Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

PROTECTIVE EFFECT OF *VIGNA ACONITIFOLIA* METHANOLIC SEED EXTRACT AGAINST SMOKELESS TOBACCO (GUTKA) CHEWING AND ON CHEMICALLY INDUCED HEPATOTOXICITY AND NEPHROTOXICITY IN RATS

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Received: 28 Oct 2013 Revised and Accepted: 02 Apr 2014

ABSTRACT

Objective: The purpose of this study was to evaluate the protective effect of *Vigna aconitifolia* methanolic seed extract against smokeless tobacco (Gutka) chewing and on chemically induced hepatotoxicity and nephrotoxicity in rats.

Methods: The experimental groups (Albino rats) treated with aqueous extract of smokeless tobacco mixed diet (AEST), Aqueous extract of smokeless tobacco mixed diet and on 21st day administration of CCl_4 (2.0ml/kg b. w.) s.c. (AEST+CCl_4) for 21 days studies. The Methanolic extract of *Vigna aconitifolia* (MEVCF) and Silymarin (Standard) were orally administered daily at 200, 400 and 25mg kg^{-1} body weight [(AEST) and (AEST+CCl_4)] for 21 days respectively. These studies to its protective and the extent of liver and kidney damages were evaluated by the activities of the liver and kidney marker enzymes and the antioxidant enzymes of liver and kidney tissues.

Results: Pretreatment with (MEVCF) seed extracts and standard drug significantly prevented physical (increased liver, kidney and body weight), biochemical (increased levels of AST, ALT, ALP and Bilirubin in liver, Creatinine, BUN, Uric acid and decreased Total protein in kidney) and Antioxidant (decreased levels of SOD, CAT, GSH and increased LPO of kidney and liver tissues) and histological changes against produced by [(AEST) and (AEST+CCl_4)] toxicities. In induced model (AEST+CCl_4)] level of changes augmented than AEST induced model.

Conclusion: Methanolic extract of *Vigna aconitifolia* seeds shows significant hepato and nephroprotective activity against [(AEST) and (AEST+CCl_4)] induced toxicities.

Keywords: *Vigna aconitifolia*, Silymarin (SIL), Aqueous extract of smokeless tobacco (AEST), Carbon tetrachloride (CCl_4), Hepatoprotective, nephroprotective.

INTRODUCTION

Hepato and nephro diseases are large public health problems in the world. [1] Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste and toxic metabolites leads sensitivity to toxicity.[2] kidney is also a target organ for a wide variety of toxic agents due to its prime function as a blood filter during the excretory process and is also sensitive to chemicals, drug induced injury.[3] As per WHO, about 80% of the population in the world relays on the traditional medicine for the treatment of various disease.[4] However, perhaps only about 1% of these are acknowledged through scientific studies to have therapeutic value when used in extract from by human. In the absence of reliable liver and kidney

protective drugs in allopathic medical practices, herbs play a role in the management of various liver and kidney disorders. The herbal drugs believed to be harmless and free from serious adverse reactions, as they are obtained from nature and are easily available. [5] In present study *Vigna aconitifolia* seed was selected for hepatoprotective and nephroprotective activity because it is having anti oxidant and free radical scavenging property. [6] The product is basically a flavored and sweetened dry mixture of areca nut, catechu, and slaked lime with tobacco. The mixture is chewed slowly, and in this process the aqueous extract due to saliva is not only absorbed locally but also ingested to enter into the systemic circulation. Smokeless tobacco use can be addictive, leading to effect the lung, liver and kidney through generating reactive oxygen species (ROS) associated with highly reactive free radicals that carcinogenesis, DNA damage and alter the antioxidant defense mechanism. When gutka chewers exposed chemicals or drugs leads to enhance the liver and kidney toxicity levels. [7] Currently, research interest has been focused on the role of antioxidant and free radical scavenging activity seed extract *Vigna aconitifolia* inhibits the ROS production in treatment of prevention of diseases mention above. It is designed to evaluate the damages imposed by the gutka and when gutka chewers exposed to chemicals (CCl_4) on liver and kidney and its protection by seed extract.

MATERIALS AND METHODS

Collection and authentication of plant material

The *Vigna aconitifolia* (Fabaceae) seeds were collected during the January -2013 from Bhimavaram, Near West Godavari (Dist),Andhra Pradesh identified, confirmed and authenticated by comparing with voucher specimen No. 1203., Dr K Madhava chetty., Assistant professor, Department of Botany, srivenkateswara university, Tirupathi- 517502, A.P.

Chemicals

All reagents used in the experiment were of analytical grade and the kits used were procured from (Robonik PVT.LTD, Navi Mumbai, India).

Preparation of seed methanolic extract

The seeds are washed with distilled water then soaked maximum 24hrs after slight-germination dry it on sunlight. Seed samples were weighed (10 g each) and reduce to course powder (#40 size mesh) and around 500gm of powder was defatted with petroleum ether and then subjected to successive hot continuous extraction with methanol (95%v/v) using Soxhlet apparatus for 18 h. The extract was air dried to evaporate solvent. [8]

Preparation of Aqueous extraction of smokeless tobacco AEST

Around 500 betel leaves were cut into small pieces and grinded with 3.5liters distilled water to make a paste like mass. 500 gm of slice cutting areca nuts and 312.5gm tobacco were powdered. Finally the

betel leaf paste, powdered areca nut, tobacco and 60gm of slaked lime were mixed well to make a paste and keep that for 6hrs. Then after 6hrs that mixture was filtered and the filtrate was freeze-dried and stored at -20^{0}C till use. [9, 10]

