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Antioxidant and Hepatoprotective Effects of Ethanolic Extracts of *Faidherbia albida* Fruits and Stem Bark against Carbon Tetrachloride Induced Liver Damage in Rats

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Abstract

Haraz tree (*Faidherbia albida*) is used in traditional medicine for some disorders such as inflammation, diarrhoea and kidney problems. In this study the ethanolic extracts of *F. albida* fruits and stem bark were evaluated for their antioxidant and hepatoprotective activities. Ethanolic extracts were prepared and the phytoconstituents of the fruit extract was investigated. The antioxidant activities of both extracts were measured using DPPH assay. CCl_4 induced hepatotoxicity was used to evaluate the activities of extracts. Thirty five albino rats were divided randomly into seven groups of five rats each; control group, CCl_4 intoxicated group, hepatoprotective standard drug, *F. albida* low and high doses of fruit extracts groups. *F. albida* stem bark low and high doses of the extract. *F. albida* fruits and stem bark extracts were administered orally at a dose of 250 and 500 mg/kg b.w daily for 5 days. The hepatotoxicity was induced by injection of CCl_4 in olive oil (1:1) at a dose of 0.2 ml/kg b.w interaperitoneally in the 2nd and 3rd day of extracts administration. Liver function was tested by measuring serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total proteins were estimated. Flavonoids, tannins, triterpenoids, saponins, coumarins, alkaloids and sterols were detected in fruit ethanolic extract. *F. albida* fruits extract exhibited high antioxidant activity against DPPH assay compared to stem bark extract. Fruits and stem bark extracts treated groups showed significantly lower ($P< 0.05$) AST, ALT and ALP values than intoxicated group suggesting the protection of hepatic cells against CCl_4 induced liver damage. The results were also compared with the hepatoprotective effect of the standard drug silymarin. Total protein was not affected ($P> 0.05$) by administration of silymarin and plant extracts, the results were comparable to intoxicated group. The results concluded that the ethanolic extracts of *F. albida* fruits and stem bark seems to possess hepatoprotective activity in rats. This effect may be due to antioxidant activity of phytoconstituents of the plant.

Keywords: Hepatoprotective activity, *Faidherbia albida*, antioxidant activity, rats, CCl_4

Introduction

The liver is a key organ of immunity, nutrition, and metabolism. It is subjected to a number of diseases such as liver cirrhosis and acute chronic hepatitis (Elagib *et al.*, 2014; Rajaratnam *et al.*, 2014). The use of herbal products is of a global importance due to their low side effects, accessibility

and affordability when compared with conventional medicine. Plants have the ability to synthesize a wide range of chemical compounds that are used to perform important biological functions, and to protect against attacks from predators such as insects, fungi and herbivorous mammals. These phytochemicals possess many beneficial effects and can be used effectively to treat various human and animal diseases (Lai and Roy, 2004; Erhirhie and Ekene, 2013). Numerous plants have been used successfully as hepatoprotective agents this is due to unsatisfactory activities of conventional drugs that used in the treatment of liver diseases, or as a result of deleterious side effects accompanied with the use of these drugs (Ali *et al.,* 2011). Haraz tree is named *Faidherbia albida* or *Acacia albida* and belongs to a large family of flowering plants *Fabaceae* which is commonly known as legume or bean family (*Leguminosae*) kingdom *Plantae* (Tutu, 2002 and Mokgolodi *et al.,* 2011)**.** The tree was originally a riverine tree of Eastern and Southern Africa which was introduced into West Africa through pastoralism and agriculture. In the Sudan, Haraz tree is distributed through the different vegetation zones from Semi-desert region to the Savannah and mountainous area. Also, the species occur along the River Nile and its tributaries, Strom banks, Valleys and on hilly slopes on the Blue Nile State, South Kordofan, Northern State and Khartoum State (Harrison and Jackson, 1958 and El-Amin, 1990; Bernard, 2002 and Moser, 2006). *F. albida* is used in folkloric medicine as a remedy for chills, bronchitis, pneumonia, cough, diarrhea, haemorrhage, postpartum complications and kidney diseases (Hammiche and Maiza, 2006 and Belayneh *et al.,* 2012). *F. albida* has been found to contain various phytochemicals such as alkaloids, tannins, saponins and terpenoids (Wurochekke *et al.,* 2013). The tree also contributes to soil conservation and soil fertility improvement (Dangasuk *et al.,* 2001). The crude aqueous extract of *F. albida* has potent anti-pyretic, anti-inflammatory and anti-diarrheal effects (Tijani *et al.,* 2008). The antitrypansomal and antimalarial effects of *F. albida* extracts were also reported (Akingbasote, 2009 and Salawu *et al*., 2010).

