Research Article

Hepatoprotective Potential of Aqueous Extract of *Vigna unguiculata (L)* Walp Seeds against Paracetamol Induced Hepatotoxicity in Rats

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ABSTRACT

Aim of the present study was to evaluate the hepatoprotective potential of the aqueous extract of *Vigna unguiculata* (L) Walp.seeds on rats against Paracetamol induced hepatotoxicity. Alteration in the levels of biochemical markers of hepatic damage like serum glutamate pyruvate tranaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin were tested in both treated and untreated groups. Paracetamol has enhanced the SGPT, SGOT, ALP and bilirubin levels, reflecting the liver injury. Pre-treatment for 9 days with aqueous extract of *Vigna unguiculata* (L) Walp.seeds at a dose of 200 and 400 mg/kg once daily has brought back the altered levels of biochemical markers to the near normal levels. Silymarin was used as standard reference drug. The hepatic antioxidant status such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) levels were reduced in the Paracetamol alone treated animals with subsequent increase in lipid peroxidation, whereas administration of the extract challenge restored the hepatic antioxidant status. The hepatoprotective activity was also supported by histopathological studies of liver tissue. The findings thus suggested aqueous extract of *Vigna unguiculata* (L) Walp.seeds afford significant protection against Paracetamol induced hepatocellular injury in rats by restoring the liver antioxidant status.

Keywords: *Vigna unguiculata* (L) Walp, Hepatoprotective, Paracetamol, Biochemical markers.

INTRODUCTION

Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost[1]. India with its mega-biodiversity and knowledge of rich ancient traditional systems of medicine provide a strong base for the utilization of a large number of plants in general healthcare and alleviation of common ailments of the people[2].

Vigna unguiculata (L) Walp is an ancient crop, which is most commonly called as “cow pea” is an edible legume of the family Fabaceae with high protein contents. It is cultivated around primary for seed, but also as a vegetable, cover crop and fodder. It is widely grown all over the world[3]. Nigeria is one of the world prime producers of Cowpea[4]. The other larger producers are India, Brazil, Haiti, Myanmar, Sri Lanka, Australia and the United States. In India mostly cultivated, in Uttar Pradesh, Chotanagpur, Assam and in southern states of Tamil Nadu, Karnataka and Andhra Pradesh. The seeds & leaves are a major source of plant proteins and vitamins for man, feed for animals. The little leaves and immature pods are eaten as vegetables. It forms part of the human diet due to it has high amount of carbohydrates (56-67%), protein (20.5-31.7%), fibre (4%) and fats (1.14-3.03%) that can fulfill the human essential nutritive necessities when complemented with cereals[5,6].

Some health benefits of cowpea include, toning the spleen, stomach and pancreas helps induce urination and relieves damp conditions like leucorrhoea. Cowpea is rich in potassium with good amount of calcium, magnesium and phosphorus. It also has small amount of iron, sodium, zinc, copper, manganese and selenium. Cowpea is rich in vitamin A & C and also has appreciable amount of thiamin, riboflavin, niacin, vitamin B6 and pantothenic acid as well as small amount of foliate. Cowpea shoots and leaves are rich sources of calcium, phosphorous and Vitamin B[7].

Medicinally it has been used as astringent, appetizer, laxative, aphrodisiac, diuretic, anti-hyperglycemic, antinociceptive, galactogogue, liver tonic. It exhibits antioxidant and free radical scavenging activities. Also exhibits Antibacterial activity against both the Gram positive and Gram negative organisms. Useful in jaundice, menstrual disorders, epilepsy, anorexia, constipation[8]. Besides its health related benefits,

beans are inexpensive, considerably cheaper and due to their physicochemical and functional attributes, legume starches can be used as nutritional ingredients in the same way as starches from cereals and tubers.

Literature review indicated that the hepatoprotective activity of *Vigna unguiculata* (L) Walp.seeds have not been clinically evaluated so far. In view of this, the present study was aimed at evaluating the hepatoprotective activity of *Vigna unguiculata* (L) Walp. seeds against Paracetamol induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

Plant material collection

The seeds of *Vigna unguiculata* (L) Walp was collected from local market of Mangaluru district and the plant was identified and authenticated by a Taxonomist Mrs. Aparna Upadhyaya, Govt. high school Hodavada, Madikeri.

