

Hepatoprotective Activity of the Methanolic Extract of the Bark of *Khaya Senegalensis* in Rats Against Paracetamol -Induced Hepatotoxicity

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Abstract: Purpose: 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 paracetamol-induced hepatotoxicity. Method: The methanolic extracts of the bark of *Khaya senegalensis* showed hepatoprotective effect against paracetamol [1&2g/kg (P.O)]- induced hepatotoxicity given one hour after injection of extract, which was evidenced by significant decrease in elevated serum of ALT and AST. Result: The methanolic extract of the bark of *Khaya senegalensis* possessed strong hepatoprotective effect, so we selected it for further biological activity -directed fractionation study. Dichloromethane and petroleum ether extracts against paracetamol-induced hepatotoxicity, didn't possess hepatoprotective effect as evidenced by failure to decrease ALT and AST. Chloroform and ethyl acetate extracts showed significant hepatoprotective effect against toxicity induced by paracetamol as evidenced by significant decrease of ALT and AST. Aqueous methanolic extract of the bark of *Khaya senegalensis* showed hepatoprotective effect against toxicity induced by paracetamol as evidence by significant decrease in ALT and AST activity. Conclusion: Result obtained from this experiment indicated that methanolic extract showed hepatoprotective effect against paracetamol (1&2g/kg (P.O) induced hepatotoxicity by decreasing the level of ALT, AST and cholesterol. The mild histopathological lesions revealed less damage in the groups treated with methanolic and chloroform extracts of the bark of *Khaya senegalensis* than the paracetamol groups, which indicated some protective effect.

Keywords: Hepatoprotective, Hepatotoxicity, Methanolic extract, Paracetamol

1. Introduction

The liver, the principal organ of metabolism and excretion is subjected to a number of diseases which may be classified as liver cirrhosis, acute chronic hepatitis (inflammatory disease) and hepatitis (non inflammatory condition), Jaundice or disease within the tissue of the liver itself. 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).

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. Other reasons are appearance of various adverse effects of synthetic chemical drugs and universal existence of these plants and their low expenses compared to synthetic drugs (2).

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. Paracetamol (Acetaminophen)

Is one of the most important drugs used for the treatment of mild to moderate pain when an anti-inflammatory effect is not necessary. Phenacetin is a pro-drug that is metabolized to acetaminophen, is more toxic than its active metabolite and has no rational indications. Acetaminophen is administered orally, absorption is related to the rate of gastric emptying, and peak blood concentration are usually reached in 30 - 60 minutes, is slightly bound to plasma proteins and is partially metabolized by hepatic microsomal enzymes and converted to acetaminophen sulfate and glucuronide which are pharmacologically inactive. A minor but highly active metabolite (N-acetyl -P- benzoquinone) is important in large doses because of its toxicity to both liver and kidney.

In therapeutic doses, a mild increase in hepatic enzymes may occasionally occur in the absence of jaundice, this is reversible when the drug is withdrawn. With larger doses, dizziness, excitement, and disorientation are seen. Ingestion of 15gm of acetaminophen may be fatal, death being caused by severe hepatotoxicity with centrilobular necrosis.

Early symptoms of hepatic damage include nausea, vomiting, diarrhea, and abdominal pain. Recent data also implicate acetaminophen in rare cases of renal damage without hepatic damage. This damage has occurred even after usual doses of acetaminophen (3).

N-acetyl- P-benzoquinoneimine is very reactive, with a high affinity for sulfhydryl groups. The amino acid glutathione provides a ready source of available sulfhydryl groups within the hepatocyte. When the liver's glutathione stores are depleted and there are no longer sulfhydryl groups available to detoxify with metabolite, it begins to react directly with the hepatocyte. Replenishing the liver's sulfhydryl capacity through the administration of N-acetyl cysteine early after ingestion of the over dose halts this process (4). N-acetyl cysteine is given in a dose of 140 mg/kg for 1 dose, followed by 70 mg/kg every 4 hours for a total of 17 doses. Damage already done can't be corrected with treatment; however, survivors usually have adequate liver function (5) (6).

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 (7). 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

- To investigate and confirm the hepatoprotective activity of the plant extract against paracetamol-induced hepatic damage.
- To study the fractions of the hepatoprotective plant extract.
- To select the most potent fraction of the plant extract.

2. Material

2.1. Animal

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, paracetamol, Silymarin, Sodium chloride, Chloroform, Methanol, Petroleum ether, Dichloromethane, Ethyl acetate.