Animals

The male wistar albino rats (150-240g) were obtained from the central animal house of Sigma institute of clinical research & administration PVT LTD, Hyderabad. The animals were housed at room temperature (22-28 ºC) for 12 hrs dark light cycles. Given aqueous extract of smokeless tobacco mixed in standard laboratory feed pellet diet and water *ad-libitum* for Aqueous extract of smokeless tobacco chewing induced group and aqueous extract of smokeless tobacco mixed in standard laboratory feed pellet diet and water *ad-libitum* for Aqueous extract of smokeless tobacco chewing and on administration of CCl_4 s.c. injection diluted 1:1 in paraffin oil on 21st day induced group of animals. Institutional Animal Ethics Committee (769/2010/CPCSEA) approved the study

Phytochemical screening

Preliminary phytochemical screening of MEVCF Methonlic extract of *Vigna aconitifolia* was carried out as described by Khandelwal. [11]

Acute Toxicity Study

toxicity studies were performed according to OECD-423guidelines. [12]

Sample collection and analysis

After the treatment period of 21 days, the animals were sacrificed on the 22nd day using Anesthetic ether. The animals were quickly dissected and blood was collected by cardiac puncture. Serum was separated by centrifugation at 25000 rpm for 15 min. The serum stored at 2-8°C, stored at freezer for one day and then subjected for the estimation of serum biochemical parameters such as AST [13], ALT [13], ALP [14], Bilirubin [15] for liver functional observation, and BUN [15], Creatinine, [17] uric acid, [15] total protein [18] for kidney functional observation using kits manufactured by Robonik (India) PVT.LTD, Navi Mumbai, India. The instrument used for this was autoanalyser Robonik (India) PVT.LTD, Navi Mumbai, India. The liver and kidney of all the experimental animals removed and processed immediately for histological investigation. [19] Livers and kidneys of some animals were homogenized with ice-chilled 10% KCl solution and centrifuged at 2000 rpm for 10minutes. Then the supernatant liquid was collected and the antioxidant parameters like Catalase [20], Super oxide Dismutase, [21] Glutathione [22] and Lipid peroxide [23] were estimated.

Physical parameters

Physical parameters of rats observed for hepatoprotective and nephroprotective activity of seed extract. In this percentage change of body weight, wet liver weights and wet kidney weights were observed.

Statistical analysis

All the data were expressed in mean ± SEM (Standard error mean). The significance of differences in mean between control and treated animals for different parameters were determined by using one way ANOVA (Analysis of Variance) and followed by Tukey's comparison test and using student, st-test (paired t- test). P values<0.05 were considered significant PVT. LTD, Navi Mumbai, India.

Table 1: Treatment design

Groups	AEST mixed diet for 21 days	AEST mixed diet for 21 days + CCl_4 on 21st day
Group I	Normal rats 0.5 ml of 0.5% Tween-80 in distilled Water orally	Normal rats 0.5 ml of 0.5% Tween-80 in distilled Water orally.
Group II	Served as hepato and nephrotoxin-I and received aqueous extract of smokeless (AEST 0.015%w/w) tobacco mixed diet.	Served as hepato and nephrotoxin-II and received aqueous extract of smokeless tobacco (AEST 0.015%w/w) mixed diet + CCl_4 s.c. (2.0ml/kg. b. w.) on the 21st day.
Group III	Served as standard control-I and received silymarin (25mg/kg b. w.) p. o. + AEST.	Served as standard control-II and received silymarin (25mg/kg b. w.) p. o. + AEST + CCl_4 s.c. (2.0ml/kg.b. w.) on the 21st day.
Group IV	Served as test group-I and received MEVCF (200mg/kg b. w.) p. o. + AEST	Served as test group -III and received MEVCF(200mg/kg b. w.) p. o + AEST + CCl_4 s.c. (2.0ml/kg. b. w.) on the 21st day.
Group V	Served as test group-II and received MEVCF (400mg/kg b. w.) p. o.+ AEST	Served as test group-IV and received MEVCF (400mg/kg b. w.) p. o.+ AEST + CCl_4 s.c. (2.0ml/kg. b. w.) on the 21st day.

Note: MEVCF- Methanolic extract of *Vigna aconitifolia*, p. o. - Per oral, s. c. – subcutaneously, b. w. – body weight.

RESULTS

Preliminary phytochemical studies revealed the presence amino acids, carbohydrates, glycosides, flavonoids, tannins and phenolic compounds, saponins, ascorbic acid etc.

Parameters assessed for liver functions

Effect of methanolic extract of *Vigna aconitifolia* (MEVCF) seeds on selected parameters in fed with AEST (0.015%w/w) mixed pellet diet without and with CCl_4 induced hepatotoxicity

Aqueous extract of smokeless tobacco (AEST) and AEST with CCl4 positive control groups of rats produced an decreased body weight (% change) and increased liver weight compared to normal. Whereas rats pretreated with silymarin and MEVCF showed a significantly decreases compared to positive control groups [Table 2]. AEST and AEST with CCl4 positive control groups resulted in significant elevation of SGOT, SGPT, ALP, Bilirubin (Total & Direct) levels and antioxidant defense parameters (LPO, SOD, CAT, GSH) compared to the normal control group. And AEST with CCl4 positive control group elevated marker levels than AEST only intoxicated positive control group. Pretreatment with silymarin and MEVCF (200, 400mg/kg p. o.) significantly prevented the biological changes [Table 4, 5] and antioxidant parameters status [Table 3] compared to positive control. Histological studies of liver AEST with CCl4 section highly damaged than AEST only intoxicated positive control group compared with normal group. Pretreated MEVCF 400mg/kg group moderately regenerate the liver disturbed architecture than silymarin (25mg/kg) and MEVCF 200mg/kg groups compared to normal [Fig1 (A-E)AEST; Fig2(A-E)AEST+CCl4].