Preparation of extract

The *Vigna unguiculata* (L) Walp seeds were dried in the shade, pulverized by mechanical grinder and passed through a 40 mesh sieve. The powder was extracted by maceration with distilled water for 24hrs. The extract was double filtered by using muslin cloth and Whattmann filter paper No.1 and concentrated by evaporation on water bath. The extract was preserved in airtight containers and kept at 4-5°C until further use[9].

Phytochemical screening

Preliminary phytochemical screening of aqueous extracts of *Vigna unguiculata* (L) Walp seeds was carried out as per the methods and tests to decipher the presence and absence of various phytoconstituents[10, 11]. These tests revealed the presence of carbohydrates, alkaloids, glycosides, saponins, tannins, flavonoids and polyphenols. The results of preliminary phytochemical screening of aqueous extracts of *Vigna unguiculata* (L) Walp seeds were showed in table 1.

Table 1: Phytochemical analysis of *Vigna unguiculata* (L) Walp seeds

1.	Carbohydrates	+
2.	Glycosides	+
3.	Alkaloids	+
4.	Flavonoids	+
5.	Tannins	+
6.	Saponins	+
7.	Steroids	-
8.	Polyphenols	+

Drugs and Chemicals

All chemicals used were of analytical grade and obtained from Himedia Laboratories. The kits for the estimation of SGPT, SGOT, ALP and Bilirubin were purchased from Agape Diagnostics LTD, Kochi. The standard drug Silymarin was purchased from Serum international Ltd, India.

Experimental animals

Healthy Wistar albino rats of either sex weighing 150-200 g were used. Animals used in the study were procured from registered breeder. The animal care and handling was carried out according to CPCSEA guidelines. Animals were acclimatized to the animal quarantine for one week prior to the experiment under controlled conditions of temperature (27 ± 2^{o}C) and were housed in sterile polypropylene cages containing paddy husk as bedding material with maximum of six animals in each cage. The rats were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Animal Ethical Committee, Srinivas College of Pharmacy, Mangalore, Karnataka (Approval No.: SCP/CPCSEA/F150/P02/2015).

Acute toxicity studies

Acute toxicity study of the aqueous extract of *Vigna unguiculata* (L) Walp plant seeds was performed as per the OECD guidelines 425 at a limit dose of 2000 mg/kg. The doses were administered by oral route in mouse as per scheduled in OECD guidelines 425. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for total 14 days for sign of toxicity and/or mortality if any. The LD50 was calculated by using OECD guidelines 425[12].

Paracetamol induced hepatotoxicity studies

Wistar albino rats were randomly assigned into five groups of six animals each. Group I served as control and received vehicle (Distilled water), Group II served as toxic control and received Paracetamol at a dose of 2 g/ kg, Group III served as reference standard and received standard drug (Silymarin 100 mg/kg), Group IV served as low dose test and received aqueous extracts of *Vigna unguiculata* (L) Walp at a dose of 200mg/kg and Group V served as High dose test and received aqueous extract of *Vigna unguiculata* (L) Walp seeds at a dose of 400mg/kg body weight. All the treatment were given orally once daily for 9 days throughout the treatment. All the four groups except group I were intoxicated by oral administration of

Paracetamol (2g/kg body weight) on 9th day of treatment.

After 48hrs of Paracetamol intoxication, blood was separated and analyzed for various biochemical parameters like ALP, SGOT, SGPT and Total Bilirubin content using enzyme analyzer. Animals were sacrificed by euthanasia; liver was dissected out and used for histopathological studies[13].

Statistical analysis

All data were expressed as mean ±SEM. The statistical significance between groups was compared using one way ANOVA, followed by Dunnett's (multiple comparisons) test.

OBSERVATION AND RESULTS

The hepatoprotective activity of aqueous extract of *Vigna unguiculata* (L) Walp seeds at doses 200 and 400 mg/kg were assessed by measuring the level of various liver biochemical parameters like SGOT, SGPT, ALP and TB and endogenous antioxidant parameters like LPO, SOD, GSH and CAT. Elevated level of biomarkers (SGOT, SGPT, ALP & TB), Lipid peroxidase and reduced levels of endogenous enzymes (SOD, GSH & CAT) in animals treated with Paracetamol is indicative of severe liver necrosis. However treatment with standard drug Silymarin and aqueous extract of *Vigna unguiculata* (L) Walp seeds has significantly decreased the serum enzyme levels indicating the protective effect of *Vigna unguiculata* (L) Walp seeds extract against hepatotoxicity. In our study, animals treated with Silymarin (100mg/kg) and 400 mg/kg of aqueous extract of *Vigna unguiculata* (L) Walp seeds significantly reduced the elevated levels of serum biomarkers (***p<0.001 & **p<0.01), lipid peroxidase and significant elevation in endogenous enzymes (***p<0.001 & **p<0.01,) when compared with the intoxicated group. However, aqueous extract at dose of 200mg/kg does not show significant reduction of serum enzymes (Table 1 & 2).

