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

A thesis Submitted in Fulfillment of The Requirements of Master Degree in
Pharmacology

By

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الآية

قال تعالى:

وَلَقَدْ أَلَّهُمَا دَاوُدٌ وَسَلَامُ مَنْ عَلَمَهُ وَقَالَا أَلْحَمَّدُ لَهُ الَّذِي فَضَّلَنَا عَلَى كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

صدق الله العظيم

(النمل الآية: ٦٠)
Dedication

I dedicate this work to Almighty Allah my creator, my strong pillar, my source of inspiration, knowledge and understanding.

I also dedicate this work to my parents, brothers, friends and my teachers.

Tamadur
First of all, thanks and praise to Almighty Allah, for giving me health and strength to accomplish this work.

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## INTRODUCTION

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List of Abbreviations

ALP - alkaline phosphatase
AST - aspartate transaminase
ALT - alanine transaminase
CCl₄ – carbon tetrachloride
DEMSO – dimethyl sulfoxide
DPPH - 1,1- diphenyl-2- picryl-hydrazyl
RNS – reactive nitrogen species
ROS - reactive oxygen species
SPSS - statistical package for social sciences
Abstract:

Haraz tree (*Faidherbia albida*) is used in traditional medicine to treat some disorders such as inflammation, skin infections and kidney problems. In this study the ethanolic extracts *F. albida* fruits and stem bark were evaluated for their antioxidant and hepatoprotective activities. Ethanolic extracts were prepared and the phytoconstituents of the fruits and stem bark extracts were investigated. The antioxidant activities of both extracts were measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Carbon tetrachloride (*CCl₄*) 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, *CCl₄* 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 *CCl₄* 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, whereas, the result of stem bark revealed the presence of flavonoids, saponins and alkaloids only. *F. albida* fruits extract exhibited high antioxidant activity against DPPH assay compared to stem bark extract. Fruit and stem bark extracts treated groups showed significantly lower (*P< 0.05*) AST, ALT and ALP values than the intoxicated group, suggesting the protection of hepatic cells against *CCl₄* 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 or the phytoconstituents of the plant.
ملخص الدراسة

نبات الحزاز (فيهيريا أليبيدا) يستخدم في الطب الشعبي لعلاج بعض الأمراض منها الالتهابات، اصابات الجلد ومشاكل الكلى. في هذه الدراسة تم قياس الأثر المضاد للأكسدة والواقعي للكلد للمستخلص الإيثيلي لثمان لحاء الجذع للحراز. تم تحضير المستخلصات الإيثيلية والتقصي عن مكونات مستخلصات الثمار ولحاء الجذع الكيميائية. تم قياس النشاطات المضادة للأكسدة للمستخلصين بواسطة فحص DPPH. استخدم رابع كلوريد الكربون المسبوب للسماة الكبدية في الجرذان لتقييم نشاط المستخلصات. تم تقسيم 35 من الجرذان عشوائيا إلى 7 مجموعات وحوت كل منها على 5 جرذان (المجموعة الضابطة، المجموعة المسمعة برابع كلوريد الكربون، مجموعة العقار القياسي الواقي للكلد، مجموعتي مستخلص ثمار الحزاز ذو التركيز المنخفض والمرتفع ومجموعتي مستخلص لحاء جذع الحزاز ذو التركيز المنخفض والمرتفع. تم إعطاء مستخلص الثمار ولحاء الجذع بالفم بجرعة 250 و 500 ملجم/كم جم يوميا لمدة 5 أيام. احدث السمبه للكلد بحقن ماده رابع كلوريد الكربون وحلها بزيت الزيتون بنسبة 1:1 بجرعة 0.2 مل/كج داخل التجويف البريتوني في اليوم الثاني والثالث من اعطاء المستخلصات. وقد اختبر وظيفة الكبد في المصل مثل AST, ALT, ALP والبروتينات الكلي. و تم الكشف عن الفلافونويد، التتريربينودس، السادس، الكومارين، القلويديات و الستيرويد في مستخلص ثمار الإيثانول، بينما أظهرت نتيجة لحاء الساق عن وجود مركبات الفلافونويد والصابونين والكربونات فقط. أظهر المستخلص الإيثانولي لثمار الحزاز نشاطا مرتفعا مضادا للأكسدة ضد فحص

