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HEPATOPROTECTIVE ACTIVITY OF *Senna Obtusifolia* SEED AQUOUS, CHLOROFORMIC AND ETHANOLIC EXTRACTS AGAINST CCL₄ INDUCED LIVER INJURY IN RATS

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Abstract

Senna Obtusifolia seeds is traditionally used as a laxative, as well as to be beneficial for the hepatitis. The aim of the present study to evaluate the hepatoprotective effect of *Senna Obtusifolia* seeds extract against carbon tetrachloride CCL₄ induced liver damage. Administrated with seed extracts in rats for 28 days significantly reduced the liver enzymes (ALP, ALT and Bilirubin) impact of CCL₄ toxicity on the serum marks of liver damage. In addition treatment of *Senna Obtusifolia* seed extract result in markedly catalase the enzymes liver in rats. Generally, the results suggest that *Senna Obtusifolia* seeds acts as potent hepatoprotective agent against CCL₄ induced hepatotoxicity in rats.

Keywords: *Senna Obtusifolia*, Hepatoprotective, Carbon tetrachloride, liver function test.

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INTRODUCTION

The liver as vital organ in the body is primarily responsible for the metabolism of endogenous and exogenous agent, it plays an important role in drug elimination and detoxification and liver damage may be caused by xenobiotics, alcohol consumption, malnutrition, infections, anaemia and medication [1]. Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent [2] hepatotoxic agent can react with the basic induce almost all types of liver lesion. Toxins and drugs are among the basic ectopathogenetic agent of acute liver failure in western countries [3] nevertheless, chemical toxins are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions [4]. Despite the fact that hepatic problems are responsible for a significant number of liver transplantations and deaths recorded worldwide, available pharmacotherapeutic options for liver diseases are very limited and there is a great demand for the development of new effective drug. A number of studies have shown that the plant extract having antioxidant activity protect against CCl₄ hepatotoxicity by enhancing antioxidant enzyme activity [5]. *Senna Obtusifolia* it grows wild in north, central and south America, Asia, Africa, and Oceania, and is considered a particularly serious weed in many place [6]. The green leaves of the plant are fermented to produce a high-protein food product called "kawal" which is eaten by many people in Sudan as a meat substitute. Its leaves, seeds, and root are also used in folk medicine, primarily in Asia. It is believed to possess a laxative effect, as well as to be beneficial for the eyes. As a folk remedy, the seeds are often roasted, then boiled in water to produce a tea. The plant's seeds are a commercial source of cassia gum, a food additive usually used as a thickener and named for the Chinese Senna's former placement in the genus cassia. Roasted and ground, the seeds have also been used as a substitute for coffee [7]. "Kawal" meat substitute from fermented *Cassia Obtusifolia* leaves [8]. The present study investigates the hepatoprotective potential of *Senna Obtusifolia* seed aqueous extract treatment against CCl₄ induced liver toxicity in rats.

MATERIALS AND METHODS

Chemicals

CCl₄ was obtained and assay kits for alanine aminotransferase (ALT) aspartate aminotransferase (AST) alkaline phosphatase (ALP) total protein and bilirubin level all of them from merck, Germany.

Preparation of the plant and extract

Fresh plant material were collected from different parts in Sudan, the plant materials were collected and dried, crushed using mortar. The dried plant was then be coarse powder 100 gm of plant sample was extracted separately in cold methanol. With sequential maceration solvent from each sample were filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extract.

Photochemical study:

Photochemical constituent

Photochemical examination were carried out for all the extract as per the standard method:

1. Detection of alkaloid:

Extracts were dissolved individually in dilute hydrochloric acid and filtered, filtrates were treated with Mayer reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids [8].

2. Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Filtrates were treated with Benedict reagent and heated gently. Orange red precipitate indicates the presences of reducing sugars [8].

3. Detection of glycosides:

Extracts were hydrolyzed with dill .HCL and then subjected to test for glycosides. Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 mints. The mixture was cooled and extracted with equal volumes benzene. the benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presences of anthranol glycosides [8].

4. Detection of saponins

0.5 gm of extract was shaken with 2ml of water. Foam produced persists for ten min indicates the presences of saponin [8].

5. Detection of phenols

Extract were treated with 3-4 drops of Ferric Chloride solution. Formation of blue or yellow colour indicates the presences of phenols [8].

6. Detection of flavonoids

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presences of flavonoids [8].

7. Detection of proteins and amino acids

The extracts were treated with few drops of conc. Nitric acid Formation of yellow colour indicates the presences of proteins [8].

8. Detection of diterpenes

Extraction was dissolved in water and treat with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presences of diterpenes [8].

Animals

Swiss albino rats weighing between 90 and 100 gm were used in this evaluation. These rats aged between 2 and 2.5 months were procured from animal house located at Khartoum city, Sudan .They were housed in well ventilated stainless-steel cages at room temperature ($24\pm 2^{\circ}\text{C}$) in hygienic condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given.

