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HEPATOPROTECTIVE ACTIVITY OF WHOLE PLANT EXTRACT OF *VIGNA MUNG* LINN AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE MODEL

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ABSTRACT

Whole plant part of Vigna mung linn were extracted using petroleum ether, chloroform, ethanol consecutively and the obtained extracts were screened for hepatoprotective acitivity using ccl_4 induced liver damage model . The activity was assessed by comparing the serum enzyme levels such as serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, total bilirubin, alkaline phosphatase of plant extracts treated group with carbon tetrachloride treated animals and results showed dose dependent activity, ethanolic extract treated group showed highly significant activity, where as chloroform extract treated group has shown the significant action but less compare ethanolic extract, petroleum ether treated group showed moderate action and petroleum ether extract at a dose of 50 mg/kg b.w showed least significant action. The results were further supported by histopathological studies.

KEYWORDS

Vigna mung, serum glutamate oxalo acetate, serum glutamate pyruvate transaminase, total bilirubin, alkaline phosphatise

INTRODUCTION

The liver is the largest organ of the body. It is located between the portal and the general circulation, between the organs of the gastrointestinal tract and the heart. The main function of the liver is to take up nutrients, to store them, and to provide nutrients to the other organs[1]. The liver is not only an important power and sewage treatment plant of the body. In fact, the liver is probably the best example for a cheap recycling system. The function of the liver as clearance organ, however, harbors the danger that the substances that should be degraded and/or eliminated lead to tissue damage. Thus, effective defense mechanisms are necessary. During the process of elimination there is chance of accumulation different kinds of toxic materials inside the hepatocytes and there is chance of liver infection, and hepatic disorders such as hepatitis. Even though different kind of allopathic molecules are available in market all of them are suffer with some are the other toxic effect, so an urgent need of developing a herbal medicine which has got both liver protecting and nutritional value is require hence an attempt has been made to screen the hepato protective acitivity of whole plant extracts of vigna mung Linn. It is commonly called as black gram which belongs to leguminosae family, in telugu it is called as minnumulu which is widely used as diet in the day to day life. It has comprised of many proteins and enzymes which has got the folklore usage in liver disorders, rheumatisim, curing infections of nervous system[2]. Roots are said to be narcotic and are used as remedy for aching bones, black gram is considered as diuretic and is used in dropsy and cephalgia[3]. So it got good values both as food and as medicine, in the hot

summer, mung bean soup are nice drinks for local folks to drive away heat[4].

MATERIALS AND METHODS:

Collection of specimens: The whole plant of *Vigna mung* Linn were collected from the nearby area of Guntur district fields in February2011 and was authentified by prof.D.Ramakanth raju retire botanist and a voucher specimen (T.S.N-001, 13/12/2011) has been deposited in pharmacognosy department Andhra university.

Preparation of plant extracts:

Collected plant material has been dried under shade and made into coarse powder passed through sieve# 20 and has been successively soxhelated using solvents like petroleum ether, chloroform and ethanol for 72 hrs. Obtained extracts were made solvent free using rota evaporator and stored in vacuum dessicator. Yield was found to be 7%, 9.5% and 13.5% respectively. Obtained extracts were tested for preliminary phytochemical screening[5]. Oral suspensions of the extracts were prepared at a dose of 50mg/ml and 100mg/ml using 5% aqueous gum acacia.

Acute toxicity studies:

Adult swiss albino mice 20-25gm were taken for acute toxicity tests. The mice were divided into control and test groups containing 6 animals each. The control group receive vehicle (5%of normal saline) and the test group receive graded doses of extracts. The animals were observed carefully up to 4 hours then occasionally up to 48 hours for sign of any behavioural changes and motility and LD 50 values were calculated[6].

Determination of hepato protective activity:

The experimental protocol was approved by the animal ethical committee of Andhra university, Visakhapatnam, which was registered with the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt of India (registration no 516/01/A/CPCSEA)

Selection of animals:

Wistar albino rats weighing 150-200gm of either sex were used. The animals were fed with balanced diet and tap water *ad libitum.* The animals were maintained at room temperature and 40-70% RH with 12hr light period (6:00-18:00). The animals were divided into control Group I received vehicle 5% aqueous gum acacia, Group II to Group IX received CCl_4 for 7 days at a dose of 0.25 ml/100gm[7], Group II serves as toxic group receives only CCl_4 , Group III serves as standard receives silymarin 50mg/kg b.w and group IV received *Vigna mung* petroleum ether extract(V.M.P.E) at a dose of 50 mg/kg b.w, group V received *Vigna mung* petroelum ether extract (V.M.P.E) at a dose of 100mg/kg b.w, Group VI received *Vigna mung* chloroform (V.M.C.E)at a dose of 50mg/kg b.w, GroupVII received *Vigna mung* chloroform extract(V.M.C.E) at a dose of 100mg/kg b.w, Group VIII received ethanolic extract(V.M.E.E) at a dose of 50mg/kg b.w, and group IX received *Vigna mung* ethanolic extract(V.M.E.E) at a dose of 100mg/kg b.w. Each group consisting of 6 animals. The vehicle and the test samples were administered orally for 7 days and the liver damage was induced in rats on the 7th day after 6 hrs of administration of drug, by giving a single oral dose of CCl_4 in olive oil (1:1 ratio). On the 8th day, the blood samples were with drawn by puncturing retro-orbital plexus[8]. The blood samples were allowed to clot for 30 min, and serum was separated by centrifuging at 2500rpm for 10 min.

Assessment of liver function:

Assessment of liver function was done by studying changes in biochemical parameters. Viz Serum glutamic oxaloacetate transaminase (SGOT)/ (AST) and serum glutamic pyruvic transminase (SGPT)/ (ALT) were estimated by

Reitman and Frankel method[9]. Total bilirubin[10], Alkaline phosphatase were also estimated[11].

Statistical analysis[12]:

The results are expressed as mean ± s.e.m and the stastical significance of difference between groups was analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. P<0.05 was considered as significant. The percentage reduction was calculated by considering the difference between mean values of toxicant and control as 100% reduction.

Histological study:

For histopathological study, liver from each animal was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and possessed for paraffin embedding using the standard microtechnique[13]. Sections (5 µm) of livers stained with hemotoxylin and eosin were observed microscopically for histopathological studies.

Table: 1 Results of preliminary phytochemical tests of the extracts:

S.no	Chemical constituents	Pet ether extract	Chloroform extract	Ethanolic extract
1	Alkaloids	+	-	+
2	Amino acids	+	+	+
3	Carbohydrates	+	+	+
4	Flavonoids	-	-	-
5	Mucilage	+	+	+
6	Proteins	+	+	+
7	Starch	+	-	+
8	Steroids and triterpenoids	+	+	+
9	Glycosides	-	-	-

+ indicates presence of constituents, - indicate

Table: 2 Effect of vigna mung whole plant extracts on serum biological parameters in ccl_4 induced liver damage model

S.No	Groups	SGOT U/L(mean±S.E.M)	SGPT U/L (mean±S.E.M)	Alkaline phosphatase (mean±S.E.M)	Total bilirubin (mg/dl) (mean±S.E.M)
1	Control group -I	120.17±05.77	106.76±03.78	190.84±06.99	1.58±0.20
2	Toxic control GROUP-II	350.49±22.16**	394.02±16.54**	440.50±13.76**	4.71±0.51**
3	Standard GROUP-III	124.23± 05.28*	122.24±05.17*	186.18±10.12*	2.05±0.19*
4	V.M.P.E GROUP-IV (50mg/kg)	361.74±12.02	391.11±05.22	426.22±14.22	3.94±0.06
5	V.M.P.E GROUP-V (100mg/kg)	340.12±07.01	356.21±16.11	407.14±04.12	3.16±0.23
6	V.MC.E Group-VI (50MG/KG)	305.43±10.26*	311.83±10.14*	399.26±07.21*	3.01±0.44*
7	V.M.C.E group-VII (100mg/kg)	291.50±18.03*	260.57±05.71*	284.16±08.99*	2.76±0.64*
8	V.M.E.E group-VIII(50mg/kg)	204.50±07.21*	201.14±09.29*	209.17±14.57*	2.43±0.23*
9	V.M.E.E group-IX(100mg/kg)	150.21±06.18*	136.24±17.22*	197.19±11.21	2.31±0.62*

Values are mean ± SEM, n=6

****P<0.01, when compared with control Group,* P<0.01, when compared with Toxic Group**

Table-3 Percentage decrease in biochemical parameters due to treatment with plant extracts of *Vigna mung*