مقارنة بمستخلص لحاء الجذع. أظهرت المجموعات المعالجة بمستخلصات الثمرة ولحاء الجذع انخفاضا (P<0.05) معنوية في مستويات ALP و ALT و AST والتي تشير إلى حماية الخلايا الكبدية ضد تلف الكبد المحدث برابع كلوريد الكربون. وتمت مقارنة النتائج أيضا مع تأثير العقار القياسي الواقي للكلد سليمان. مستوى البروتين الكلي لم يتأثر (P>0.05) بأعطاء السليمان و مستخلصات النبات وهي شبيهة بنتائج المجموعة المسمعة. وخلصت النتائج إلى أن المستخلصات الإيثانولية لثمار الحزاز ولحاء الجذع يبدو أنها تمتلك نشاطا واقعا للكبد في الجرذان. قد يكون هذا التأثير بسبب النشاط المضاد للأكسدة أو المركبات النباتية.
INTRODUCTION

The use of herbal products is of a global importance due to their low side effects, accessibility and affordability when compared with conventional drugs (Erhirhie and Ekene, 2013). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans and can be used to effectively treat human diseases (Lai and Roy, 2004). The world health organization estimates that 4 billion people, 80% of the world population, presently use herbal medicines for some aspect of their primary health care (Akerele, 1992).

Recently there is a tremendous increase in the research on medicinal plants as an alternative source of medicines to treat various liver diseases (Osman et al., 2013). Numerous plants have been used successfully as hepatoprotective agents this is due to insufficient activities of conventional drugs that are used in the treatment of liver problems, or as a result of deleterious side effects accompanied with the use of these drugs (Ali et al., 2011).

In the Sudan many scientific studies have been performed to evaluate the protective effect of some Sudanese medicinal plants such as Capparis decidua, Khaya senegalensis, Lepidium sativum and Solanum nigrum (Abuelgasim et al., 2008; Ali et al., 2009; Elhag et al., 2011 and Ali et al., 2011). The results indicated promising activities of these plants as hepatoprotective agents.
Hence the present study was conducted due to the failure activities of conventional drugs that are used to treat liver diseases as well as the significant adverse effects associated with the use of these drugs.

**Objectives**

a) To evaluate the hepatoprotective activity of *F. albida* fruits and stem bark ethanolic extracts against CCl₄ induced hepatotoxicity in rats.

b) To investigate the *invitro* antioxidant activity of *F. albida* fruits and stem bark ethanolic extracts using DPPH assay.
CHAPTER ONE

1. LITERATURE REVIEW

1.1 Importance of the Liver
The liver; the largest internal organ in the body, is the principal organ of immunity, nutrition, metabolism, glycogen storage, plasma protein synthesis, detoxification and production and secretion bile into intestinal lumen (Elagib et al., 2014 and Rajaratnam et al., 2014).

Liver diseases can be inherited such as haemochromatosis or caused by a variety of factors that damage the liver such as viruses, bacteria, parasites and toxins. It is subjected to a number of diseases such as liver cirrhosis, hepatitis (caused by various viruses A, B, C, some poisons and autoimmune hepatitis), haemochromatosis (a hereditary disease causing the accumulation of iron in the body), Wilson’s disease (a hereditary disease which causes the body retain copper), Liver cancer and Glycogen storage type11. Jaundice is a common sign of liver diseases, It is defined as a yellowing of skin, mucous membranes and sclera due to the high bilirubin level in the body. On the basis of causes Jaundice can be classified into three types: Pre-hepatic jaundice, hepatic jaundice and post hepatic jaundice (Radostitis et al., 2007 and Mohit et al.,2011).