3. Methods

3.1. Preparation of drugs

The drug solutions were freshly prepared daily, 1% carboxy methyl cellulose in normal saline and paracetamol dissolved in 1% carboxy methyl cellulose. The drugs were administered by oral route (P.O) or Intraperitoneal (I.P).

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 (8).

3.3.1 Assessment of hepatoprotective activity against paracetamol-induced hepatotoxicity

Hepatotoxicity was induced by paracetamol (1000 mg/kg suspended in 1% carboxy methyl cellulose orally) (9). Hepatotoxin was given orally 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.

3.3.2. Assessment of hepatoprotective activity against paracetamol (2g/kg P.O) -induced hepatotoxicity

Hepatotoxicity was induced by paracetamol (2g/kg suspended in 1% carboxy methyl cellulose orally). The dose of plant extract given once daily for 7 consecutive days, and paracetamol (2g/kg P.O) was administered on 5th day of the extract administration (10). After 48 hours of paracetamol dosing the blood samples were drawn from orbital plexuses under light ether anesthesia. Serum was separated. The serum samples assayed for ALT, AST, ALP, total protein, total bilirubin, albumin and cholesterol. Livers samples were kept in formaldehyde for histopathology.

3.4. Histopathology

The specimens of the liver were collected immediately after slaughter or death of animals and fixed in 10% formalin, embedded in paraffin was sectioned at 5 µm and stained routinely with haematoxylin and eosin (11).

3.5. Estimation of ALT, AST, ALP, total bilirubin, total protein, albumin 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 ± 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 the tested plant revealed presence of flavonoids, tannins, triterpenoids, and saponins. The extract is devoid of coumarins, unsaturated sterols and alkaloid.

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

4.1.1 Effect of petroleum ether and dichloromethane extracts against paracetamol-induced hepatotoxicity

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

4.1.2. Effect of chloroform extract against paracetamol-induced hepatotoxicity

Oral administration of chloroform extract in a dose of 25 and 50 mg/kg against paracetamol-induced hepatotoxicity caused significant (P < 0.05) hepatoprotective activity by decreasing the elevated level of ALT, and significant (P < 0.05) decrease of elevated level of AST activity at a dose of 50 mg/kg, but no significant decrease of AST at a dose of 25 mg/kg (table 1).

4.1.3. Effect of ethyl acetate extract against paracetamol-induced hepatotoxicity

Intraperitoneal administration of ethyl acetate extract in a dose of 25 and 50 mg/kg against paracetamol-induced hepatotoxicity caused significant (P < 0.05) hepatoprotective activity by decreasing the elevated level of ALT and significant (P < 0.05) decrease of elevated level of AST activity at a dose of 25 mg/kg, but no significant decrease of AST at a dose of 50 mg/kg (table 1).

4.1.4. Effect of aqueous methanolic extract against paracetamol-induced hepatotoxicity

Intraperitoneal administration of aqueous methanolic extract in a dose of 50 mg/kg significantly (P < 0.05) protect the liver against toxicity induced by paracetamol as evidenced by decreasing ALT, AST and ALP activity as shown in (table1).

Table 1: Effect of intraperitoneal administration of tested fractions of methanolic extract of the bark of *Khaya senegalensis* and silymarin on liver enzymes after administration of paracetamol.

Treatment	ALT (IU/ml)	AST (IU/ml)	% of inhibition of ALT
Control	65.6 ± 4.84	164 ± 13.98	-
Paracetamol 1g/kg	149 ± 15.02**	372.6 ± 104.6**	-
Silymarin 12.5 mg/kg	76.6 ± 7.3*	141 ± 8.47*	48.5%
Silymarin 25 mg/kg	61.2 ± 20*	156.2 ± 3.2*	58.9%
Petroleum ether 25mg/kg	163.6 ± 28.2	228.2 ± 14.9	-

Petroleum ether 50mg/kg	390 ± 76.1	508.4 ± 124.5	-
Dichloromethane 25mg/kg	284.4 ± 12.3	571 ± 260.6	-
Dichloromethane 50mg/kg	423 ± 167.9	783 ± 244.2	-
Chloroform 25 mg/kg (P.O)	95.8 ± 12.2*	236.8 ± 13.3	35.7%
Chloroform 50 mg/kg (P.O)	69.2 ± 5.3*	195.8 ± 14.5*	53.5%
Ethyl acetate 25mg/kg	98.2 ± 6.7*	169 ± 8.3*	34%
Ethyl acetate 50mg/kg	81.4 ± 5.2*	225.4 ± 8.2	45.36%
Aqueous methanolic 50mg/kg	63.4 ± 2.99*	180 ± 8.97*	57.4%