Table 2: Effect of Silymarin & Aqueous extract of *Vigna unguiculata* (L) Walp on Serum SGPT, SGOT, ALP & Total bilirubin in PCM induced liver toxicity

Groups	Treatment	ALP (U/l)	SGOT (U/l)	SGPT (U/l)	TB (mg/dl)
Vehicle control	Distilled water 1ml/ Kg	141.5±0.5	103.3±0.61	85.5±0.92	0.62±0.08
Toxic control	PCM 2g/Kg p.o	364.2±4.17[a]	345.8±6.38[a]	208.7±6.81[a]	2.47±0.19[a]
Standard	Silymarin 100mg/Kg, p.o	150.5±4.46***	119.5±2.22***	97.0±1.65***	0.8±0.07***
Low dose	*Vigna unguiculata* (L) Walp 200mg/Kg, p.o	213.0±6.45*	198.8±2.62*	148.5±2.11*	1.48±0.07*
High dose	*Vigna unguiculata* (L) Walp 400mg/Kg, p.o	173.0±2.91**	156.8±2.64**	123.3±2.89**	0.98±0.14**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. [a]p< 0.001 when compared with vehicle treated control group.*p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Table 3: Effect of Silymarin and Aqueous extract of *Vigna unguiculata* (L) Walp on LPO, GSH, SOD & CAT in PCM induced liver toxicity

Groups	Treatment	LPO (Abs at 535 nm)	SOD (Abs at 560 nm)	GSH (Abs at 412nm)	CAT (Abs at 620 nm)
Normal Control	Distilled water 1 ml/Kg	3.5± 0.42	23.67± 0.8	28.83± 0.6	44.5± 0.67
Toxic control	PCM 2g/Kg p.o.	18.33± 0.76[a]	14.33± 0.42[a]	20.0± 1.03[a]	31.0± 1.39[a]
Standard	Silymarin 100mg/Kg p.o	6.5± 0.56***	22.0± 1.21***	25.0± 0.58***	43.67± 0.42***
Low dose	*Vigna unguiculata* (L) Walp 200mg/Kg p.o	12.16± 0.6*	16.0± 0.86*	21.5± 0.96*	32.83± 1.07*
High dose	*Vigna unguiculata* (L) Walp 400mg/Kg	10.17± 0.6**	18.5± 0.34**	22.83± 0.48**	36.83± 0.79**

	p.o				
All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test, [a]p<0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.					

Histopathalogical Studies

Histopathalogical profile of liver of control animals showed normal liver architecture (Fig.1.1), whereas the liver section of animals treated with Paracetamol showed distorted liver architecture with more hepatocytes showing degenerative changes and necrosis (Fig.1.2). The liver section of animal treated with standard drug (Silymarin) shows complete restoration of normal cellular population size of liver and absence of liver damage (Fig. 1.3). The liver section of animals treated with *Vigna unguiculata* (L) Walp (200 mg/kg & 400 mg/kg) showed normal hepatocytes and absence of necrosis (Fig 1.4 & 1.5). The above reports confirmed the hepatoprotective effect of *Vigna Unguiculata (L)* Walp seeds in paracetamol induced hepatotoxicity in rats.

DISCUSSION

The present study demonstrate the hepatoprotective effect of *Vigna unguiculata* (L) Walp seeds extract against paracetamol induced liver toxicity in Wistar albino rats. Hepatotoxicity usually occurs by poor drug habits, alcohol and prescribed or over-the-counter drugs, which can eventually lead to the liver ailments like hepatitis and cirrhosis. According to the latest WHO data published in May 2014 Liver Disease Deaths in India reached 216,865 or 2.44% of total deaths and ranks 61 in the world. Hepatotoxicity was induced by administering paracetamol (2 g/kg), which is widely used as analgesic and antipyretic drug, which can produce acute liver damage at higher doses. Acute paracetamol poisoning is one of the common causes of liver failure[14]. Overdose of Paracetamol causes centrilobular hepatic necrosis which can be fatal. In overdoses, Paracetamol metabolically get activated by Cytochrome P-450 enzymes to a reactive metabolite N-acetyl-p-benzo-quinoneimine (NAPQI) that depletes glutathione (GSH). NAPQI covalently binds to cellular macromolecules and initiates cell damage[15]. Hepatic cellular damage may result in leakage of enzymes like Serum Glutamate oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), which can be measured as indicators of cell damage. Their levels are markedly elevated in hepatitis and other acute liver damage. SGPT level is most commonly used to determine hepatic damage than SGOT[16]. The seed extract *Vigna unguiculata* (L) Walp shows dose dependent decrease in the elevated serum biomarkers (SGPT, SGOT, ALP and bilirubin), lipid peroxidation, and significant increase in endogenous enzymes (GSH, SOD, CAT).