Parameters assessed for kidney functions

Effect of methanolic extract of *Vigna aconitifolia* (MEVCF) seeds on selected parameters in fed with AEST (0.015%w/w) mixed pellet diet without and with CCl_4 induced nephrotoxicity

Aqueous extract of smokeless tobacco (AEST) and AEST with CCl4 positive control groups of rats produced an decreased body weight (% change) and increased kidney weight compared to normal.Whereas rats pretreated with silymarin and MEVCF showed a significantly decreases compared to positive control groups [Table 2].

AEST and AEST with CCl4 positive control groups resulted in significant elevation of serum creatinine, Blood urea nitrogen (BUN), uric acid, total protein levels and antioxidant defense parameters (LPO, SOD, CAT, GSH) compared to the normal control group. And AEST with CCl4 positive control group elevated marker levels than AEST only intoxicated positive control group. Pretreatment with silymarin and MEVCF (200, 400mg/kg p. o.) significantly prevented the biological changes [Table 7] and antioxidant parameters status [Table 6] compared to positive control.

Histological studies of kidney section AEST with CCl4 section highly damaged than AEST only intoxicated positive control group compared with normal group.

Pretreated MEVCF 400mg/kg group moderately regenerate the liver disturbed architecture than silymarin (25mg/kg) and MEVCF 200mg/kg groups compared to normal. [Fig3(A-E)AEST;[Fig4(A-E)AEST+CCl4].

Table 2: Effect of methanolic extract of *Vigna aconitifolia*(MEVCF) seeds on selected physical parameters in rats fed with AEST(0.015%w/w) mixed pellet diet without and with CCl_4 induced hepato and nephrotoxicity.

Groups	Body weight % change		Wet liver Weight (g/100g)		Wet kidney Weight (g/100g)	
	Without CCl_4	With CCl_4	Without CCl_4	With CCl_4	Without CCl_4	With CCl_4
Normal	8.775±1.182	9.08±1.138	4.083 ± 0.239	4.058± 0.202	0.545±0.0241	0.494±0.024
AEST(0.015%w/w)	-30.375±1.645[a]	-37.43±1.712[a]	8.288 ± 0.235[b]	11.733±0.222[a]	0.817±0.0249[b]	1.099±0.039[a]
Std Silymarin(25mg/kg)	-2.998±0.269***	-3.28± 0.217***	5.588± 0.268***	7.66±0.243**	0.605±0.0268***	0.845±0.029***
MEVCF(200mg/kg)	-3.023±0.270***	-3.318 ± 0.217***	5.665± 0.267***	7.682± 0.245**	0.614±0.0237***	0.851±0.026***
MEVCF (400mg/kg)	-1.488± 0.266***	-2.328 ± 0.263***	4.582± 0.255***	5.775± 0.254***	0.558 ±0.0238***	0.576±0.026***

All values are expressed as a mean ± SEM, n=6, ns= not significant;

Table 3: Effect of methanolic extract of *Vigna aconitifolia*(MEVCF) seeds on selected antioxidant defense parameters status in rats fed with aqueous extract of smokeless tobacco AEST(0.015%w/w) mixed pellet diet without and with CCl_4 induced hepatotoxic rats of liver tissue.

Groups	LPO nmoles /mg Of protein		SOD (U/mg of protein)		GSH(mg/g of tissue)		CAT(µmoles/mg Of protein)	
	WithoutCCl$_4$	With CCl_4	WithoutCCl$_4$	With CCl_4	WithoutCCl$_4$	With CCl_4	WithoutCCl$_4$	With CCl_4
Normal	108.847±2.36	105.648±2.518	35.287±2.420	37.765±2.265	8.325±0.566	9.088±0.736	37.245±2.454	41.253±2.4
AEST (0.015% w/w)	267.058±2.40[a]	295.05±2.510[a]	20.8±2.374[a]	17.615±2.41[a]	1.802±0.601[a]	1.803±0.58[a]	18.80±2.364[a]	16.7±2.33[a]
Std(SIL) (25mg/kg)	149.58±2.42***	156.0±2.17***	27.3±1.86***	25.24±2.39***	5.14±0.76***	4.6±0.62***	32.18±2.44***	32±2.5***
MEVCF (200mg/kg)	149.73±2.42***	156.3±2.20***	27.24±1.86***	25.23±2.397***	5.113±0.77***	4.602±0.61***	32.16±2.4***	32±2.5***
MEVCF (400mg/kg)	135.60±2.46***	142.2±2.29***	29.34±2.40***	28.278±2.399***	6.558±0.62***	6.495±0.66***	34.145±2.54***	36.6±2.3***

All values are expressed as a mean ± SEM, n=6, ns= not significant;

Table 4: Effect of methanolic extract of *Vigna aconitifolia*(MEVCF) seeds on selected serum biochemical parameters in rats fed with AEST(0.015%w/w) mixed pellet diet without and with CCl_4 induced hepatotoxic rats.

