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Hepatoprotective activity of *Ficus sycomorus* L. against N-nitrosodiethylamine and CCl₄-induced hepatocarcinogenesis in experimental rats

Samia M. El-Sayad¹, Makboul A. Makboul¹, Rofida M. Ali¹, Jasmine O. El-Amir², Salwa F. Farag^{1*}

¹Faculty of Pharmacy, Pharmacognosy Department, Assiut University, Assiut 71526, Egypt

²Faculty of Veterinary Medicine, Pathology Department, Assiut University Assiut 71526, Egypt

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*For Correspondence

Prof. Dr. Salwa Farouk Farag, Faculty of Pharmacy, Pharmacognosy Department, Assiut University, Assiut, Egypt.

E-mail: farag_s@yahoo.com

Phone: +02 088 2080711,
Fax number: +02 088 2080774

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ABSTRACT

The hepatoprotective activity of the different extracts of *Ficus sycomorus* L. (FS) against N-nitrosodiethylamine (NDEA) and CCl₄-induced hepatocarcinogenesis (HCC) in rats has been investigated for the first time. Histological observations of liver tissues demonstrated that, both wood (FSWE) and leaf extracts (FSLE) possess significant hepatoprotective activity and stem bark extract (FSBE) shows moderate activity while fruit extract (FSFE) is not significantly effective.

INTRODUCTION

Ficus sycomorus L. (*F. Moraceae*) is grown in Egypt and called sycamore or g'immeiz^[1] The fruit is edible and the different parts of the plant are used in various African countries for the treatment of many diseases and disorders such as diarrhea, dysentery, skin infections, stomach disorders, liver diseases, jaundice, chest conditions, cough and scrofula, tuberculosis, inflammations, throat pain, fungal diseases, epilepsy, lactation disorders, helminthiasis, mental disorders, sickle cell disease, infertility and sterility^[2-7]

Concerning the antitumor properties of the plant, the previous biological studies revealed that, the fruit extract exhibited antitumor activity in the potato disc bioassay^[8,9] Also, root bark extract showed hepatoprotective activity against CCl₄-induced hepatotoxicity in rats^[3] In addition, the extracts of the leaves demonstrated hepatoprotective and antipathological effect on the liver of mice infected with *Shistosoma mansoni*^[10]

In the present study, we investigated the hepatoprotective activity of the different extracts of *F. sycomorus* L. against N-nitrosodiethylamine and CCl₄-induced hepatocarcinogenesis in experimental rats. This may be promising at stopping hepatocarcinogenesis, delaying its progress, minimizing the damage to liver cells or reducing its complications.

MATERIALS AND METHODS

Material

Plant Material

Fresh samples of *Ficus sycomorus* L. including leaves, stems and unripe fruits were collected in the period of February to April 2010, from the Experimental Station of Ornamental Plants, Faculty of Agriculture, Assiut University and kindly identified and authenticated by the late Prof. Dr. Naeem E. Keltawy, Professor of Ornamental Horticulture and Floriculture, Faculty of Agriculture, Assiut University. A voucher sample (no. 2010 FS) has been deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Animals

Adult Albino rats (100-150 g) of either sex were used for acute toxicity. Male albino rats weighing 150-200g (4-5 weeks old) were used for hepatoprotective effect. The animals were bred and housed under standardized environmental conditions in Pre-clinical Animal House, Pharmacology Department, Faculty of Medicine, Assiut University. They were fed with standard diet and allowed free access to drinking water. The animals were randomly assigned to groups according to the experimental design.

Chemicals

N-Nitrosodiethylamine (NDEA) was purchased from Sigma-Aldrich Company, Steinheim, Germany. Carbon tetrachloride (CCl₄) was obtained from El-Faronia Company, Cairo, Egypt. Tween 80 was purchased from Sigma Chemical Co., St. Louis, USA. Normal saline 0.9% was obtained from (El-Nasr pharmaceutical and chemical Co., Egypt). All other chemicals used were of analytical grade and purchased locally.

Preparation of extracts

The air-dried powdered leaves (1.0 kg), stem bark (0.5 kg), wood (1 kg) and fresh unripe fruit (1.5 kg) of *F. sycomorus* (FS) were separately extracted with 70% ethanol by maceration at room temperature. Each extract was concentrated under reduced pressure to obtain crude extract weight 60 g, 60g, 60g and 50 g, respectively.