The present study was aimed to evaluate the antioxidant and hepatoprotective activities of ethanolic extracts of *F. albida* fruits and stem bark against CCl_4 induced liver damage in albino rats.

Materials and Methods

Plant materials: *Faidherbia albida* fruits and stem bark were purchased from Nyala market in Southern Darfour in March 2017. Authentication was done at Medicinal and Aromatic Plants, Traditional Medicine and Research Institute, (MAPTMRI) National Centre of Research, (NCR), Khartoum, Sudan. The plant materials were dried at room temperature, cleaned and powdered.

Extraction: Ethanolic extracts of both parts were prepared according to the method described by Harborne (1984). Approximately a weight of 200 g of each plant materials were extracted by soxhlet apparatus at a temperature (40 – 45 °C) using ethanol 70%. The filtrates were then collected and evaporated using rotavaper.

Phytochemical screening: General phytochemical screening of the fruits of *F.albida* was carried out to investigate the active constituents of the extracts (Harborne, 1984).

Antioxidant activity: The DPPH radical scavenging assay was performed according to the method of Shimada *et al.* (1992) with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl

stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as 300µM. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, the decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. The percentage of radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate**.**

Experimental animals: Wistar albino rats (100-166g) were obtained from Nile Pharma Company, Cairo, Egypt. They were kept in cages and housed in standard environmental conditions, controlled temperature (22±2°C) and relative humidity (60%) with free access to water and standard laboratory food. The rats were housed for one week before the start of the treatments for adaptation.

Experimental design*:* Thirty five Wistar albino rats were divided into 7 groups of 5 rats each. Group 1: normal control, rats were given distilled water only orally. Group 2: CCl_4 intoxicated group, rats were administrated CCl_4 at a dose of 0.2 ml /kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2nd and 3rd day of extract administration. Group 3: Served as hepatoprotective standard drug; animals were given CCl_4 at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2nd and 3rd day of extract administration and at the same time were single dosed daily with silymarin used as a hepatoprotective standard drug at a dose of 100 mg/kg orally for 5days. Group 4: Served as of *F. albida* low dose extract; rats were injected CCl_4 at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2nd and 3rd day of extract administration with daily administration of *F. albida* fruits ethanolic extract at a single dose of 250 mg/kg b.w orally for 5 days. Group 5: *F. albida* high dose extract; animals were injected CCl_4 at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2nd and 3rd day of extract administration with simultaneous daily administration of *F. albida* fruits ethanolic extract at a single dose of 500mg/kg b.w orally for 5 days. Group 6: Served as *F. albida* low dose extract; rats were injected CCl_4 at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2nd and 3rd day of extract administration with concurrent administration of *F. albida* stem bark ethanolic extract at a single dose of 250 mg/kg b.w orally for 5 days. Group 7: Served as *F. albida* high dose extract; rats were injected CCl_4 at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2nd and 3rd day of extract administration with simultaneous administration of *F. albida* stem bark ethanolic extract at a single dose of 500mg/kg b.w orally for 5 days.

Sampling: Blood samples were collected in clean tubes after sacrificed the rats under anaesthesia at the end of experiment. Blood was centrifuged after clotting for 10 minutes at 2500rpm. Serum was separated and stored at - 20˚C for biochemical analysis.

Biochemical analysis: *Spectrophotometric method* using standard kits (Stanbio laboratory Inc., San Antonio, TX, USA) was used. The activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were measured according to the method of King (1965) and Bergermeyer *et al.* (1986). The total protein was also estimated according to the method of Henry *et al.* (1957).

Statistical analysis: The data were analyzed using SPSS (Statistical Package for Social Sciences) version 20. The results were expressed as mean ± standard error. The analysis was performed using (analysis of

variance) ANOVA. Values with P <0.05 were considered to be statistically significant (Mendenhall, 1971).

Results

Phytochemical screening of *F. albida* fruits extract:

Phytochemical screening of *F. albida* fruits ethanolic extract revealed the presence of flavonoids, tannins, triterpenoids, saponins, coumarins, alkaloid and sterols as active constituents.

Antioxidant activity of ethanolic extracts of *F. albida* fruits and stem bark:

The ethanolic extracts of *F. albida* fruits displayed potent antioxidant activity (87±0.04%) when tested using DPPH radical scavenging assay. The fruits showed antioxidant activity comparable to that seen by propyl gallate used as a standard antioxidant agent (91±0.01%). However, the stem bark of *F. albida* exhibited low antioxidant activity (17±0.06%) compared with *F. albida* fruits and propyl gallate.