Groups	SGOT/AST (IU/L)		SGPT/ALT (IU/L)		ALP (IU/L)	
	Without CCl_4	With CCl_4	Without CCl_4	With CCl_4	Without CCl_4	With CCl_4
Normal	61.876±2.439	54.92±2.431	27.933±5.061	35.94± 2.475	74.038± 24.7	69.13±2.1975
AEST(0.015%w/w)	368.99 ±3.34[a]	464.79± 2.44[a]	301.295±2.44[a]	394.53±2.29[a]	284.953±2.44[a]	325.96±2.43[a]
Std SIL (25mg/kg)	115.19 ±1.70***	122.23±2.42**	59.00 ±2.40***	66.86±3.89***	88.595±2.29***	91.98±2.41**
MEVCF(200mg/kg)	114.73 ±1.66***	121.93±2.413**	59.02 ±2.3806***	66.04±4.13***	89.858±2.408***	93.018±2.45**
MEVCF(400mg/kg)	82.3 ±2.329***	94.48±2.228***	46.907 ±2.476***	54.903±2.502***	75.59 ± 2.419***	76.608±2.415***

All values are expressed as a mean ± SEM, n=6, ns= not significant;

Table 5: Effect of methanolic extract of *Vigna aconitifolia*(MEVCF) seeds on selected serum biochemical parameters in rats fed with AEST(0.015%w/w) mixed pellet diet without and with CCl_4 induced hepatotoxic rats.

Groups	Bilirubin levels Without CCl_4 (mg/dl)		Bilirubin levels With CCl_4 (mg/dl)	
	Total	Direct	Total	Direct
Normal	0.216±0.022	0.182 ± 0.0148	0.196±0.0185	0.187±0.020
AEST(0.015%w/w)	2.598±0.048[a]	3.352 ±0.586[a]	3.173±0.116[a]	3.46± 0.238[a]
Std SIL (25mg/kg)	0.700±0.0189***	0.713 ± 0.0233***	0.873±0.027***	0.82±0.0168***
MEVCF(200mg/kg)	0.718±0.0169***	0.714 ± 0.0237***	0.875± 0.024***	0.828± 0.016***
MEVCF(400mg/kg)	0.495 ± 0.0241***	0.517± 0.0246***	0.652 ± 0.026***	0.698± 0.027***

All values are expressed as a mean ± SEM, n=6, ns= not significant;

Table 6: Effect of methanolic extract of *Vigna aconitifolia*(MEVCF) seeds on selected antioxidant defense parameters status in rats fed with aqueous extract of smokeless tobacco AEST(0.015%w/w) mixed pellet diet without and with CCl4 induced nephrotoxic rats of kidney tissue.

Groups	LPO nmoles /mg Of protein		SOD (U/mg of protein)		GSH (mg/g of tissue)		CAT (µmoles/mg Of protein)	
	WithoutCCl	With CCl_4	Without CCl_4	With CCl_4	WithoutCCl$_4$	With CCl_4	WithoutCCl$_4$	With CCl_4

	[4]							
Normal	101.26±2.272	96.143±2.29	38.282±1.895	39.337±2.430	7.217±0.754	8.287±0.552	35.242±2.408	37.078±1.889
AEST (0.015% w/w)	256.6 ±2.270[a]	277.145±2.45a	19.387±2.517[a]	20.405±2.530[a]	2.093±0.500[a]	1.985±0.539[a]	16.283±2.185[a]	17.853±2.377[a]
Std(SIL) (25mg/kg)	140.1±2.6***	153.8±2.37**	29.3±2.4198***	28.45±2.47***	4.1±0.779**	4.21±0.730***	29.31±2.41***	27.082±2.39***
MEVCF (200mg/kg)	140.7±2.4***	153.8±2.37**	31.26±2.427***	28.28±2.40***	4.1±0.780**	4.18±0.731***	29.298±2.41***	30.34±2.42***
MEVCF (400mg/kg)	129.2±2.2** *	138.6±2.4** *	33.33±2.441** *	30.29±2.40** *	5.61±0.614** *	5.54±0.628** *	31.41±2.426** *	32.378±2.43** *

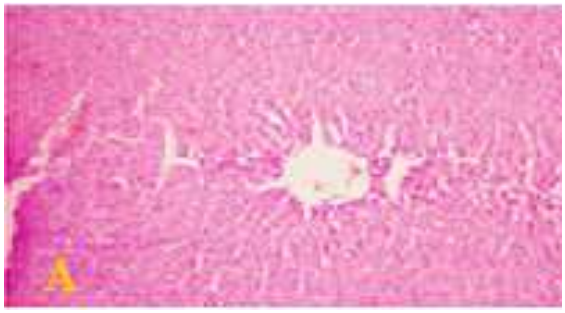
All values are expressed as a mean ± SEM, n=6, ns= not significant;

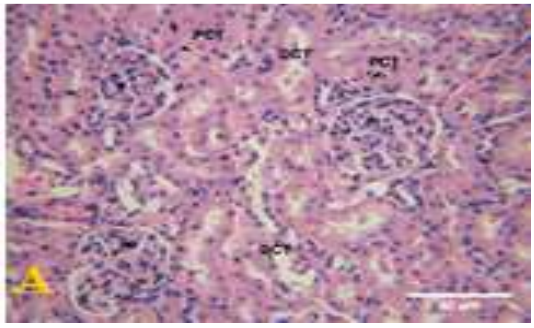
Table.7: Effect of methanolic extract of *Vigna aconitifolia* (MEVCF) seeds on selected serum biochemical parameters in rats fed with AEST(0.015%w/w) mixed pellet diet without and with CCl4 induced nephrotoxic rats.