Preparation of the extracts for administration

A specific weight (2.5 g for acute toxicity and 40 g for hepatoprotective activity studies, respectively) of each dried extract of the leaves (FSLE), stem bark (FSBE), wood (FSWE) and fresh unripe fruits (FSFE) was prepared as an emulsion in normal saline containing 3% v/v Tween 80.

Acute toxicity studies

The acute toxicity study was conducted in accordance with Lorke's method^[11, 12] The study was conducted in two phases using a total of twenty five rats. In the first phase, thirteen rats were divided into 5 groups. Groups I-IV, animals were given intraperitoneally 10, 100 and 1000 mg/kg body weight (b.w.) of each extract, respectively (one rat per dose) to possibly establish the range of doses producing any toxic effect. In addition, a fifth group of one rat was set up as a negative control and injected intraperitoneally by 3 % v/v Tween 80 in normal saline.

In the second phase, further specific doses (1600, 2900 and 5000 mg/kg b.w.) of each extract were administered to three rats (one rat per dose) to further determine the correct LD₅₀ value. The extracts were given *via* intraperitoneal route. All animals were observed frequently on the day of treatment. The acute lethal effect of the different extracts of FS on rats showed that no animal died within 24 hours after treatment with extract (Table 1). The major signs of toxicity noticed within 24 hours included difficulty in breathing, loss of appetite, general weakness, irritability, writhing, hypothermia, loss of motor coordination, muscle relaxation, sedation and deep sleep. These signs were not seen in 100 mg/kg b.w. dose group but progressed and became increasingly pronounced as the dose increased towards 5000 mg/kg b.w. The LD₅₀, being greater than 5000 mg/kg b.w.

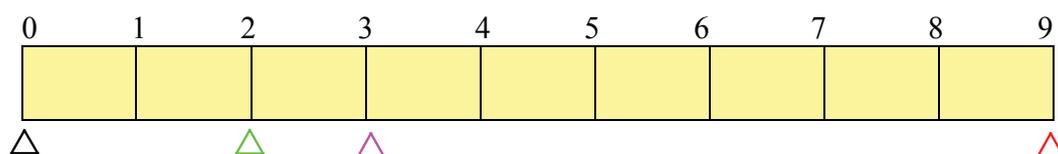
EXPERIMENTAL DESIGN

The hepatoprotective action was performed as described by the method of Sundaresan and Subramanian 2003^[13]. Fifty five male rats were randomly divided into 6 groups (as shown in Figure 1); each group was comprised of ten animals except group I which was comprised of 5 animals. The experimental design and treatment protocol were as follows: Group I: Normal control rats fed with standard diet for 9 weeks. Group II: Negative control rats and received oral dose of 3% v/v Tween 80 in normal saline (1 ml/kg b.w. daily) for two weeks before the administration of NDEA and continued till the end of the experiment (i.e. 9 weeks). Groups III-VI: Rats received oral dose of the tested extract of *F. sycomorus* L. (FSLE, FSBE, FSWE and FSFE, respectively) (400mg/Kg b.w. daily) for two weeks before the administration of NDEA and continued till the end of the experiment. Then animals of groups II-VI were given a single intraperitoneal (i.p.) injection of NDEA (200 mg/kg b.w.). After one week, they were given CCl₄ subcutaneously once weekly in a dose of 3 ml/ kg b.w. for 6 weeks to ensure induction of HCC. At the end of 9th week of the experiment, all the animals were sacrificed by cervical decapitation, the livers were excised and liver specimens were taken

from each lobe of the liver. The specimens were at once immersed in 10% buffered formalin solution for fixation, dehydrated in ascending grades alcohol, cleared in methyl benzoate, embedded in paraffin, sectioned at 4 microns thickness and stained with Hematoxylin (H) and Eosin stain (E) for histopathological examination according to standard protocols. In this experiment, proliferation of oval shaped cells was observed in rats exposed to NDEA and CCl₄. These cells were characterized by a small oval blue staining nucleus and a faintly basophilic cytoplasm.

