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Preliminary phytochemical screening and hepatoprotective activity of methanol extract of *Artocarpus hirsutus* leaves.

Jitendra Patel[1*], Venkateshwar Reddy[2], GS Kumar[3]

***Corresponding author:**

Jitendra Patel

[1]Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500072, TS, India.
[2]Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad -500001, TS, India.
[3]Department of Life Sciences, School of Pharmacy, International Medical University, Kuala Lumpur 57000. Malaysia.

Abstract

The objective of the present work was investigating the preliminary phytochemical screening and hepatoprotective activity of methanol extract of the leaves of *Artocarpus hirsutus*.

Group I served as vehicle control, Group II served CCL_4 (2ml/kg, s.c.), Group III served as standard Silymarin (50 mg/kg, p.o.) Group IV and V served as methanolic extracts of *Artocarpus hirsutus* (MEAH) at the dose level (250 and 500 mg/kg, p.o.). The degree of protection was determined by measuring level biochemical marker like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin, total bilirubin and Cholesterol. The histopathology study also showed the hepatic protection of extracts. The preliminary phytochemical screening was performed to find out the phytoconstituents responsible for the activity.

The marker biochemical level such as ALT, AST, ALP, Direct bilirubin, Total bilirubin and Cholesterol were significantly raised in CCL_4 treated rats when compared with the normal group ($p<0.05$), but the MEAH (500 mg/kg, bw) treated rats exhibited maximum depletion. The histopathology study also showed the hepatic protection of extracts. Preliminary phytochemical screening showed the presence of glycosides, flavonoids, Tannins, triterpenoids, carbohydrates and steroids.

The results of *in vivo* hepatoprotective activity showed that the methanol extract of *Artocarpus hirsutus* exhibit significant hepatoprotective activity. This might be due to flavonoids and tannins; which was confirmed their presence in phytochemical tests.

Keywords : *Artocarpus hirsutus*; Methanolic extract; CCL_4; Hepatoprotective and Silymarin.

Introduction

Liver is the very important and massive visceral organ found into substantial portion of abdomen, which plays important role in regulating different physiological process in body. It involved in many vital functions like metabolism, secretion, storage, and detoxification and synthesizes useful principles. It also removes toxic materials from blood [1]. Liver diseases are mainly caused by excess consumption of alcohol, autoimmune disorders, toxic chemicals and infections [2]. It has become one of the major causes of mortality and morbidity all over the world. Many chemicals and drugs cause different types of liver toxicity that are highly variable. All over the world, the researchers have been in continuous search for some effective remedy for restoring the functions. The plant kingdom is indubitably one of the valuable sources of new medicinal agents. Numerous herbs and plants play a major role in the management of different liver disorders [3]. *Artocarpus hirsutus* belongs to the family Moraceae and this comprises 50 varieties of species. They are deciduous and evergreen tall tree grows up to 75 meters in height in southern regions of India. This species occurs wild and is also cultivated for its edible fruits, leaves, bark and also timber. It is known by a variety of names such as Aani, Aini, Aini-maram, Anjili and Anhili found in Karnataka, Kerala and Tamil Nadu. The other *Artocarpus* genus like *Artocarpus altilis* (bread fruit), *Artocarpus heterophyllus* (jack fruit) have medicinal value of their source as an edible aggregate fruit [4]. *Artocarpus hirsutus* (Wild jack fruit) is been used in antimicrobial activity [5] anti-ulcer activity [6] traditional medicine, food and industry [7]. Plants are vital for the remedies as well as existence for human disease because they contain components of therapeutic value [8].

Materials and Methods

Plant material

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The leaves of the plant collected from Tirumala Hills, Chittoor district (A.P.). The plant materials were identified and authenticated by Dr. K. Madhav chetty Assistant professor, S.V. University, Tirupati India. The authentication letter (Voucher specimen No.-1116) has been deposited in Pharmacognosy department, KVK College of Pharmacy, Surmaiguda, Hyderabad (TS).

