration of the bark of *Khaya senegalensis* is used in treatment of malaria, hepatitis, dysentery and sinusitis. Also leaves of plants were used to treat dermatological disorders, abdominal diseases and trachoma. It is commonly used for wound healing and malaria (13). The present findings revealed that various biochemical alterations produced by CCl₄ were prevented by the therapy of silymarin.

The results in this study revealed, that the dose of 12.5 and 25 mg/kg of silymarin possessed significant hepatoprotective activity against CCl₄ by reducing the increase in the levels of serum transaminase. The administration of CCl₄ -induced alterations in the biochemical parameters of the livers is due to its hepatotoxic

properties (14). Our findings were in agreement with the result of (15) when used similar route of administration and doses and (16) used silymarin 25 mg/kg (P.O) for 9 days and carbon tetrachloride 1 ml/kg (S.C) for 48 hours.

The preclinical studies using different hepatotoxic substances showed that silymarin has multiple actions as a hepatoprotective agent. The anti-oxidant property and cell regenerating functions as a result of increased protein synthesis are considered as most important (17). The methanolic extract of the bark of *Khaya senegalensis* showed a significant hepatoprotective activity in reducing the increase level of ALT, AST, ALP quite similar in all doses (12.5, 25 and 50 mg/kg) against carbon tetrachloride-induced hepatotoxicity. The percentages of inhibition for (ALT) were almost similar. Moreover, acute intoxication with CCl₄ is characterized especially by systematic central-lobular necrosis and swollen and lipid-laden cell injuries in the middle area of hepatic lobule (18). There was no significant change in the activities of bilirubin level, total protein, albumin and cholesterol in the rats treated with CCl₄ in acute intoxication in a dose of 800 mg/kg .

5.1 Fractionation of methanolic extract of the bark of *Khaya senegalensis*

Effect of petroleum ether and dichloromethane extract on carbon tetrachloride -induced hepatotoxicity at a dose of 25 and 50 mg/kg, both extracts failed to reduce (P > 0.05) the increase in serum concentrations of ALT and AST induced by intraperitoneal administration of carbon tetrachloride . In the present study, both silymarin (12.5 & 25 mg/kg), ethyl acetate extracts (25 and 50 mg/kg) and chloroform extracts (25 and 50 mg/kg) reduced significantly (P < 0.05) the increase in levels of serum transaminases induced by intraperitoneal injection of carbon tetrachloride. ALT that is known to be very sensitive to cytotoxic hepatic injury and AST that is particularly sensitive to carbon tetrachloride poisoning were measured as indices of hepatotoxicity (19). CCl₄ is reported to cause liver damage due to free radical formation, which in turn causes peroxidation of cellular membranes leading to necrosis (20). In the present

investigations chloroform extract at a dose of (25 and 50 mg/kg) possessed hepatoprotective activity against CCl₄-induced hepatotoxicity by reducing the level of ALT, significantly (P <0.05). In the present study the activity of chloroform extract was more potent than ethyl acetate extract. The qualitative phytochemical investigation on the methanolic extract of the bark of *Khaya senegalensis* also showed positive results for flavonoid. The present findings revealed that various biochemical alterations produced by CCl₄ were prevented by a dose of 25 and 50 mg/kg of ethyl acetate extract. Similarly (21) demonstrated that ethyl acetate extract of *Sarcostemma brevistigma* is hepatoprotective. This may be due to the presence of flavonoids in ethyl acetate extract of *Sarcostemma brevistigma*. Various flavonoids have been reported for their hepatoprotective activity (22).

6. Conclusion

On the light of the present study it is concluded that the methanolic extract of the bark of *Khaya senegalensis* could protect liver against oxidative damages and could be used as an effective protector against CCl₄-induced hepatic damages

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