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

P < 0.05** significant difference from control

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

4.2.1. Effect of petroleum ether and dichloromethane extracts against paracetamol-induced hepatotoxicity

Intraperitoneal administration of petroleum ether and dichloromethane extracts in a dose of 25 and 50 mg/kg against paracetamol-induced hepatotoxicity didn't possess hepatoprotective activity (P > 0.05). It failed to reduce the elevated level of ALT & AST (table 2)

4.2.2. Effect of chloroform extract against paracetamol-induced hepatotoxicity

Oral administration of chloroform extract in a dose of 25 and 50 mg/kg against paracetamol-induced hepatotoxicity caused significant (P < 0.05) hepatoprotective activity by decreasing the elevated level of ALT, and significant (P < 0.05) decrease of elevated level of AST activity at a dose of 50 mg/kg, but no significant decrease of AST at a dose of 25 mg/kg. The percentage of inhibition of ALT was 35.7% and 53.5% respectively as shown in (table 2).

4.2.2.3. Effect of ethyl acetate extract against paracetamol-induced hepatotoxicity:

Intraperitoneal administration of ethyl acetate extract in a dose of 25 and 50 mg/kg against paracetamol-induced hepatotoxicity caused significant (P < 0.05) hepatoprotective activity by decreasing the elevated level of ALT. and significant (P < 0.05) decrease of elevated level of AST activity at a dose of 25 mg/kg, but no significant decrease of AST at a dose of 50 mg/kg. The percentage of inhibition of ALT was 34% and 45.36% respectively as shown in (table 2).

4.2.2.4. Effect of aqueous methanolic extract against paracetamol-induced hepatotoxicity

Intraperitoneal administration of aqueous methanolic extract in a dose of 50 mg/kg significantly (P < 0.05) protect the liver against toxicity induced by paracetamol as evidenced by decreasing ALT and AST activity. The percentage of inhibition of ALT was 57.4% as shown in (table 2).

Table 2: Effect of intraperitoneal and oral administration of methanolic extract of the bark of *Khaya senegalensis* on liver enzymes after administration of paracetamol - induced hepatotoxicity

Treatment	ALT (IU/ml)	AST (IU/ml)	% of inhibition of ALT
Control	65.6 ± 4.84	164 ± 13.98	-
Paracetamol 1g/kg	149 ± 15.02**	372.6 ± 104.6**	-
Silymarin 12.5 mg/kg	76.6 ± 7.3*	141 ± 8.47*	48.5%
Silymarin 25 mg/kg	61.2 ± 20*	156.2 ± 3.2*	58.9%
Petroleum ether 25mg/kg	163.6 ± 28.2	228.2 ± 14.9	-
Petroleum ether 50mg/kg	390 ± 76.1	508.4 ± 124.5	-
Dichloromethane 25mg/kg	284.4 ± 12.3	571 ± 260.6	-
Dichloromethane 50mg/kg	423 ± 167.9	783 ± 244.2	-
Chloroform 25 mg/kg (P.O)	95.8 ± 12.2*	236.8 ± 13.3	35.7%
Chloroform 50 mg/kg (P.O)	69.2 ± 5.3*	195.8 ± 14.5*	53.5%
Ethyl acetate 25mg/kg	98.2 ± 6.7*	169 ± 8.3*	34%
Ethyl acetate 50mg/kg	81.4 ± 5.2*	225.4 ± 8.2	45.36%
Aqueous methanolic 50mg/kg	63.4 ± 2.99*	180 ± 8.97*	57.4%

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

4.3. Effect of intraperitoneal and oral administration of methanolic extract of the bark of *Khaya senegalensis* after paracetamol -induced hepatotoxicity

Effect of methanolic extract at a dose of 50 mg/kg and paracetamol on the activities of ALT, AST, ALP, total bilirubin, total protein, albumin and cholesterol in rats' serum are shown in tables (3, 4 and 5). In rat treated with paracetamol the activities of serum enzymes ALT, AST and cholesterol significantly increased (P < 0.05) when compared with the untreated control at day 7. There were no significant changes in the activities of ALP, total bilirubin, total protein and albumin. However treatment with intraperitoneal and oral administration with methanolic extract resulted in significant (P < 0.05) decrease in the level of ALT, AST and cholesterol at day 7 when compared with rat treated with paracetamol group. Intraperitoneal and oral administration of methanolic extract of the bark of *Khaya senegalensis* alone showed no toxicity throughout the experimental period.