1.2 Standard Drug Silymarin
Silymarin is one of the herbal medicines that have been extensively studied, both clinically and chemically, for the treatment of major liver diseases. The active ingredients of the plant are obtained from the dried seeds of Silybum marianum. The plant containing four flavonolignan isomeric components (silybin, isosilybin, silychristin, and silydianin), Silybin, which is the most active compound of Silymarin, is the major contributor of the hepatoprotective-ness of the medicine. Silymarin is taken orally and is mainly excreted through bile as conjugates. It has been claimed that silymarin has clinical applications in the treatment of toxic
hepatitis, fatty liver, cirrhosis, ischaemic injury, radiation toxicity and viral hepatitis as a result of its anti-oxidative, anti-lipid-peroxidative, antifibrotic, anti-inflammatory, immunomodulating, and even liver regenerating effects, it promotes protein synthesis, helps in regenerating liver tissue, controls inflammation, enhances glucuronidation and protects against glutathione depletion (Rajaratnam et al., 2014 and Elagib et al., 2014).

1.3 Oxidative stress

Oxidative stress is used to describe the steady state level of oxidative damage in a cell, tissue, or organ caused by excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Free radicals and other “reactive oxygen species” are formed by a variety of normal processes within the body (including respiration and immune and inflammatory response) as well as by elements outside the body, such as air pollutants, sunlight, and radiation. ROS include free radicals such as superoxide (\(\bullet \text{O}_2^-\)), hydroxyl (\(\bullet \text{OH}\)), peroxyl (\(\bullet \text{RO}_2\)), hydroperoxyl (\(\bullet \text{HRO}_2^-\)) as well as non-radical species such as hydrogen peroxide (\(\text{H}_2\text{O}_2\)) and hydrochlorous acid (\(\text{HOCl}\)). RNS include free radicals like nitric oxide (\(\bullet \text{NO}\)) and nitrogen dioxide (\(\bullet \text{NO}_2^-\)), as well as non-radicals such as peroxynitrite (\(\text{ONOO}^-\)), nitrous oxide (\(\text{HNO}_2\)) and alkyl peroxynitrates (RONOO) (Turko et al., 2001; Evans et al., 2002 and Maritim et al., 2003).

1.4 Oxidation and Antioxidant

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death. When the chain reaction occurs in a purified monomer, it produces a polymer resin, such as
a plastic, a synthetic fiber, or an oil paint film. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Oxidation reactions are crucial for life, and can also be damaging to a variety of cells. Plants and animals contain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill cells. Antioxidants are widely used as ingredients in dietary supplements and have been investigated for the prevention of different diseases such as cancer, coronary heart disease, liver and kidney diseases. Antioxidants may promote health in medicine and these compounds have many industrial uses, such as preservatives in food, cosmetics and preventing the degradation of rubber and gasoline (Sies, 1997; Bjelakovic et al., 2007; Linster et al., 2007 and Lawal, 2012).

1.5 Hepatoprotective Plants

Numerous plants have been claimed to posses potential antioxidant and hepatoprotective activities such as *Epaltes divaricata, Mimosa pudica*, *Calotropis procera, Carthamus oxyacantha, Xylopia aethiopica* and *Pseudocedrela kotschyi* (Hewawasam et al., 2004; Rajendran et al., 2009; Jain et al., 2013; Bukhsh et al., 2014; Adewale et al., 2014 and Nchouwet et al., 2017). These plants were evaluated as antioxidant and hepatoprotective agents in experimental animals using different experimental models such as CCl₄ and paracetamol to induced hepatotoxicity.

Several Sudanese medicinal plants were used successfully in traditional medicine in the Sudan to treat liver diseases such as *Capparis deciduas* and *Solanum nigrum* (Ali et al., 2009 and Elhag et al., 2011). Many Sudanese plants were evaluated experimentally to support their protective effect.
against various hepatotoxic chemicals such as CCl₄ and paracetamol in experimental animals. Results indicated that *Capparis deciduas* and *Solanum nigrum* aqueous and methanolic extracts have potential hepatoprotective activities against CCl₄ induced liver damage (Ali *et al.*, 2009; Elhag *et al.*, 2011 and Ali *et al.*, 2011). The aqueous extracts produce potent hepatoprotective effect than the methanolic extracts in reducing ALT, ALP, AST levels and bilirubin concentration. The methanolic extracts of *Lepidium sativum* seeds and *Khaya senegalensis* bark were also evaluated by Abuelgasim *et al.* (2008) and Elagib *et al.* (2014). They reported that, the methanolic extracts protect rat liver from the harmful effect of CCl₄ as evident by the significant decrease in the levels of serum enzyme ALT, ALP and AST liver enzymes compared to CCl₄ intoxicated group. Bilal *et al.* (2016) studied the hepatoprotective activity of ethanolic and ethyl acetate extracts of *Sterculia setigera* stem bark, the extracts protect the liver against CCl₄ from damage in rats.