Table 1. Acute lethal effect of *F. sycomor* L. extracts

Experiment Treatment	Phase 1			Phase 2		
	Dose (Mg/kg b.w.)	No. of dead rats after 24 hrs.	Treated rats after 24 hrs.	Dose (Mg/kg b.w.)	No. of dead rats after 24 hrs.	Treated rats after 24 hrs.
Negative control	0	0/1	0/1			
FSLE	10	0/1	0/1	1600	0/1	0/1
	100	0/1	0/1	2900	0/1	0/1
	1000	0/1	0/1	5000	0/1	0/1
FSBE	10	0/1	0/1	1600	0/1	0/1
	100	0/1	0/1	2900	0/1	0/1
	1000	0/1	0/1	5000	0/1	0/1
FSWE	10	0/1	0/1	1600	0/1	0/1
	100	0/1	0/1	2900	0/1	0/1
	1000	0/1	0/1	5000	0/1	0/1
FSFE	10	0/1	0/1	1600	0/1	0/1
	100	0/1	0/1	2900	0/1	0/1
	1000	0/1	0/1	5000	0/1	0/1



△ FS extracts administration in groups III-VI till the end of experiment

△ NDEA administration in groups II-VI (single i.p. injection)

△ Confirmation of the induction of HCC for 6 weeks

△ Dissection of rats

Figure 1: The experimental design and treatment protocol diagram

RESULTS AND DISCUSSION

No mortalities were observed in animals of groups I, IV and V, however the mortalities were 40%, 40% and 30% in animals of groups II, III and VI, respectively. Histological studies (Figure. 2A) revealed that liver sections from normal control rats (group I) showed normal architecture, characterized by polyhedral shaped hepatocytes and cytoplasm granulated with small uniform nuclei. In group II, it was shown that administration of NDEA and CCl₄ induced preneoplastic foci of different sizes, oval cell proliferation and bile duct hyperplasia. Ppreneoplastic foci were observed in the hepatic lobule. No pressure atrophy on the surrounding normal cell was observed. The typical trabecular arrangement of liver cells was lost and the sinusoids were collapsed. The cytoplasm was abundant and more eosinophilic than normal and had a "ground glass appearance". The cells were larger and had large nuclei with prominent nucleoli (Figure. 2B). A varied number of vacuolated large hepatocytes within the preneoplastic foci were observed (Figure. 2C). The vacuolated cells had a plant cell like (clear cell) in which ballooned cytoplasm and hyperchromatic, pleomorphic nuclei were observed. Proliferation of oval cells which extended among the hepatic parenchyma was observed (Figure. 2D). These cells were characterized by a small oval vesicular nucleus and a faintly basophilic cytoplasm. Bile duct hyperplasia was characterized by elongated bile ducts lined by one or several layers of biliary epithelial cells (Figure. 2E). Data in Table 2 revealed that, the preneoplastic foci, oval cell proliferation and bile duct hyperplasia were inhibited in group V. The preneoplastic foci and bile duct hyperplasia were inhibited and the percentage of oval cell proliferation was decreased to 16.6% in group III. In group IV,

the percentage of preneoplastic foci and oval cell proliferation was decreased to 10%, while bile duct hyperplasia was inhibited. The occurrence of preneoplastic foci and bile duct hyperplasia were not significantly decreased in group VI.