Table 3: Effect of intraperitoneal and oral administration of methanolic extract of the bark of *Khaya senegalensis* on liver enzymes after administration of paracetamol - induced hepatotoxicity

Group	ALT (IU/ml)		AST (IU/ml)		ALP (IU/ml)	
	Day (0)	Day (7)	Day (0)	Day (7)	Day (0)	Day (7)
Control	75.2 ± 5.59	74.7 ± 4.94	161.4 ± 6.3	Control	75.2 ± 5.59	74.7 ± 4.94
Paracetamol 2g/kg	81.2 ± 3.333	459.8 ± 113.3**	195.6 ± 7.6**	Paracetamol 2g/kg	81.2 ± 3.333	459.8 ± 113.3**
Methanolic extract 50 mg/kg (P.O) alone	65.6 ± 4.84	72.0 ± 2.88	164.0 ± 13.98	Methanolic extract 50 mg/kg (P.O) alone	65.6 ± 4.84	72.0 ± 2.88
Methanolic extract 50 mg/kg (I.P) alone	65.8 ± 8.19	59.0 ± 8.4	180.2 ± 12.66	Methanolic extract 50 mg/kg (I.P) alone	65.8 ± 8.19	59.0 ± 8.4
Methanolic extract 50 mg/kg (P.O) + Paracetamol	77 ± 13.89	103.2 ± 16.8*	160.8 ± 30.55*	Methanolic extract 50 mg/kg (P.O) + Paracetamol	77 ± 13.89	103.2 ± 16.8*
Methanolic extract 50 mg/kg (I.P) + Paracetamol	94 ± 3.7	70.4 ± 70.0*	210.8 ± 22.43*	Methanolic extract 50 mg/kg (I.P) + Paracetamol	94 ± 3.7	70.4 ± 70.0*

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

Table 4: Effect of intraperitoneal and oral administration of methanolic extract of the bark of *Khaya senegalensis* on liver function (Total bilirubin, total protein and albumin) after administration of paracetamol - induced hepatotoxicity

Group	Total bilirubin (mg/dl)		Total protein (g/dl)		Albumin (g/dl)	
	Day (0)	Day (7)	Day (0)	Day (7)	Day (0)	Day (7)
Control	0.18 ± 0.02	0.18 ± 0.02	7.32 ± 0.139	7.3 ± 0.149	3.64 ± 0.8	3.62 ± 0.07
Paracetamol 2g/kg	0.16 ± 0.24	0.18 ± 0.00	7.64 ± 0.16	7.94 ± 0.32	3.56 ± 0.12	3.58 ± 0.09
Methanolic extract 50 mg/kg (P.O) alone	0.14 ± 0.24	0.14 ± 0.02	7.48 ± 0.42	7.46 ± 0.14	3.8 ± 0.25	3.68 ± 0.09
Methanolic extract 50 mg/kg (I.P) alone	0.1 ± 0.00	0.24 ± 0.07	6.8 ± 0.21	6.88 ± 0.29	3.46 ± 0.06	3.02 ± 0.251
Methanolic extract 50 mg/kg (P.O) + Paracetamol	0.12 ± 0.02	0.1 ± 0.00	7.52 ± 0.32	7.12 ± 0.64	3.98 ± 0.15	3.24 ± 0.28
Methanolic extract 50 mg/kg (I.P) + Paracetamol	0.24 ± 0.06	0.1 ± 0.00	6.32 ± 0.32	6.26 ± 0.18	2.96 ± 0.14	3.06 ± 0.08

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

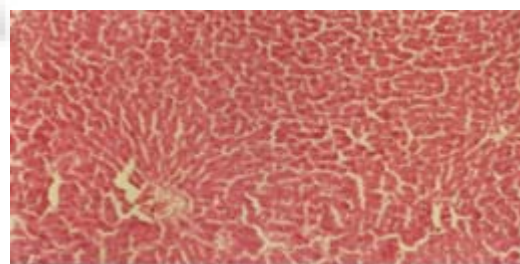
Table 5: Effect of intraperitoneal and oral administration of methanolic extract of the bark of *Khaya senegalensis* on cholesterol after administration of paracetamol - induced hepatotoxicity