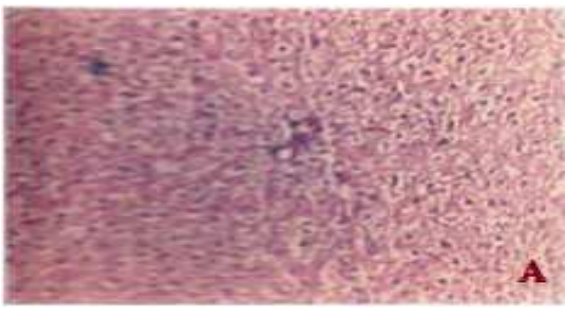
Groups	Creatinine (mg/dl)		Uric acid (mg/dl)		Blood ureanitrogen(BUN)		Total Protein	
	Without CCl_4	With CCl_4	Without CCl_4	With CCl_4	Without CCl_4	With CCl_4	Without CCl_4	With CCl_4
Normal	0.615±0.024	0.799 ±0.0242	1.907±0.238	2.0583±0.212	20.185±2.225	22.795±2.462	7.88 ±0.245	7.2±0.236
AEST (0.015% w/w)	1.992±0.244[b]	3.612±0.239[a]	8.776±0.242[a]	11.197±0.237[a]	66.645±2.086[a]	87.107±2.209[a]	3.438±0.227[a]	2.89±0.248[a]
Std(SIL) (25mg/kg)	1.58±0.249***	1.69±0.246***	4.885±0.246***	6.64±0.269***	43.92±2.38***	59.68±1.95***	6.52±0.242***	4.74±0.247***
MEVCF (200mg/kg)	1.59±0.249***	1.70±0.24***	4.882±0.24***	6.628±0.247***	43.933±2.394***	59.7±1.956***	6.49±0.246***	4.72±0.241***
MEVCF (400mg/kg)	1.35±0.193***	1.4±0.238***	4.407±0.249***	5.697±0.248***	35.953±2.456***	45.83±2.459***	6.912±0.23***	5.69±0.242***

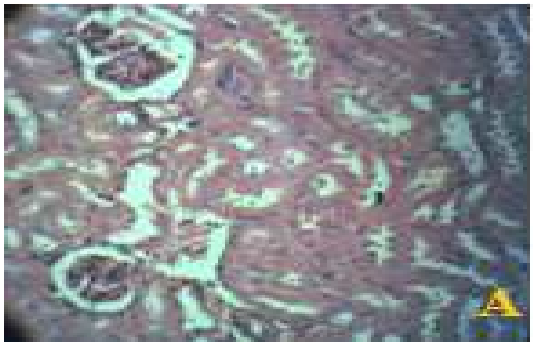
All values are expressed as a mean ± SEM, n=6, ns= not significant; One-way Analysis of Variance(ANOVA)fallowed by tukey's multiple comparison tests,*p<0.05,**p<0.01,***p<0.001 as compared to control and a p<0.001.b p<0.01,c p<0.05 as compared with normal group. AEST-Aqueous extract of smokeless tobacco, MEVCF-Methanolic extract of *Vigna aconitifolia*, CCl4- Carbon tetrachloride, SIL- Silymarin.

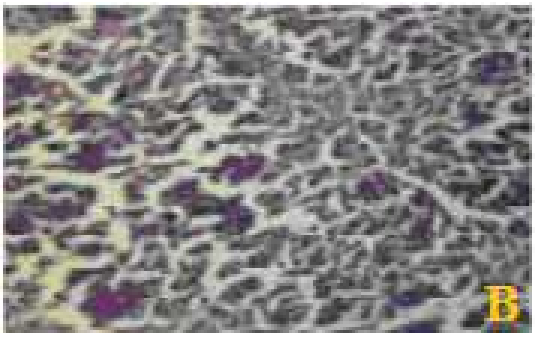
Rats liver histopathology: Rats kidney histopathology