Biochemical profile of liver of *F. albida* fruits and stem bark ethanolic extracts against CCl_4 induced liver damage in rats:

Rats intoxicated with CCl_4 (group 2) at a dose of 0.2 ml /kg b.w dissolved in olive oil (1:1) showed a significant (P< 0.05) increase in the levels of serum AST, ALT and ALP, indicating the occurrence of severe hepatocellular damages as compared to normal animals. Rats treated with silymarin standard hepatoprotective drug displayed significant hepatoprotective activity. This was proved by a significant decrease of the serum ALP, ALT and AST compared to intoxicated group. These results confirm the hepatoprotective effect of silymarin. Animals treated with *F. albida* fruits and stem bark ethanolic extracts at a dose of 250 and 500 mg/ kg b.w were significantly decreased (P > 0.05) the levels of ALP, ALT and AST suggesting the protection of hepatic cells against CCl_4 induced liver damage when compared to rats given CCl_4 only. There was significant difference (P < 0.05) between total protein levels in control rats and other test groups. There were no significant differences (P > 0.05) between total protein levels in CCl_4 group, silymarin and *F. albida* fruits and stem bark ethanolic extracts at a dose of 250 and 500 mg/ kg b.w. The effect of *F. albida* fruits and stem bark extracts on liver enzymes and total proteins against CCl_4 induced liver damage in rats are presented in table (1).

Table (1): The Effect of *F. albida* fruits and stem bark extracts on liver enzymes and total proteins against CCl_4 induced liver damage in rats

Groups	TP (g/dl)	ALP (U/I)	ALT (U/I)	AST (U/I)
Group 1	7.46 ± 0.29^{a}	82.60 ± 1.66^{e}	5.60 ± 1.60^{b}	110.00 ± 0.00^{b}
Group 2	6.68 ± 0.15^{b}	191.00 ± 2.79^{a}	72.33 ± 21.67^{a}	245.00 ± 44.11^{a}
Group 3	6.36 ± 0.32^{b}	150.00 ± 2.34^{b}	22.20 ± 7.28^{b}	84.50 ± 32.05^{b}
Group 4	6.50 ± 0.12^{b}	105.80 ± 3.95^{d}	22.80 ± 6.34^{b}	136.25 ± 3.75^{b}
Group 5	6.40 ± 0.14^{b}	198.20 ± 3.58^{a}	10.00 ± 2.95^{b}	102.00 ± 19.42^{b}
Group 6	6.26 ± 0.20^{b}	118.40 ± 2.27^{c}	13.50 ± 2.18^{b}	126.25 ± 5.15^{b}
Group 7	5.97 ± 0.19^{b}	79.17 ± 3.38^{e}	10.80 ± 2.92^{b}	102.40 ± 21.00^{b}

a,b: means within the same column followed by different superscripts are significantly (p< 0.05) different. Values are expressed as mean ± standard error, n = 5 rats in each group.

Discussion

The present study was conducted to evaluate the antioxidant and hepatoprotective activities of *F. albida* fruits and stem bark ethanolic extracts on liver injury induced by CCl_4 in albino rats. This plant is used traditionally in the treatment of various disorders such as pneumonia, cough,

diarrhoea, haemorrhage, postpartum complications and kidney diseases (Hammiche and Maiza, 2006 and Belayneh *et al.*, 2012). The CCl_4 is well known as a potent hepatotoxin producing centrilobular necrosis and fatty changes due to increase in oxidative stress which lead to liver injury. The hepatotoxic effects of CCl_4 are largely due to a highly reactive free radicals produced by cytochrome P_{450} mixed function oxidase system (Gupta *et al.*, 2004; Ali *et al*., 2009 and Bukhsh *et al.*, 2014). The oxidative stress caused by free radicals may induce peroxidation and damage to biomolecules such as lipid protein and nucleic acids, which may further leads to aging, cancer and many other diseases in the body (Abuelgasim *et al*., 2008; Ali *et al*., 2011). The toxicity of CCl_4 is also characterized by significant increase in the levels of hepatic enzymes such as AST, ALT, and ALP due to leakage of these enzymes in the blood which is attributed to the damaged structural integrity of the liver, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (Manjunatha *et al.*, 2008; Ali *et al.*, 2010 and Bukhsh *et al.*, 2014).

In this study the ethanolic extracts of *F. albida* fruits and stem bark at dose of 250 and 500 mg/kg b.w exhibited significant decrease in the levels of serum enzyme ALP, ALT and AST compared to the CCl_4 intoxicated rats. This activity could be attributed to the hepatoprotective action of the plant against CCl_4 hepatotoxicity. The extracts of *F. albida* fruits and stem bark showed comparable activities to that observed by standard drug silymarin. The levels of total proteins in all treated groups including CCl_4 were found to be lower than normal control. However, these levels seem to be lower than that observed by normal control. The antioxidant effect of the ethanolic extract of *F. albida* fruits and stem bark was also performed. The ethanolic extract of *F. albida* fruits exhibited high antioxidant activity compared to that seen by stem bark.