Group	Cholesterol (mg/dl)	
	Day (0)	Day (7)
Control	56.4 ± 5.18	56.0 ± 4.8
Paracetamol 2g/kg	43 ± 1.78	78.7 ± 5.47**
Methanolic extract 50 mg/kg (P.O) alone	53 ± 6.14	47.6 ± 3.05
Methanolic extract 50 mg/kg (I.P) alone	46.8 ± 2.15	33.6 ± 1.6
Methanolic extract 50 mg/kg (P.O) + Paracetamol	46.8 ± 3.3	56.2 ± 7.35*
Methanolic extract 50 mg/kg (I.P) + Paracetamol	43.6 ± 3.9	47.6 ± 5.73*

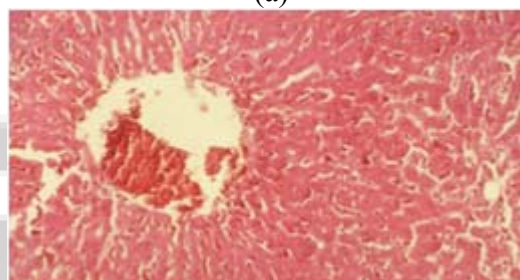
Values were expressed as mean ± SEM (n = 5), P < 0.05* significant difference from paracetamol. P < 0.05** significant difference from control.

4.4 Histopathological Findings

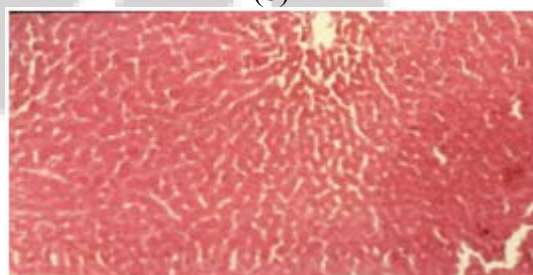
Histopathological sections of the livers of this experiment were shown in figure (1). Histopathological profile of the control animals showed normal hepatocytes. Groups treated with paracetamol exhibited intense centrilobular necrosis, vacuolization and fatty change. The section of liver taken from the animals treated with standard drug silymarin showed normal hepatocytes similar to that of control.



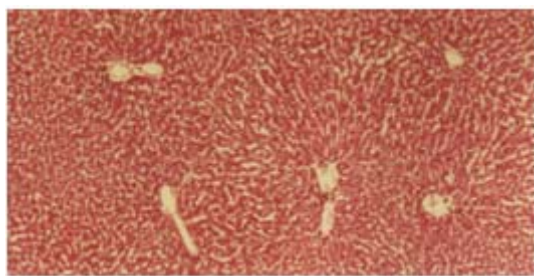
(a)



(b)



(c)



(d)

Figure 1: Liver cells of rats treated with methanolic and chloroform extracts of the bark of *khaya senegalensis* (50mg/kg) and paracetamol (2gm/kg).

(a) Section of control group showing normal cellular architecture with distinct hepatic cell, sinusoidal spaces and central vein. (b) Section of paracetamol group showing vacuolation, congestion, sinusoidal haemorrhages and dilatation. (c) Section of methanolic extract treated rats showing few vacuolation, reduced sinusoidal dilatation and no congestion. (d) Section of chloroform extract treated rats showing few vacuolation, reduced sinusoidal dilatation and no congestion

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 (12).

In the present study silymarin at a dose of 12.5 and 25 mg/kg reduced significantly the increase in serum concentration of ALT, AST, ALP and total bilirubin induced by oral administration of paracetamol. The percentage of inhibition is a dose dependent.

Paracetamol liver injury is not due to the drug itself but to the formation of the toxic metabolite N-acetyl P-benzoquinoneimine generated through the cytochrome P₄₅₀ drug metabolizing system. Normally, hepatic stores of glutathione combine with the toxic metabolite and prevent liver cell injury. When glutathione stores are depleted by over production of this metabolite, however, the reactive metabolite binds to liver cell proteins and causes hepatic necrosis (13). Silymarin has been studied for its protective action against acetaminophen induced toxicity in animal models. (14) *In vitro* studies on rat hepatocyte showed that silymarin treatment normalized the elevated biochemical parameters of liver and serum caused by acetaminophen, by its stabilizing action on plasma membrane.