**1.6 Phytoconstituents as Hepatoprotective agents**

Plants contain a variety of active principle compounds called phytochemicals or phytoconstituents and these are, flavonoids, triterpens, alkaloid, saponin, cyanogenic glycosides, tannin and coumarins (Abuelgasim *et al.*, 2008; Ali *et al.*, 2009; Elagib *et al.*, 2014 and Nchouwet *et al.*, 2017). Many phytochemicals have been reported to have significant hepatoprotective and antioxidant effects such as flavonoids, tannins, carotinoids and steroid (Nchouwet *et al.*, 2017).

**1.7 Models Used to Evaluate Hepatoprotective Plants**

Several hepatotoxic agents to induce hepatotoxicity are used as an experimental models, such as carbon tetrachloride (CCl₄), galactosamine, thioacetamide, alcohol and paracetamol. CCl₄ is widely used in the biological research, to evaluate hepatoprotective activity of new plants. In the body, CCl₄ produces trichloromethyl free radicals, which react...
react with other molecules in the cell and stimulate a series of reactions. These reactions lead to initiate the peroxidation of membrane lipids and hence liver damage (Mohit et al., 2011 and Bukhsh et al., 2014).

1.8 Plant Used in This Study Haraz Tree

1.8.1 Classification of Haraz Tree

Kingdom Plantae
Class Magnoliopsida
Order Fabales
Family Fabaceae
Sub-family Mimosoideae
Genus Acacia/ Faidherbia
Species *Faidherbia albida* Lawal (2012). (Fig 2. 3 and 4).

1.8.2 Local Names

The tree has different names in the different languages and countries. In Arabic it is known as "Haraz", while in English it is known as Apple ring Acacia, but in French it is known as Arbre blane (Tutu, 2002; Moser, 2006 and Gibreel, 2008).

1.8.3 Description

In Sudan, the tree is a large thorny tree (4 - 30 meter high) with one main stem or sometimes it is shrub–like buttressed. The crown is ranging between rounded to irregularly spreading branches in the open areas. The trunk usually single with diameters often up to 2 m. The bark is dark brown or dull grey, rough, deeply and scaly in mature trees, smooth in young trees and fissured when old. The young 4 branches are distinctive white in zigzag pattern, while the branchlets are light grey, spiny only at nodes, spines, straight and brown in colour with white base. The leaves are shed at start of rainy season and bipinnate, (2 - 12 pairs of pinnae) with a single conspicuous gland on the rachis, gland on the rachis to oval, yellow or reddish – brown to black, while the leaflets are grey - green, oblong (up to
1 cm long), hairy and unequal at base (Tutu, 2002; Hyde and Wursten, 2010 and Oluwakanyinsola et al., 2010).

Fruit is an unusual pod, bright orange to reddish-brown, thick, indehiscent, characteristically and conspicuously curled and twisted; large, up to 25 × 5 cm. Each pod contain 10-29 dark brown, ovoid, with shiny seeds, each measuring 10 × 6.0 mm and separated by thin septum. The seed coat is tough, leathery and water proof. The wood is light sapwood streaky grey white while, the heartwood is yellow (Tutu, 2002 and Barnes and Fagg, 2003).