The presence of flavonoids, triterpens, tannins and coumarins in *F.albida* fruits may explain its role in hepatoprotection due to antioxidant properties or inhibition of the free radicals mediated damage (Gupta *et al.*, 2004; Manjunatha *et al.*, 2008; Abuelgasim *et al*., 2008; Ali *et al*., 2009 and Ali *et al*., 2011). The hepatoprotective activities of certain flavanoids, tannins and coumarins are known (Abuelgasim *et al*., 2008). The hepatoprotective activity of the plant may be due to antioxidant activity which act as scavengers and remove the free radicals formed, these antioxidants also have the ability to prevent the process of peroxidation and improve the health of hepatocytes (Bukhsh *et al.*, 2014).

From these finding, it can be concluded that the ethanolic extract of *F. albida* fruits and stem bark may possess hepatoprotective activity in rats. This may be due to phytoconstituents or antioxidant activity of the plant. Further studies should be done to confirm the hepatoprotective activity of *F. albida* and to determine the exact phytoconstituent(s) responsible for the hepatoprotective effect of *F. albida* fruits and stem bark.

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الاثار المضاد للأكسده والواقية للكبد للمستخلصات الإيثانولية لثمار وجذع الحراز من تلف الكبد المستحث بواسطة رابع كلوريد الكربون في الجرذان

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المستخلص

نبات الحراز (فيدهيربيا ألبيدا) يستخدم في الطب الشعبي لعلاج بعض الأمراض منها الإلتهابات ،الاسهال و مشاكل الكلى. في هذه الدراسة تم تقييم الأثر المضاد للأكسده والواقي للكبد للمستخلص الإيثيلي لثمار ولحاء الجذع للحراز. تم تحضير المستخلصات الايثلية والتقصى عن مكونات النبات الكيميائية. تم قياس النشاطات المضادة للاكسدة للمستخلصين بواسطة فحص DPPH. تم استخدام ماده رابع كلوريد الكربون المسببه للسميه الكبدية لتقييم نشاط المستخلصات. تم تقسيم 35 جرذ عشوائيا الى 7 مجموعات وحوت كل منها على 5 جرذان (المجموعة الضابطة، المجموعة المسممة برابع كلوريد الكربون , مجموعة العقار القياسى الواقى للكبد، مجموعتى مستخلص ثمار الحراز ذو التركيز المنخفض والمرتفع ومجموعتى مستخلص لحاء جذع الحراز ذو التركيز المنخفض والمرتفع. تم اعطاء مستخلص الثمار ولحاء الجذع بالفم بجرعة 250 و 500 مل/كجم يوميا لمده 5 أيام. تم احداث السميه للكبد بحقن ماده رابع كلوريد الكربون المحلولة بزيت الزيتون بنسبه 1:1 بجرعة 0.2 مل/كج داخل التجويف البريتوني للجرذان في اليوم الثاني والثالث من اعطاء المستخلصات. وقد تم اختبار وظيفة الكبد في المصل مثل AST, ALT, ALP والبروتين الكلى. و تم الكشف عن الفلافونويد ، التانينات ، التريتربينويدس ، السابونين ، الكومارين ، القلويدات و الستيروييد في مستخلص ثمارالايثانول. أظهر المستخلص الايثانولى لثمار الحراز نشاطًا مرتفعا مضادًا للأكسدة عن طريق فحص DPPH مقارنة بمستخلص لحاء الجذع. أظهرت المجموعات المعالجة بمستخلصات الثمرة ولحاء الجزع انخفاضا ($P< 0.05$) معنويا فى مستويات AST و ALT و ALP مقارنة بالمجموعة المسممة والتي تشير إلى حماية الخلايا الكبدية ضد تلف الكبد المحدث برابع كلوريد الكربون. وتمت مقارنة النتائج أيضا مع تأثيرالعقار القياسى الواقى للكبد سليمارين. مستوى البروتين الكلى لم يتأثر ($P> 0.05$) باعطاء السليمارين و مستخلصات النبات وهى شبيهة بنتائج المجموعة المسممة. وخلصت النتائج إلى أن المستخلصات الايثانولية لثمار الحراز ولحاء الجذع يبدو أنها تمتلك نشاطا واقيا للكبد في الجرذان. قد يكون هذا التأثير بسبب النشاط المضاد للأكسدة للمركبات النباتية.