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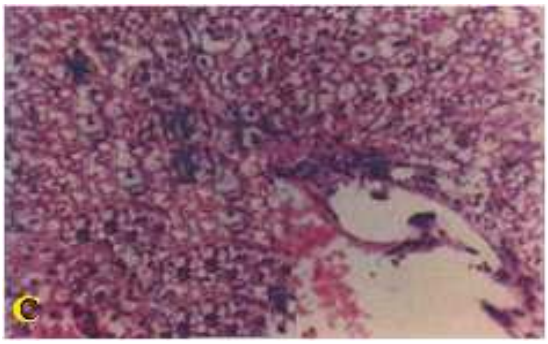
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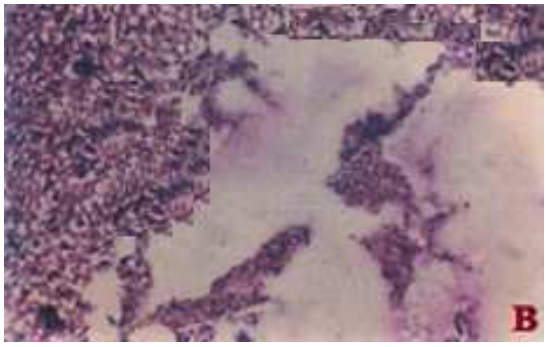
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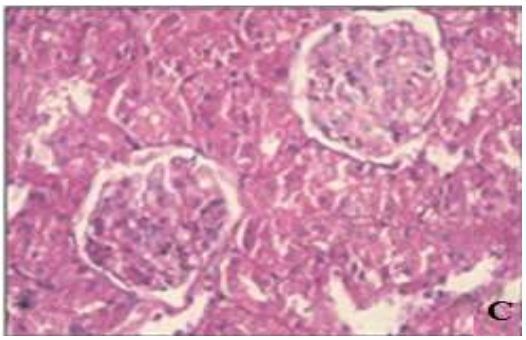
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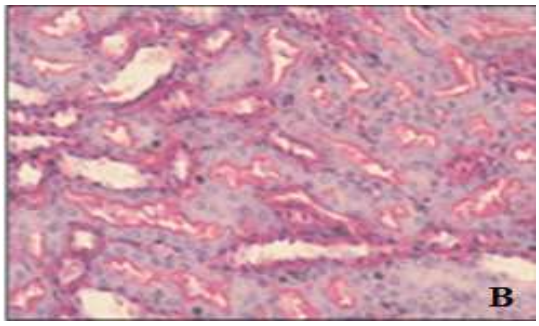
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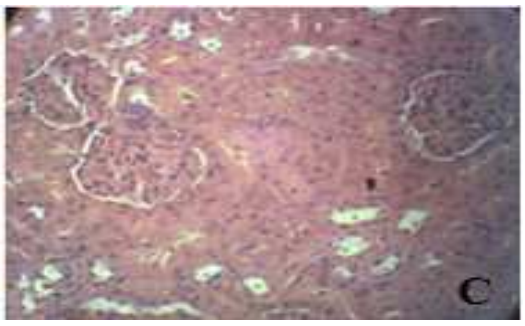
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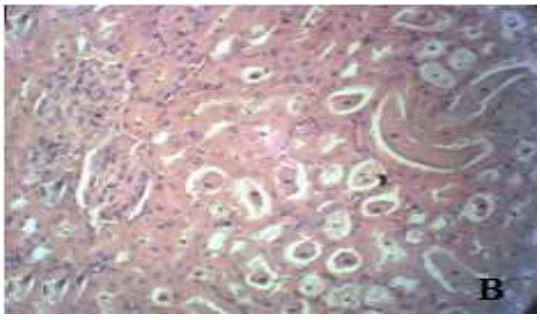
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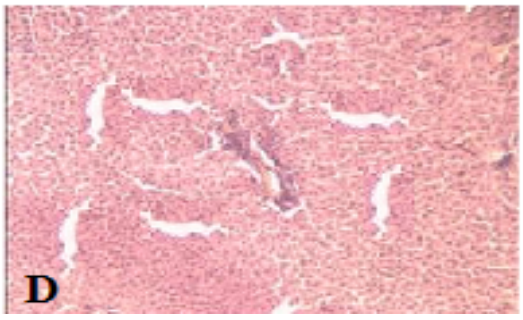
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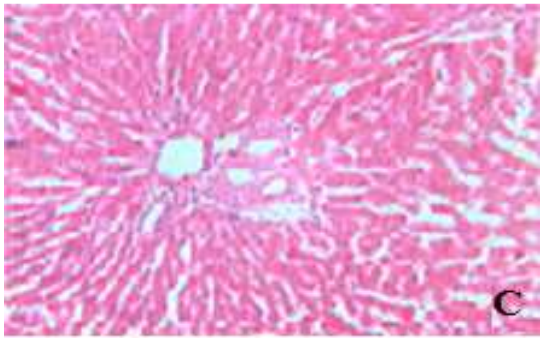
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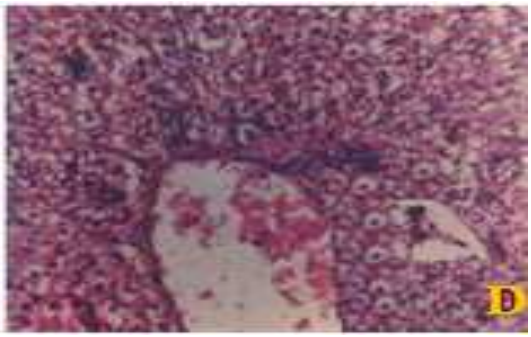
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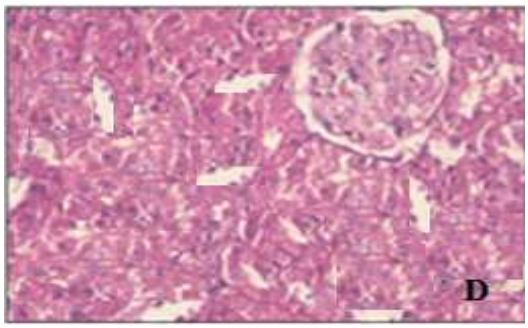