1.8.4 Distribution

Haraz tree (F. albida) is originated in the Sahara prior to its desertification. The tree was originally a riverine tree of eastern and southern Africa which was introduced into West Africa through pastoralism and agriculture (Bernard, 2002 and Moser, 2006). F. albida is widely spread in semi-arid tropical Africa into the Middle East and Arabia, from 270 m below sea level in Palestine up to 2500 m above sea level in Jebel Marra, Sudan (Joker, 2000). 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).
Figure 1: Geographical distribution of *F. albida* (Boffa, 1999)
1.8.5 Phenology
Haraz tree is an unusual tree as it sheds leaves at the start of the rainy season, while, the flowering of individual tree is often not uniform (Tutu, 2002). The mature *F. albida* usually spread its branches and a rough, dark brown or greenish-grey bark that is often light grey and smooth when young (Oluwakanyinsola *et al.*, 2010). In contrast to all other native "acacias", *F. albida* has a peculiar inverse phenology, an unusual habit of retaining its leaves during the dry season and dropping them during the rains (Tijani *et al.*, 2008 and Hyde and Wursten, 2010). According to Hyde and Wursten (2010), the flowering period of *F. albida* is between May and September, In Sudan the flowering occurs from November to January and fruiting from December to April. However, not all *F. albida* trees flower every year but in certain areas the flowering may occur twice a year (Joker, 2000).

1.8.6 Phytochemical Constituents of Haraz Tree
*F. albida* contain different chemical constituents in its different botanical parts and that is called secondary metabolites such as alkaloids, tannin, saponins, glycosides and terpenoids (Wurochekke *et al.*, 2013 and Kashimawo *et al.*, 2017).

1.8.7 Nutritional Value of Haraz Tree
According to Lawal and Kabiru (2007), the dry matter, ash, crude protein, crude lipid, crude fiber and available carbohydrate in Haraz fruits were found to be 93.3 %, 6.7 %, 19.5 %, 3.3 %, 13.3 % and 50.5 %, respectively on dry basis.
Figure 2: Haraz tree (from Zalingi).
Figure 3: *F. albida* Fruits
Figure 4: *F. albida* Stem bark
1.8.8 Utilization of Haraz Tree

The seeds of *F. albida* are reported to be eaten as famine food by humans in Ghana, Nambia, Zambia and Zimbabwe (Palmer and Pitman, 2002 and Pardy, 2004). The seeds can be also pounded and baked into cakes or mixed with maize meal. Furthermore, in the dry season, people eat the seeds and pods (cooked or raw). The pods may be also used as flavouring agent or as condiment (Marunda, 2002 and Maundu and Botengnas, 2005). In addition the branches of Haraz tree (*F. albida*) are usually as fodder (Bernard, 2002). In the arid and semi-arid regions of sub-Saharan West Africa, for instance, seasonal variations in the availability and quality of pastures affect livestock production (Castillo-Caamal et al., 2003). Therefore, Haraz tree is found as a valuable alternative and provides free nutritious fodder particularly during dry periods (Gassama-Dia et al., 2003). Other uses of *F. albida* also, is maintained and protected on farms to shade coffee trees and to provide shade for livestock in the dry season (Maundu and Botengnas, 2005). Haraz tree is considerable as a useful ornamental tree for gardens and avenues. It uses as boundary, barrier, support and lopping of branches is common in many areas lopped for fencing compounds and livestock (Maundu and Botengnas, 2005). Haraz gum that spontaneously exudes from the trunk is sometimes collected like gum arabic, but it does not have the same properties. The timber, although straight grained, dense and weighty, is soft and fibrous. It is used for building animal enclosures, huts and dug-out canoes, as well as for making many household objects and tools. In Nigeria, the bark is pounded and used as a packing material for goods carried on pack animals (Bernard, 2002).

1.8.9 Traditional Uses

*F. albida* is used traditionally to treatment diarrhea, leprosy, pneumonia and cough (Kashimawo et al., 2017). Also, it is used as a treatment for dysentery, inflamed eyes, skin infections, hemorrhage, rheumatism, and
vomiting (Bernard, 2002; Moser, 2006; Kubmarawa et al., 2007 and Tijani et al., 2009). Extracts of the bark, gum and the roots are used as a gum wash to stop bleeding. The leaves are used as an astringent for teeth and may contain fluorine. Medicinal uses specifically for the gum are given as an emollient for inflammation, haemorrhage, diarrhoea and ophthalmia (Wickens et al., 2009). The powdered pods and seeds are widely used to stupefy or poison fish in pools (Timberlake et al., 1999).