Test of hepatoprotective activity

The rats were divided randomly into 9 groups of 5 rats each. The hepatoprotective activity of the plant extracts was tested using CCl₄ model. Group I (normal control) received neither the plant extract nor CCl₄ for 72 hours that is they receive only food and water only; Group II (induction control) was given a single intraperitoneal dose (0.24). Group III –VI was subdivided into further two subgroups such as Group 1A, Group 1B and 1C group. Group IIA, Gr. IIB, group , IIC group , received crude (aqueous chloroformic and ethanolic) extracts of plant materials of *Senna Obtusifolia* seed respectively at an intraperitoneal dose of 200mg/kg and 400mg/kg. wt as a fine suspension made by adding normal saline after adding two dose of CCl₄ (0.27) and (0.25) respectively . Subgroups of the same groups received the extract suspension at remaining two groups. IB, IIB group received only tow an intraperitoneal dose of 200mg/kg and 400mg/kg. Wt& a single dose of CCl₄ (0.25) (0.28) respectively and IC, IIC received only tow an intraperitoneal dose of 200mg/kg and 400mg/kg. Wt& a single dose of CCl₄ (0.26) (0.29) respectively, the suspensions of test samples were administered to rats 1hr, 24 hrs and 48 hrs after CCl₄ injection.

Assessment of hepatoprotective activity:

In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 72 hours of drug treatment, the animals were dissected under ether an aesthesia. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labelled centrifuging tubes and allowed to clot for 30min at room temperature. Serum

was separated by centrifugation at 3000 rpm for 15 mins. The separated serum was used for the estimation of some biochemical parameters like Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT), Bilirubin and Total protein.

RESULT AND DISCUSSION

The present study had been attempted to demonstrate the role of hepatoprotective activity of crude aqueous extracts of plant materials of *Senna Obtusifolia* seed, induced hepatotoxicity at different doses of carbon tetrachloride. The results of hepatoprotective activities of crude aqueous extracts of these plants at a dose of 200 mg/kg b.wt and 400mg/kg b.wt. On rats intoxicated with carbon tetrachloride were illustrated in the table 2. The table also showed the comparison of effects among the untreated (normal control) and carbon tetrachloride treated (induction control or standard) group with the drug treated group of rats. The results were represented as Mean \pm 1. The statistical significance was computed using student's 't' test. Carbon tetrachloride group significantly increased the serum level of ALT (73 to 171), AST (229 to 353), Bilirubin (0.4 to 1.025) and ALP (939 to 1439). Total protein (4.74 to 4.85) shown in table 2. The 't' value obtained for exceeded the limit for significance. That is in all cases, 'p' was less than 0.0001. The plant extracts of *Senna Obtusifolia* seed dose of 400 mg/kg b.wt. Showed very significant changes rather than 200mg/kg produced toxicity compared to normal group. That is percent of decrease of Bilirubin, Total protein and ALT after treated with aqua's extract 400mg/kg b. wt. dose *Senna Obtusifolia* seed extract showed highest percent of recovery in Bilirubin level among the four tested extracts. Such results indicate that the activity of *Senna Obtusifolia* extract may be dose specific and more work is needed to know the mechanism of action of its anti-hepatitis effect. One important parameter, Bilirubin level was found significant changes in all cases, it should be noted that in all cases CCl₄ and plant extracts (Table 2). Liver damage induced by CCl₄ is commonly used model for the screening of hepatoprotective the injured liver to normal after 72 hrs at a dose of 400 mg/kg b.wt which indicate that *Senna Obtusifolia* has antihepatotoxic effect. In addition, the possible antihepatotoxic mechanism of *Senna Obtusifolia* have not been reported yet. It is assumed that the effect of *Senna Obtusifolia* extract on liver protection is related to glutathione-mediated detoxification as well as free radical suppressing activity. In conclusion from the overall result of the biochemical and histopathological examinations, it could be inferred that *Senna Obtusifolia* showed the highest hepatoprotective activity. The result

could also be further study on the plants could be extended for the isolation and structure determination of the hepatoprotective principle or principles.

Table 1: Preliminary photochemical investigation of *Senna Obtusifolia*

Phytoconstituent	Plant seed extract		
	Chloroform extract	Ethanol extract	Water extract
alkaloid	+	+	+
Saponin	+	+	+
Flavinoid	+	-	+
Amine	+	+	+
Diterpine	+	-	+
Glycoside	-	-	+
Phenol	-	+	+
Carbohydrate	+	+	+

Key: (+) detected (-) not detected

Table 2: Serum biochemical parameters

Groups	Serum biochemical parameters				
	ALT	AST	ALP	Total protein	Bilirubin
Normal rates	73	229	4939	4.74	0.4
Control (-) rates received does of ccl4	171	353	5439	4.85	1.2
Control (+) treated with silymarin	303	272	4898	5.3	0.85
200mg/kg of aqoues extract	173	261	4030	4.6	0.73
400mg/kg of water extract	181	317	4600	5.4	0.54
200mg/kg of chloroform extract	265	273	4423	4.6	0.72
400mg/kg of chloroform extract	206	321	4662	4.5	1.1
200mg/kg of ethanol extract	260	390	5670	5.5	1.2
400mg/kg of ethanol extract	309	367	5778	4.9	1.00

Key: (ALT)alanine aminotransferase, (AST) aspartate aminotransferase, (ALP) alkaline phospatase.

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