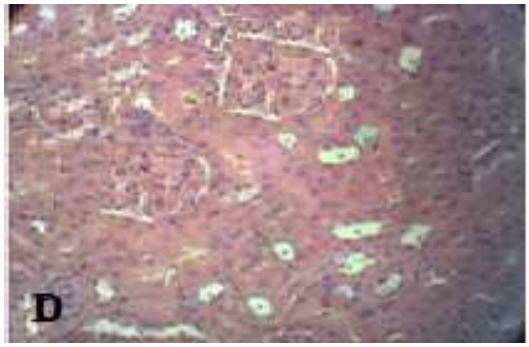

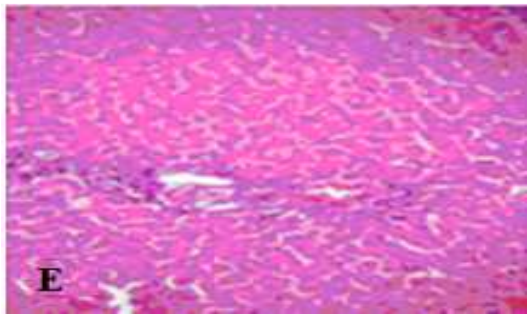

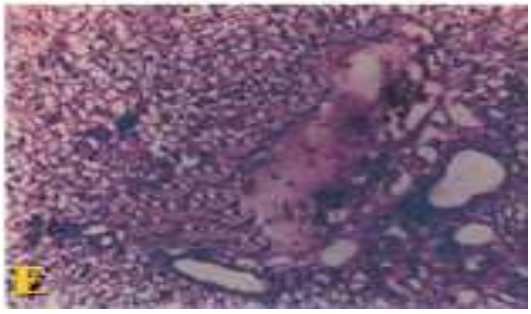

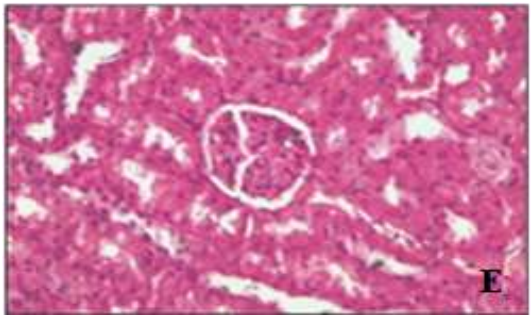

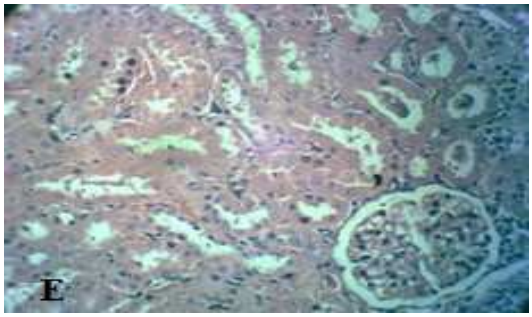

Fig. 1: (A-E) AEST; Fig-2 (A-E) AEST+CCl_4 Figure-3(A-E) AEST; Fig-4 (A-E) AEST + CCl_4

Rats liver histological studies: Fig-1(A-E) (H&EX400) AEST:1A-Normal control group: Normal Control rats showing normal hepatic cells with well preserved cytoplasm; well brought out central vein; prominent nucleus and nucleolus.1B- Control group AEST (0.015%w/w): rats liver section showing disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration. 1C- Silymarin 25mg/kg +AEST (0.015%w/w): rats liver section showing regeneration of hepatic cells and reducing mild necrosis, vacuolization of cytoplasm. 1D- MEVCF (200mg/kg) +AEST (0.015%w/w: Rats liver section showing regeneration of hepatic cells arrangement and reducing necrosis, vacuolization of cytoplasm. 1E- MEVCF (200mg/kg) +AEST (0.015%w/w): Rats liver section showing moderate regeneration of hepatic cells arrangement and moderate reducing necrosis, vacuolization of cytoplasm and resembling nearer to normal group. Fig-2(A-E) (H&EX10) AEST + CCl_4: 2A- Normal control group: Rats liver section showing normal hepatic cells each with well defined cytoplasm, prominent nucleus, and nucleolus and well brought out central vein. 2B- Control group AEST (0.015%w/w) + CCl_4: Rats liver section showing total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia, crowding of central vein and apoptosis. 2C- Silymarin 25mg/kg +AEST (0.015%w/w) + CCl_4: Rats liver section showing regeneration hepatic architecture and mild necrosis, reducing vacuolization, apoptosis. 2D-MEVCF (200mg/kg) +AEST (0.015%w/w) +CCl_4: Rats liver section showing regeneration hepatic architecture and mild reducing necrosis, vacuolization, apoptosis. 2E- MEVCF (200mg/kg) +AEST (0.015%w/w) +CCl_4: Rats liver section showing moderate regeneration hepatic architecture and moderate reducing vacuolization, apoptosis, congestion of sinusoids.

Rats kidney histological studies: Fig-3(A-E) AEST: 3A- Normal control group: Sections of normal rat kidney renal corpuscles showing normal glomerulus, brush borders of proximal and distal convoluted tubules. 3B- Control group AEST (0.015%w/w): Hydropic changes seen in tubular epithelium. 3C- Silymarin 25mg/kg +AEST (0.015%w/w): Showing regenerative Bowman's capsule (RBC) and vacuolization. 3D-MEVCF (200mg/kg) +AEST (0.015%w/w): Showing regenerative Bowman's capsule (RBC) and vacuolization AEST+ MEVCF (200mg/kg). 3E- MEVCF (200mg/kg) +AEST (0.015%w/w): Showing moderate regenerative Bowman's capsule (RBC) and tubular epithelial cells, reduced vacuolization AEST + MEVCF (400mg/kg). Fig-4(A-E) AEST + CCl_4: 4A- Normal control group: Rat kidney section showing normal glomeruli with an intact Bowman's capsule, proximal and distal convoluted tubules. 4B- Control group AEST (0.015%w/w) + CCl_4: rat kidney section showing severe glomerular degeneration and severe hydropic tubular degeneration and severe tubular cast and obliteration of the tubular lumen. 4C- Silymarin 25mg/kg +AEST (0.015%w/w) + CCl_4: Rats kidney section showing some degree of mesengial proliferation with mild hydropic tubular degeneration and mild tubular cast interposed with normal proximal and distal convoluted tubules. 4D- MEVCF (200mg/kg) +AEST (0.015%w/w) +CCl_4: Rat kidney section showing some degree of mesengial proliferation with mild hydropic tubular degeneration and mild tubular cast interposed with normal proximal and distal convoluted

tubules. 4E- MEVCF (200mg/kg) +AEST (0.015%w/w) +CCl_4: A rat kidney at showing moderate tubular regeneration with normal glomeruli and Bowman's capsule.