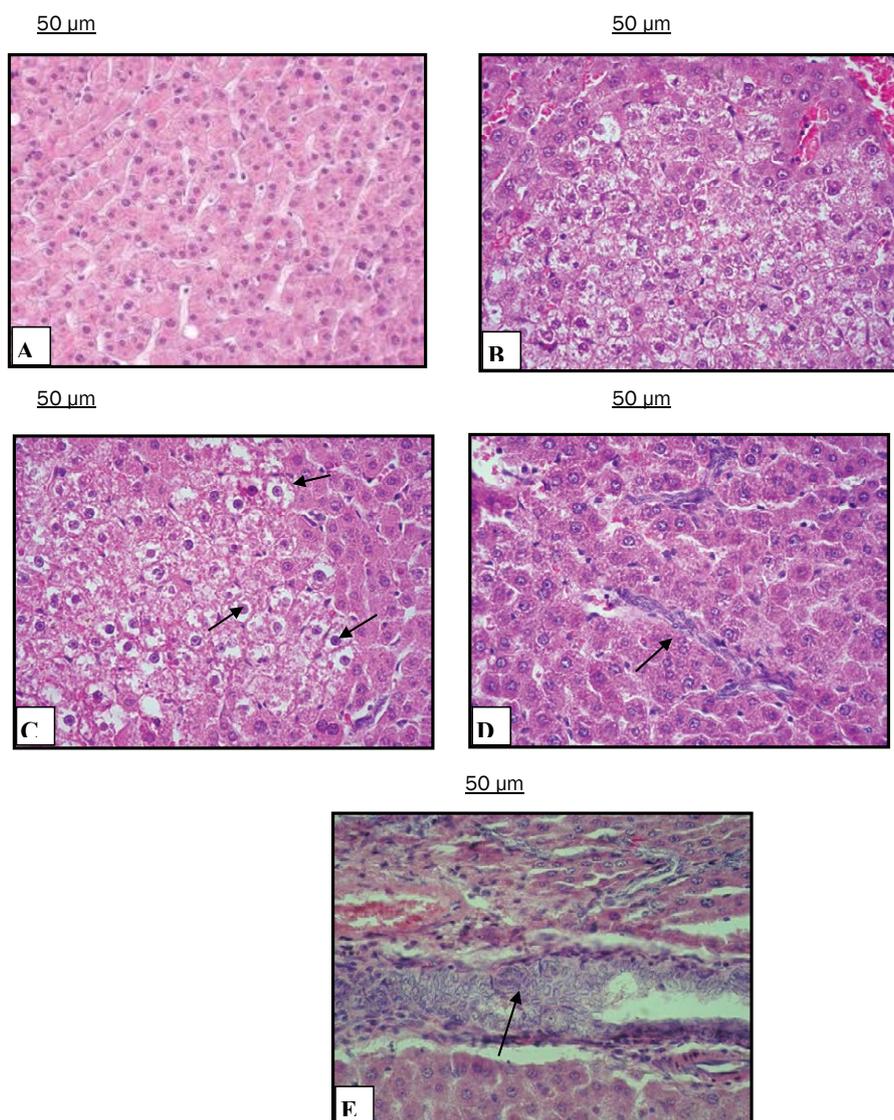


Figure 2: Histopathological photomicrographs of the liver of rats

A: Liver from normal control rats (group I).

B: liver from rats of group II showing preneoplastic foci, no pressure atrophy on the surrounding normal cell, abundant cytoplasm and "ground glass appearance" and larger cells with large nuclei and prominent nucleoli.

C: Liver from rats of group II showing vacuolated cells had a plant cell like (clear cell) (arrows).

D: Liver from rats of group II showing proliferation of oval cells extended among the hepatic parenchyma (arrows).

E: Liver from rats of group II showing bile duct hyperplasia characterized by elongated bile ducts lined by one or several layers of biliary epithelial cells (arrows). H & E staining.

Table 2: Effect of *F. sycomorus* extracts on different proliferating lesions of hepatic parenchyma of NDEA and CCl₄-induced hepatocarcinogenesis in rats

Groups	Treatment	No. of rats at the end of experiment	Preneoplastic foci	Oval cell proliferation	Bile duct hyperplasia
I (Normal control)	Untreated	5/5	0	0	0
II (Negative control)	NDEA + CCl ₄	6/10	3 (50%)	3 (50%)	4(66.6%)
III	FSLE + NDEA + CCl ₄	6/10	0	1 (16.6%)	0
IV	FSBE + NDEA + CCl ₄	10/10	1 (10%)	1 (10%)	0
V	FSWE + NDEA + CCl ₄	10/10	0	0	0
VI	FSFE + NDEA + CCl ₄	7/10	2 (28.6%)	3 (42.9%)	1 (14.8%)

The exact mechanism(s) of *F. sycomorus*-mediated prevention of NDEA-induced hepatocarcinogenesis are not known. It has

been reported that, a possible mechanism of the MeOH leaf extract of *F. carica* Linn. as hepatoprotective in rats with liver damage induced by CCl₄ at an oral dose of 500 mg/kg may be due to its anti-oxidant effect or inhibition of cytochrome P450s which impair the bio activation of CCl₄ into their corresponding reactive species.^[14] Moreover, the aqueous root bark extract of *F. sycomorus* L. at an oral dose of 640 mg/kg showed hepatoprotective effect against CCl₄ induced hepatotoxicity in rats due to presence of a mixture of α -amyrin and flavonoids.^[3] From these findings, it can be concluded that, *F. sycomorus* L. may suppress the formation of NDEA and CCl₄ induced hepatotoxicity in rats by scavenging reactive oxygen species.

Flavonoids, coumarins, tannins and steroids/triterpenoids and their glycosides have been reported as constituents of this genus.^[15] Since these constituents are free radical scavengers and have hepatoprotective activity, it may be speculated that these constituents of *F. sycomorus* L. are responsible for the observed protective effects.^[3, 14,16]

CONCLUSION

From the present study we can conclude that, both wood and leaf extracts possess significant hepatoprotective activity against NDEA and CCl₄-induced hepatocarcinogenesis in rats while, the stem bark shows moderate activity and the unripe fruits extract is not significantly effective. This is the first report for the hepatoprotective effect of the extracts of *F. sycomorus* leaves, stem bark, wood and unripe fruits in animal models.

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