Groups	% Decrease In Levels Of			
	SGOT	SGPT	AKLP	Total Bilirubin
Standard Silymarin	98.42%	94.68%	102.04%	85.02%
V.MP.E (50mg/kg)	4.58%	1.04%	5.62%	24.61%
V.M.P.E (100mg/kg)	4.86%	13.22%	13.25%	49.54%
V.M.C.E (50mg/kg)	19.6%	28.89%	16.47%	54.34%
V.M.C.E (100mg/kg)	25.74%	46.64%	62.67%	62.33%
V.M.E.E (50mg/kg)	63.69%	67.18%	92.80%	72.88%
V.M.E.E (100MG/KG)	87.2%	89.81%	97.62%	76.71%

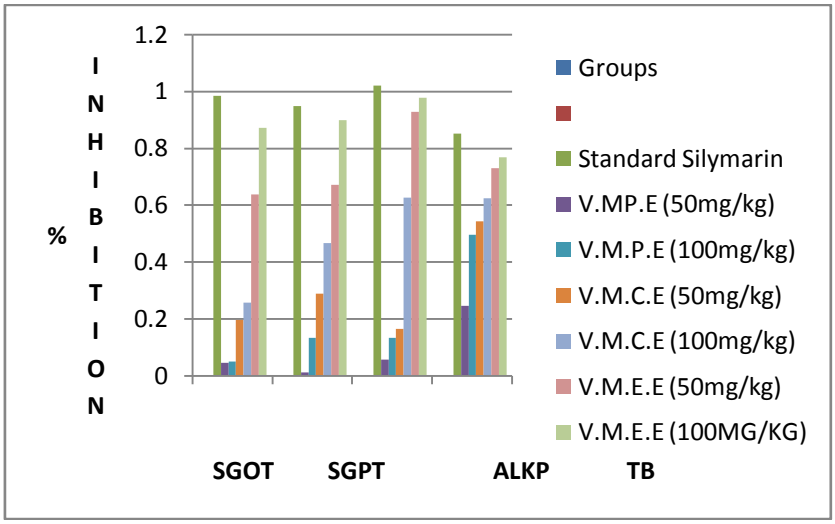
RESULTS AND DISCUSSION:

This work is an attempt made for the validation of rational usage of Vigna mung Linn as a heptoprotective agent in liver infections. In acute toxicity study no mortality was found up to 1000mg/kg p.o of *Vigna mung* plant extracts treated animal group. The LD_{50} was not determined and $1/10^{th}$ of the tested proven safe concentration is taken as our experimental dose.

CCl_4 is a hepatoxin commonly used for the production of experimental liver toxicity[14]. The serum transaminase level is most widely used as a measure of hepatic injury, due to its ease of measurement and high degree of sensitivity. It is useful for the detection of early damage of hepatic tissue and requires less effort than that required for a histological analysis, moreover without sacrifice of animal. From the results, it was observed that there is a significant increase in the levels of SGOT, SGPT & total bilirubin in the toxicant group. Pretreatment with plant extracts and silymarin in test groups and standard group respectively daily for seven days showed significant($p<0.01$) protective effect against CCl_4 induced hepatotoxicity when compared to toxicant group. From the results, it was observed that the percentage reduction in silymarin pretreated group in the biochemical parameters, SGOT, SGPT, ALKP, TB were found to be 98.42, 94.68,102.04 and 85.02% respectively , where as in the *Vigna mung* ethanolic extract at a dose of 100mg/ kgb.w has shown higly significant action as 87.2%, 89.81%, 97.62% and 76.71% respectively. Chloroform extract pretreated group showed significant reduction in biochemical parameters, where as petroleum ether extract showed moderatly significant action when compared to other extracts.

Graph: 1

PERCENTAGE INHIBITION OF LIVER BIOCHEMICAL PARAMETERS OF V.MUNG EXTRACTS IN CCL_4 INDUCED HEPATTOTOXICITY MODEL



Hence the ethanolic extract, chloroform extract of *V.mung* at the dose of 100mg/kg and 50mg/kg were found to have significant hepatoprotective activity. The hepato protective activity of *V.mung* could be due to the presence of alkaloids[15], proteins, and mucilage in case of chloroform extract. Whereas ethanolic extract possess proteins and mucilage[16] which also are reported to have hepato protective and antioxidant properties.

HISTOPATHOLOGICAL SECTIONS OF LIVER IN RATS

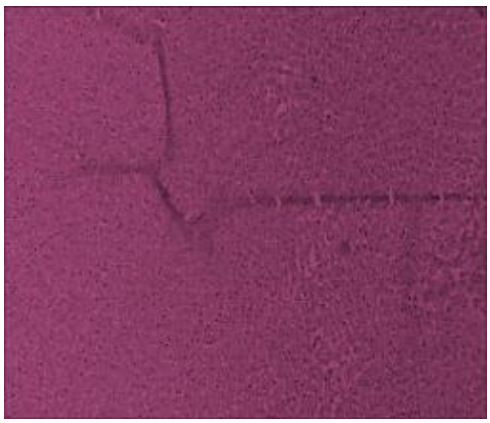


Fig:1 Hitopathological section of liver in Animals treated as Control Group-I shows normal cellular structure.