1.8.10 Pharmacological Activities of *Faidherbia albida*

The crude aqueous extract of *F. albida* was evaluated by Tijani et al. (2008) for it is anti-pyretic, anti-inflammatory and anti-diarrheal effects. The extract showed significant activity in rats.

The effect of hydro-alcoholic extract of the stem bark of *F. albida* on some biochemical parameters of rats infected with *Trypanosoma brucei brucei* was evaluated by Tijani et al. (2009). The results indicated that the *F. albida* extract has anti-tripanosomal activity and effective in the management of anaemia induced by *Trypanosoma brucei brucei* in rats.

Lawal (2012) investigated the anti-diabetic and antioxidant effects of *F. albida* root bark extract were investigated in rats. The extract revealed a significant hypoglycemic effects on alloxan induced diabetic rats as well as antioxidant activity on DPPH assay.

1.9.11 Toxicological Studies

The toxicity profiles of ethanolic stem bark extract of *F. albida* were evaluated in Wistar albino rats by Salawu et al. (2010). The study revealed that the stem bark of the plant safe when used in sub-acute dosage of 125, 250 and 500 mg extract/kg body weight.
CHAPTER TWO

2. MATERIALS and METHODS

2.1 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.

2.2 Experimental Animals

Wistar albino rats (100-166g) were obtained from Nile Pharma company, Cairo, Egypt. They were kept in cages and housed, in the National Centre of Research Egypt 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 treatment for adaptation.

2.3 Instruments and Chemicals

Rotavaper (BUCHI-Germany).
Centrifuge (USA).
Insulin syringes (Changzhou-Jiangsu-China).
Balance (JICA-Japan).
Blood container (CRSI-China).
Capillary tubes (Superfit Continental Private Limmitted-India).
Rat’s cages (Egypt).
Gum arabic (local-Sudan).
CCl₄ (ADWIA- Egypt).
Olive oil (DIC- India).
Silymarin (MUP- Egypt).
Ethanol (Tedia-USA).
Ethylether (Tedia-USA).
2.4 Extraction
The ethanolic extracts of both parts of the plant 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.

2.5 Phytochemical Screening
General phytochemical screening of the fruits and stem bark of *F.albida* was carried out to investigate the active constituents of the extract (Harborne, 1984). The procedure is based on the addition of specific reagents to the ethanolic extract of *F.albida* fruits and observing changes of the solution colour.

2.5.1 Test for Alkaloids
Half gram 0.5g of the plant material filtrate was treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow deposit indicates the presence of alkaloids.

2.5.2 Test for Flavonoids
Half gram (0.5g) of the extract was treated with few drops of lead acetate solution. The formation of yellow color precipitate indicates the presence of flavonoids.

2.5.3 Test for Saponins
Approximately 0.3g of the extract was shaken with 2 ml of water. Production of foam that persists for 10 minutes indicates the presence of saponins.

2.5.4 Test of Tannins
Half gram (0.5g) of the extract was dissolved in distilled water, boiled for 5 minutes then 2 drops of FeCl₃ was added. Production of greenish precipitate indicates the presence of tannins.
2.5.5 Test for Cumarins
Two hundred milliliter gram (0.2g) of the extract was dissolved in 10 ml distilled water in test tube and a filter paper was covered the test tube to be saturated with a vapor after spots of 0.5N KOH was put on a filter paper, then the filter paper was examined under UV light, the presence of coumamins was indicated if the spot adsorbed the UV light.

2.5.6 Test of Sterols and Triterpenes
Half gram 0.5g of the extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and triterpenes (pink to purple) in the sample.