Preparation of extracts

The collected leaves were shade-dried at room temperature and powdered. The coarse powder (100 gms) were extracted by using successive soxhlet extraction using solvent in increasing order of polarity such as petroleum ether, chloroform, methanol and distilled water for 72 hrs. After completion extracts were filtered and solvent evaporated in rotary evaporator [9].

Phytochemical analysis

The extracts were liable to preliminary phytochemical screening for chemical constituents such as Flavonoids, tannins, glycosides, alkaloids, triterpenoid, sterols, saponins, phenolic compounds, carbohydrates, gum and mucilage [10-12].

Acute oral toxicity

The acute toxicity of MEAH was determined as per OECD guideline no.420, based on the cut-off value of the median lethal dose (LD^{50}), the effective dose (ED^{50}) was determined. [13]

Animals

The 150-250gm healthy albino rats selected and kept in cages with standard rat chow diet and water *ad libitum* assimilated to surroundings for one week prior to study. Animals are maintained on light and dark cycle at constant temperature (25˚C ±3˚C) and relative humidity (50±20 %). The experiment has done in Nishka scientific and reference laboratory, Uppal, Hyderabad. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Hepatoprotective activity

The rats were randomly divided into 5 groups. Group I served 1 ml distilled water p.o., for 5 days. Group II also served 1 ml distilled water p.o. for 5 days. Group III served with standard silymarin 50mg/kg p.o., for 5 days. Group IV served with 70% methanolic extract 250mg/kg p.o. for 5 days. Group V served 70% methanolic extract 500mg/kg p.o. for 5 days. On 2nd and 3rd day Group I served only olive oil (1 ml/kg) s.c. route but group II, III, IV and V given CCL_4 : olive oil (1:1) at a dose 2ml/kg s.c. just after half hr. of vehicle administration. The animals were sacrificed on the 6th day of study under mild ether anesthesia. The blood sample was collected by retro orbital route for analysis of ALT, AST, ALP, Total bilirubin, direct bilirubin and cholesterol [14]. The liver were dissected out and washed with normal saline solution and stored in 10% formalin. The livers were processed for histopathology to evaluate the hepatic architecture microscopically [15-17]. The results are given in table 1.

Histopathology

The livers were implanted in paraffin wax and sections were cut into 5-6 micron thickness. The sections were stained with hematoxylin and eosin. The stained sections were observed under microscope for liver architecture. Compare the architecture of CCL_4 damaged with Group III, IV and V. The results are given in figure-2

Statistical analysis

The statistical significance were determined by using one way ANOVA followed by Dunnett's multiple comparison test by using Graph p Instat software. The values were represented as Mean ± SEM, (n=6). Less than 0.05 value of P was considered to be statistically significant. *P<0.5 **P<0.01 and ***P<0.001, when compared with control and toxicant group as applicable.

Table 1: Effect of *Artocarpus hirsutuson* enzyme ALT, AST, ALP, Total bilirubin, direct bilirubin and Cholesterol levels in blood serum of CCl_4 induced hepatotoxicity.

Groups	Treatment	ALT IU/L	AST IU/L	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl	Cholesterol mg/dl
Group I	Normal	48.60±7.131	110.03±9.72	86.07±4.79	0.53±0.02	0.23±0.2	134.00±3.27
GroupII	CCl4	172.30±7.67	227.91±14.36	133.56±6.79	1.75±0.11	1.70±0.01	215.5±1.66
GroupIII	Silymarin	67.35±4.40**	126.59±4.17**	87.30±4.40**	1.00±0.10**	0.66±0.02**	171.67±1.22**
GroupIV	MEAH 250mg/kg	92.35±5.01*	146.24±9.25**	97.98±7.69	1.07±0.14**	0.82±0.007**	203.33±1.06**
GroupV	MEAH 500mg/kg	70.00±3.82**	116.75±10.73 **	89.77±5.74**	0.59±0.10**	0.65±0.009**	192.00±1.06**

Values are expressed in Mean ± SEM of 6 rats in each group. *p<0.01, **p<0.001 when compared with respective CCl_4treated group