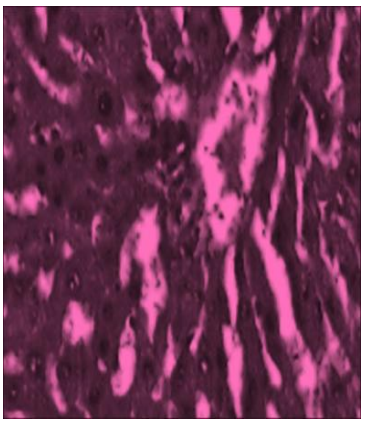


Fig:2: Histopathological section of liver inGroup II animals treated as Toxic group, treated with carbon tetrachloride showing central lobular vein with high amount of necrosis

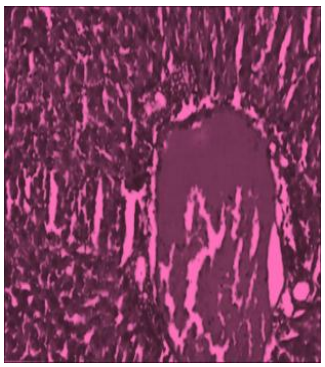


Fig: 3 Histopathological section of liver inGroup III animals treated as standrad group, treated with carbon tetrachloride +Silymarin at a dose (10mg/kg b.w).

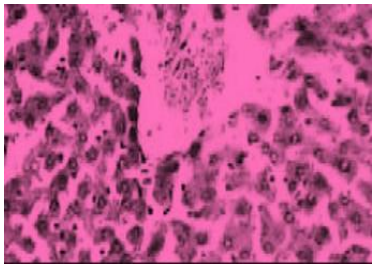


Fig: 4 histopathological section of liver inGroup V animals treated as standard group, treated with carbon tetrachloride + pet ether extract of *V.mung* at a dose (100mg/kg b.w).

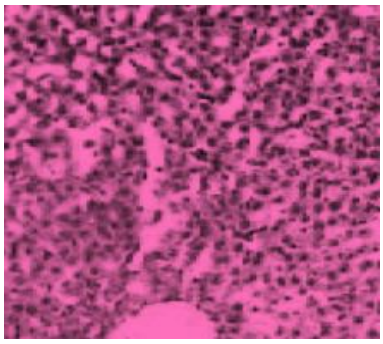


Fig :5 Histopathological section of liver in Group VII animals treated as standard group, treated with carbon tetrachloride + chloroform extract of *V.mung* at a dose (100mg/kg b.w).

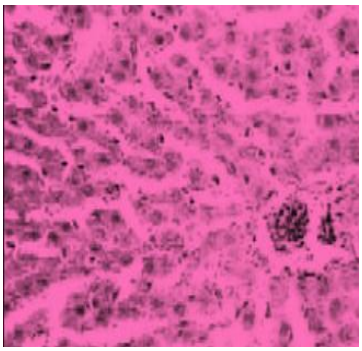


Fig: 6 Histopathological section of liver in Group IX animals treated as standrad group, treated with carbon tetrachloride + ethanolic extract of *V.mung* at a dose (100mg/kg b.w).

Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animal exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus, and nucleolus and well brought out central vein whereas that of CCl_4 intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia[17], crowding of central vein and apoptosis. Treatment with petroleum extract of *V.mung* at a dose of 100 mg/kg b.w. showed moderate to weak activity in protecting the liver cells from CCl_4-injury. Among the plant extract, treatment with chloroform extract returned the injured liver to quite normal. And in case of animal treated with ethanolic extract almost it is equivalent with the standard group liver. Now, it could be decided that the hepatoprotective activity was dose and time dependent. Out of three plant extracts, the ethanol extract of *V.mung* had shown very high significant potential heptoprotective activity at a dose of 100 mg/kg. b.w. Even chloroform extract had shown significant protection against ccl_4 induced liver toxicity.

CONCLUSION:

From this work we can conclued that the folklore usage of *Vigna mung* as a hepatoprotective drug has been validated, it is useful in treating different liver infections and diseases

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