2.6 Antioxidant Activity
The antioxidant activity of F. albida fruits and stem bark were evaluated by DPPH (1,1'-diphenyl-2- picryl-hydrazyl) radical scavenging according to the method of Shimada et al. (1992) with some modification, propyl gallate was used as standard antioxidant agent. 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 dimethyl sulfoxide (DMSO) while DPPH was prepared in ethanol. After incubation, the decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. The percentages of radical scavenging activity of F. albida fruits and stem bark and propyl gallate were determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

2.7 Experimental Design
Thirty five Wistar albino rats were divided into 7 groups of 5 rats each. Group 1: Normal control and was given distilled water 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 2$^{nd}$ day and 3$^{rd}$ day.

**Group 3:** Hepatoprotective standard drug; rats were given CCl$_4$ at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2$^{nd}$ and 3$^{rd}$ days of silymarin administration. Silymarin was given at a dose of 100 mg/kg orally for 5days.

**Group 4:** Low dose of *F. albida* fruits extract; rats were injected CCl$_4$ at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2$^{nd}$ and 3$^{rd}$ days of extract administration. *F. albida* fruits ethanolic extract was administrated at a dose of 250 mg/kg b.w orally for 5 days.

**Group 5:** High dose of *F. albida* fruits extract; rats were injected CCl$_4$ at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2$^{nd}$ and 3$^{rd}$ days of extract administration. *F. albida* fruits ethanolic extract was given at a dose of 500 mg/kg b.w orally for 5 days.

**Group 6:** Low dose of stem bark of *F. albida* extract; rats were injected CCl$_4$ at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2$^{nd}$ day and 3$^{rd}$ day of extract administration.

*F. albida* stem bark ethanolic extract was given at a dose of 250 mg/kg b.w orally for 5 days.

**Group 7:** High dose of stem bark of *F. albida* high extract; rats were injected CCl$_4$ at a dose of 0.2ml/kg b.w dissolved in olive oil (1:1) intraperitoneally in the 2$^{nd}$ day and 3$^{rd}$ day of extract administration.

*F. albida* stem bark ethanolic extract was given at a dose of 500 mg/kg b.w orally for 5 days.

**2.8 Preparation of Blood Samples**

Blood samples were collected (after sacrificed the rats under anaesthesia) on day five and blood was collected in clean containers. Blood was
centrifuged after clotting for 10 minutes at 2500rpm. Serum was separated and stored at -20°C for biochemical analysis.

**2.9 Biochemical analysis**

Spectrophotometric method using standard kits (Stanbio laboratory Inc., San Antonio, TX, USA) was used to measure the activities of aspartate transaminase (AST), alanine transaminase (ALT) according to the method of Bergermeyer *et al.* (1986), alkaline phosphatase (ALP) following the method of King (1965), total protein described by Henry *et al.* (1957).

**2.10 Statistical analysis**

The data were analyzed using SPSS (Statistical Package for Social Sciences). 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).
CHAPTER THREE

3. RESULTS

3.1 Phytochemical screening of *F. albida* fruits and stem bark extract

Preliminary phytochemical screening of *F. albida* fruits ethanolic extract revealed the presence of alkaloid, flavonoids, saponins, tannins, coumarins, sterols and triterpenoids, as active constituents. Whereas, the result of stem bark revealed the presence of flavonoids, saponins and alkaloids only. The phytochemical result of *F. albida* fruits and stem bark ethanolic extracts are presented in table (1).

3.2 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 to *F. albida* fruits and to the standard antioxidant agent (propyl gallate). The results are shown in (Table 2).

3.3 Effect of *F. albida* fruits and stem bark ethanolic extracts on the biochemical profile of CCl₄ induced liver damage in rats.

Rats intoxicated with CCl₄ (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 group 1 normal control rats.

Rats in group 3 that were treated with silymarin standard hepatoprotective drug, displayed a significant hepatoprotective activity. This was proved by a significant decrease (P< 0.05) of the serum ALP, ALT and AST as
compared to intoxicated rats in group 2. 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, significantly decreased (P < 0.05) the levels of ALT and AST suggesting the protection of hepatic cells against CCl₄ induced liver damage when compared to rats given CCl₄ only. The results were found to be comparable to group 3, standard drug. There was a significant difference (P < 0.05) between total protein levels in control rats and the other test groups. However there were no significant differences (P > 0.05) between total protein levels in CCl₄ group, silymarin and *F. albida* fruits and stem bark ethanolic extracts at a dose of 250 and 500 mg/ kg b.w. However ALP levels were significantly decreased (P < 0.05) in group 3,4,6 and 7 compared to CCl₄ group 2. The levels of ALP in group (high dose of *F. albida* fruits) were similar to group 2.