Table 2: Preliminary phytochemical screening of MEAH:-

S.N.	Test	MEAH	S.N.	Test	Leaves powder of AH
I	**Flavonoids**		IV	**Glycosides**	
	$Fecl_3$ test	(+) ve		Borntrager's test	(+) ve
	NAOH Solution test	(+) ve		Modified Borntrager's test	(+) ve
	Lead acetate test	(+) ve		Keller kiliani test	(+) ve
	Mineral acid test	(+) ve		Baljit test	(+) ve
	Alkali-acid test	(+) ve	V	**Gums and Mucilage**	
II	**Tannins**			Ppt with 90% alcohol	(-) ve
	$Fecl_3$ test	(+) ve Galli ellagi tannins present	VI	**Triterpenoid**	(+) ve
				Saponins	
	Dil $Fecl_3$ test	(+) ve both type of tannin present		Foam test	(+) ve
	Gelatine test	(+) ve		Haemolysis test	(-) ve
	Match stick test	(-) ve	VII	**Sterol**	
	Chlorogenic acid test	(+) ve		Lieberman burchard reaction	(+) ve
III	**Alkaloids**		VIII	**Carbohydrates**	
	Mayer's reagent	(-) ve		Molischs test	(+) ve
	Wagner's reagent	(-) ve		Fehling's test	(+) ve
	Hager's reagent	(+) ve			

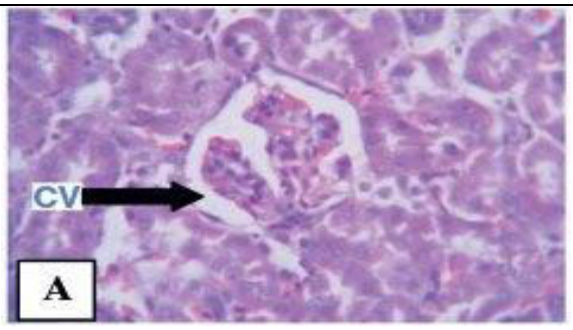

Figure A(control): liver section with normal structure and architecture

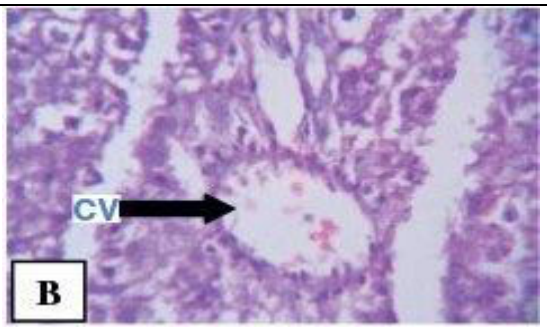

Figure B: (CCl4 treated): showing extensive area of necrosis, profound inflammation and congestion.

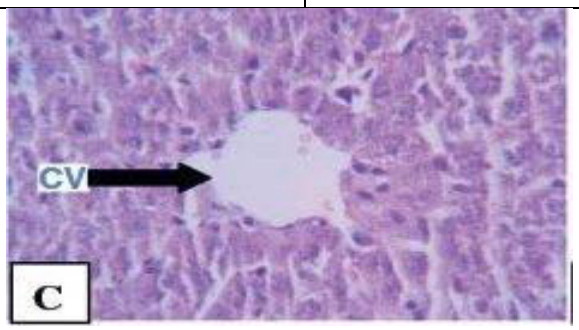

Figure C: (pretreatment with Silymarin): reduced inflammation and degenerative changes

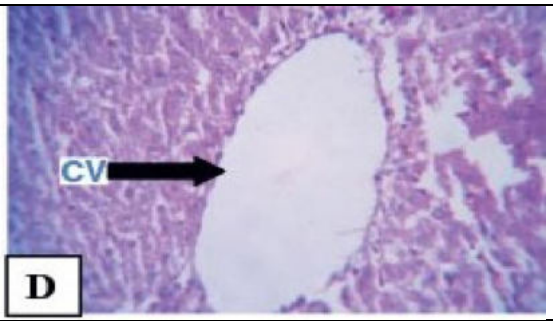

Figure D: (pretreatment of extract at low dose of 250 mg/kg bw): reduced inflammation, degenerative changes