Also the methanolic extract of the bark of *Khaya senegalensis* showed a significant hepatoprotective activity against paracetamol- induced hepatic damage in rats by reducing the increase level of ALT at the doses 12.5, 25 and 50 mg/kg and AST at the doses 25 and 50 mg/kg. The percentages of inhibition for ALT were variable against paracetamol. Also (15) investigated the hepatoprotective effect of aqueous extracts of stem bark of *Balanites aegyptiaca*, *Khaya senegalensis*, *Prosopis Africana* and *Vitellaria paradox* against paracetamol-induced

hepatotoxicity. Their results indicated that the different plant extracts caused significant inhibition of elevated serum ALT, AST and ALP levels.

In the assessment of liver damage by paracetamol or any other hepatotoxin, the determination of enzyme levels such as ALT and AST is largely used (16). Necrosis or membrane damage releases the enzyme into circulation, therefore, high levels of AST indicate liver damage, such as that due to viral hepatitis, cardiac infarction and muscle injury. Serum ALP level on the other hand, is related to the function of hepatic cell. Increase in serum level of ALP is due to increase synthesis of the enzyme, in presence of increasing biliary pressure (17).

The histology of the liver, also the dosage of serum transaminases, particularly ALT, specific markers of hepatocellular necrosis (18), can be used to measure the activity required. Also methanolic extract of the bark of *Khaya senegalensis* in a dose of 50 mg/kg alone for 7 days after oral and intraperitoneal administration did not show any difference between (I.P) and (P.O) administration and there was no difference between control group and the group that received the plants extract alone. There was significant difference ($P < 0.05$) in decreasing the level of ALT and AST against paracetamol on the 5th day (48 hours) induced hepatotoxicity and the group of rats treated with 50mg/kg (P.O or I.P) plant extract and paracetamol. Cholesterol was also significantly reduced in the serum of the plant extract treatment.

There was no significant change in the activities of marker enzymes, bilirubin level, total protein, albumin and cholesterol in the rats treated with methanolic extract of the bark of *Khaya senegalensis* alone as compared to the control, there by showing the absence of any adverse toxic effects of extracts.

Effect of petroleum ether and dichloromethane extract on paracetamol-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 and by oral administration of paracetamol. 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 oral administration of paracetamol.

The aqueous methanolic extract at a dose of (50 mg/kg) reduced significantly ($P < 0.05$) the increases in serum ALT and AST induced by paracetamol (P.O). The anti-hepatotoxic activity of aqueous methanolic extract against paracetamol (P.O) could either be due to inhibitory effect on microsomal enzymes or lipid peroxidation or free radical scavenging effect. In the present study the activity of chloroform extract was more potent than ethyl acetate extract. The present findings revealed that various biochemical alterations produced by paracetamol were prevented by a dose of 25 and 50 mg/kg of ethyl acetate extract. Similarly (19) 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 (20). Concerning the important hepatoprotective role of the bark of *Khaya senegalensis*, the toxicological effects of this plant in rats were tested. There were no toxicological reports encountered. (21) reported that *M. senegalensis* had no significant toxic effects where as *S. cornatus* *T. indica* and *K. senegalensis* had a stimulatory impact on lymphocyte cells.

Also methanolic extract alone at a dose of 50mg/kg/day (P.O and I.P) and chloroform extract at a dose of 50 mg/kg/day (P.O) a lone for 14 days were tested. The effect was more pronounced with methanolic extract. This is probably due to content of flavonoids. Administration of methanolic and chloroform extract of the bark of *Khaya senegalensis* a lone showed no toxicity on the level of the liver function biochemistry. Cholesterol was also significantly reduced in the serum of rats recieved the plant extract alone. Many phytochemical reports revealed that *Khaya senegalensis* was found to contain tannin, phlobatannin, anthraquinone, cardiac glycoside and saponin. (15). The presence of tannins in the bark of *Khaya senegalensis* may play a role in its hepatoprotective activity. This is supported by (22) who found out that foliage tannins have potent anti-oxidant and anti-inflammatory activities. The possible mechanism of the hepatoprotective action of *Khaya senegalensis* might be least partly due to its antioxidant effect.

The histopathological changes revealed less damage in the groups treated with methanolic and chloroform extracts of the bark of *Khaya senegalensis* than paracetamol groups, which indicated some protective effects as evidenced by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration.

6. Conclusion

It was concluded that, the methanolic extract of the bark of *Khaya senegalensis* possessed potent protection of liver against toxicity caused by paracetamol.

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