DISCUSSION

Hepatoprotective and nephroprotective activity

The present study similar to the Protective effect of *Bacopa monnieri* leaf extract against oxidative stress induced hepatotoxicity in rats. [24] But our AEST and AEST with CCl4 have also possessed production of nephrotoxicity, our seed extract has hepato and nephroprotective activity may be due to presence of high levels of antioxidants like *Vigna* spieces. [25] Dose selection of AEST diet was 0.015%. [9] The results of this work indicate that AEST treatment caused a significant increase in the levels of liver serum markers, physical parameters and kidney serum markers indicates liver and kidney damage. This was further augmented on exposure to a single assault of CCl_4. The antioxidant defense parameters in kidney and liver tissues also impairs of AEST with CCl_4 than AEST alone. The standard antioxidant silymarin (25 mg/kg p. o.) and MEVCF (200, 400 mg/kg p. o.) were very effective in reducing the levels and restores the impairment of antioxidant defense status to the control levels. Histological studies of liver and kidney sections positive controls also hepatic architecture damaged and increased necrosis and sinusoidal congestion in liver and glomeruli damage, tubular epithelium, increased vacuolization, necrosis in kidney [26] showed AEST with CCl_4 than AEST alone. The habit of using gutka is increasing because of the cheapness, bright pouches, easy availability, sweet taste and forceful misleading advertisements, highly addictive. Smokeless chewing tobacco significantly leads to cause cardiac disorders, oral cancer and other organ disorders such as liver, kidney, and lungs.[7] Its chemical analysis mixture of arecanut, tobacco, lime have shown the presence of poly-aromatic hydrocarbons, nitrosamines and toxic elements. The intoxication by these AEST can induce the microsomal cytochrome P450, a source of ROS. Superoxide anion and hydrogen peroxide are the ROS, cause lipid peroxidation and oxidative damage. [27] In addition CCl_4 is one of the most is metabolized to the oxidative moiety by the cytochrome commonly used hepatotoxins and has nephrotoxic activity. The oxidant used to induce stress in vivo was CCl_4 which P450cytochrome P**450** 2E1 (CYP2E1) isoenzyme. CCl_4 treatment generates free radicals that trigger a cascade of events resulting in hepatic fibrosis. [24] The mechanism by which CCl4 causes cell oxidative injury involves cytochrome P450 system that transforms CCl4 into CCl3 and then transformed into more reactive CCl3O2. CCl3O2 causes lipid peroxidation, disturbs Ca^{2+} homeostasis and eventually kills cells. [28] The liver and kidney also possesses a CYP450 enzyme that participates in drug metabolism. An MDA level in LPO is of AEST and AEST with CCl4 increases levels. MDA is a major reactive aldehyde resulting from the peroxidation of biological membrane polysaturated fatty acids, is used as an indicator of tissue damage by a series of chain reactions. Oxidative stress induced lipid peroxidation effects of AEST on the hepatic pulmonary and kidney antioxidant defense; it had more deleterious effects on liver than kidney. These effects highly increase with CCl_4. In recent times, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing tissue injuries caused by free radicals. It has been proved that to decrease elevated liver serum markers and kidney serum markers. And restoring of antioxidant parameters status levels such as antioxidant activity and possesses hepatoprotective and nephroprotective activity of *Vigna mungo, Coriandrum sativum, Dashamula, Allanblackia gabonensis, Curcumin* and vitamin E. Present study of *Vigna aconitifia* seeds possess an antioxidant activity and diuretic activity because of the presence of saponins in *Vigna* species. Phenolic compounds have high levels of antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species.

Flavonoids, tannins, and saponins have been reported in vitro and in vivo stabilizing effect on the Lysosomes of experimental animals. Tannins and saponins stabilize the erythrocyte membrane by binding cations and other bimolecules. [29] In present study, from the above reports of research shows the *Vigna aconitifolia* seeds posses antioxidant and diuretic activity. Our results have protective activity against AEST and AEST with CCl_4.and.significantly.restoring.the.levels.

CONCLUSION

The *Vigna aconitifolia* seeds methanolic extract, produced adequate hepatoprotective and nephroprotective activity on albino wistar rats as evidenced by physical, biochemical, histological, and antioxidant defense parameters of liver and kidney, respectively. The *Vigna aconitifolia* seed extract effectively increased with dose. From the above discussion it conclude that the *Vigna aconitifolia* seeds methanolic extract (MEVCF) at high doses (200, 400 mg/kg) exhibited significantly hepatoprotective and nephroprotective activity against AEST and AEST with CCl_4 induced hepatotoxicity and nephrotoxicity, respectively due to the presence of strong antioxidants such as total phenolic compounds, tannins, and flavonoids, phytic acid, glycosides, saponins.

ACKNOWLEDGEMENT

The first author very grateful to her mother, co-authors, and Dr K. Madhava chetty, who had authenticated and all authors are thankful to the management of Nimra College of Pharmacy, Jupudi, Vijayawada, for providing facilities.

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