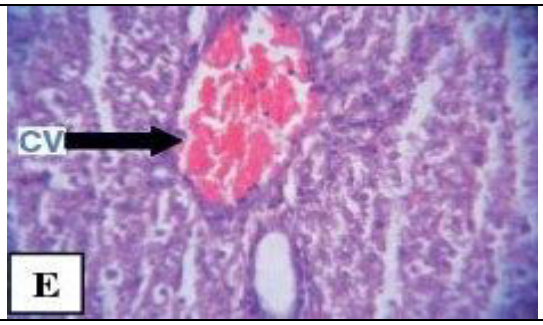

Figure E: (pretreatment of extract at low dose of 500mg/kg bw): reduced inflammation, degenerative changes

Figure 1: Images of liver architecture in CCl_4 induced hepatotoxicity in rats. CV: central vein

Results

Acute toxicity (LD50) studies

The lethal dose (LD 50) was identified for MEAH. The acute toxicity studies were performed according to OECD guidelines No. 420. Hence, no lethality was observed at 2000mg/kg in rat since it was considered the cut off dose. The 250mg/kg and 500mg/kg dose was given as effective dose.

Hepatoprotective activity

Rats treated with CCL_4 (2ml/kg s.c.), significant developing ($P<0.05$) in serum ALT, AST, ALP, Total Bilirubin, direct bilirubin and cholesterol as compared to normal. Pretreatment with MEAH (250 mg/kg and 500mg/kg) for 5 days; it significant declined ($P<0.05$) as compare to CCL_4 treated group. Pretreatment with standard Silymarin produced significant declined decreased ($P<0.05$) as compare to CCL_4 treated group (table 1).

Histopathological studies

Histopathological examinations of liver sections of normal rats revealed normal histological characters but CCL_4 treated group conceded various degree of fatty degeneration like ballooning of liver cells, inflations of lymphocytes and the loose of cellular membranes. The high dose of MEAH (500mg/kg, p.o.) significantly regularizes this damage in histological architecture of liver (Figure 2).

Discussions

The CCL_4 hepatotoxicity is most popular used model for the hepatoprotective activity. The increasing in serum ALT, AST, Total bilirubin, direct bilirubin and cholesterol has been marked to the damaged structure character of liver. They are found in cytoplasm and released in blood after liver damage. CCL_4 induces hepatotoxicity by metabolic activation thus it selectively causes toxicity in liver cells maintaining semi normal metabolic functions. CYP450 dependent mixed oxidase in ER activates metabolically to CCL_4. It forms trichloromethyl free radicals, which attached with lipids and proteins of cells in presence of oxygen to increase lipid peroxidation [18]. The formation of highly reactive tricolor free radical directly attacks to the poly unsaturated fatty acids of endoplasmic reticulum and it leads to over production of ALT, AST, ALP, total bilirubin, direct bilirubin and cholesterol [19]. All these leads to change in ER structure, membrane, activation of metabolic enzymes, reduction of protein synthesis and activation of glucose-6 phosphatase [20]. CCL_4 noted to cause marked elevation in serum transaminase. In the present study, pretreatment with MEAH (250mg/kg and 500mg/kg) deflated the increases in activities of enzymes ALT, AST, ALP, Total bilirubin, direct bilirubin and cholesterol was noted to be lower than the CCL_4 treated group. Silymarin is well known hepatoprotective agent obtained from *Silybum marianum* is described to have effects on liver plasma membrane and possess many action against hepatotoxins. The antioxidant property and cell regenerating are the results of increases protein synthesis were considered important actions. Antioxidant protections of MEAH might be due to presence of flavonoids and tannins that regenerate liver cells and fix the membrane. The study shows that the MEAH at higher dose (500 mg/kg p.o.) is close to standard. The histopathological study shows that constituents like tannins and flavonoids in extract showed superb protection to liver architecture almost to the level of the Silymarin treated group. The extracts also show the significant liver protection activity in dose dependent mode by reducing enzymes. Thus 70% MEAH proven hepatoprotective activity may be due to flavonoids and tannins.

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Conflict of Interest

The authors declare no conflict of interest.

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