The effect of *F. albida* fruits and stem bark extracts on liver enzymes and total proteins against CCl₄ induced liver damage in rats are presented in table (3).
**Table 1: Phytochemical screening of ethanolic extracts of *F. albida* fruits and stem bark.**

<table>
<thead>
<tr>
<th>Plant material</th>
<th>FL</th>
<th>TA</th>
<th>TR</th>
<th>SA</th>
<th>CO</th>
<th>AL</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stem bark</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Key words: FL= flavonoids, TA= tannins, TR= triterpenoids, SA= saponins, CO= coumarins, AL= alkaloid and ST= sterols. + = found, - = not found.
Table 2: Antioxidant activity of *F. albida* fruits and stem bark ethanolic extracts of using DPPH radical scavenging assay

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>%RSA ±SD(DPPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fruits</td>
<td>87±0.04</td>
</tr>
<tr>
<td>2</td>
<td>Stem bark</td>
<td>17±0.06</td>
</tr>
<tr>
<td>3</td>
<td>Propyl Gallate</td>
<td>91±0.01</td>
</tr>
</tbody>
</table>
Table 3: The Effect of *F. albida* fruits and stem bark extracts on liver enzymes and total proteins against CCl₄ induced liver damage in rats

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

<sup>a,b</sup>: 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.
CHAPTER FOUR
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 rats. *F. albida* 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). Liver damage induced by CCl$_4$ is a common model used to evaluate the activity of new hepatoprotective plants (Zhao et al., 2018). 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 (Abuelgasim et al., 2008). The hepatotoxic effects of CCl$_4$ are largely damaging to hepatocytes due to production of a highly reactive free radicals 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 tissue damage, aging, cancer and many other diseases in the body (Abuelgasim et al., 2008 and 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 the 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 injection of CCl$_4$ at a dose of 0.2 mg/kg in olive oil (1:1) significantly increased the levels of serum ALT, ALP and AST, due to
destruction of liver cells by CCl$_4$. Previous reports indicated similar finding (Ali et al., 2009; Elhag et al., 2011 and Bukhsh et al., 2014).

The hepatoprotective activity of Silymarin was also proved in this study by the significant decrease of liver enzymes compared to CCl$_4$ group. These levels were found to be comparable to the levels of normal control especially ALT and AST levels. This activity could be attributed to the hepatoprotective action of the plant against CCl$_4$ hepatotoxicity, which is due to presence of the secondary metabolites (flavanoids, tannins, carotinoids and steroids).

Oral administration of *F. albida* fruits and stem bark at dose of 250 and 500 mg/kg b.w daily for 5 days significantly lowered the serum levels of ALP, ALT and AST compared to the CCl$_4$ intoxicated rats. 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 phytochemical analysis of the ethanolic extracts of *F. albida* fruits and stem bark revealed that the extract of the fruits is rich in chemical constituents compared to that seen by stem bark.

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 propyl gallate used as a standard agent. However, the stem bark of *F. albida* showed less antioxidant activity compared to the fruits. 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 liver 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 due to exposure to toxins. These antioxidants also have the ability to prevent the process of peroxidation and improve the health of hepatocytes (Bukhsh et al., 2014).
CONCLUSION AND RECOMMENDATIONS

Conclusion

The ethanolic extract of *F. albida* fruits and stem bark may possess hepatoprotective activity in rats. This activity may be due to phytoconstituents or antioxidant activity of the plant.

Recommendations

- More studies are required to confirm the hepatoprotective activity of this extract.
- Further investigations should be performed to determine the exact phytoconstituent(s) responsible for the hepatoprotective effect of *F. albida* fruits and stem bark.
- Further investigations should be done to assess the toxicological effects and other biological activities of *F. albida*. 
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