The preliminary phytochemical studies confirmed the presence of Carbohydrates, Alkaloids, Glycosides, Polyohenols, tannins, flavonoids, saponins and proteins. Phytoconstituents like the flavonoids[17], triterpenoids[18], saponins[19] and alkaloids[20] are known to possess hepatoprotective activity. The presence of flavanoids in our extract may be responsible for its antioxidant[8] and thus hepatoprotective activity. Numerous studies have suggested that flavonoids commonly function as antioxidants and may protect plants against oxidative stress caused by suboptimal environmental conditions[21, 22]. The antioxidant capacity of flavones is attributed to the high reactivity of the hydroxyl substituent, with the number of hydroxyl groups on the B-ring being correlated with ROS scavenging capability[23]. It was also supported by the histopathological examination carried out on isolated liver.

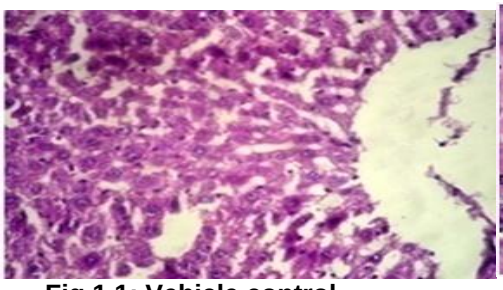

Fig.1.1: Vehicle control

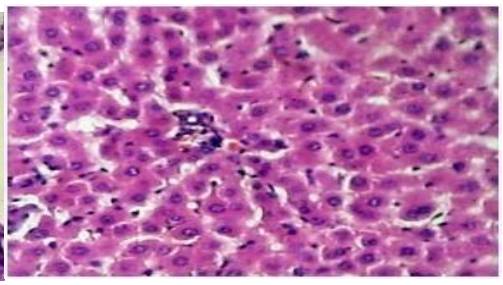

Fig. 1.2: Toxic control

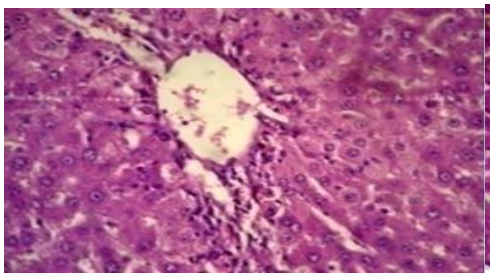
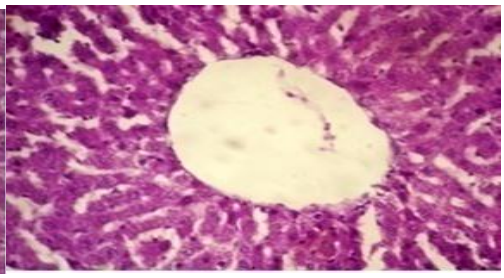

Fig. 1.3: Standard (Silymarin) Fig. 1.4: Vigna unguiculata (L) walp (200 mg/kg)

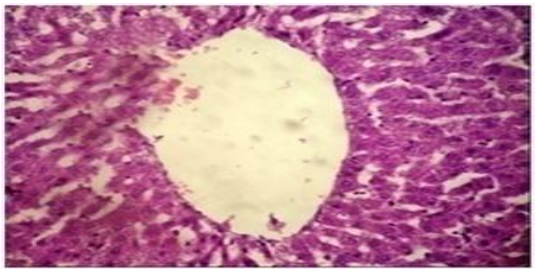

Fig. 1.5: *Vigna unguiculata* (L) walp (400 mg/kg)

CONCLUSION

The results of this study demonstrate that *Vigna unguiculata* (L) Walp seed was effective for the prevention of Paracetamol induced hepatotoxicity in rats. Our results show that the hepatoprotective effects of *Vigna unguiculata* (L) Walp seeds extract may be due to both an increase in the activity of the antioxidant-defense system and an inhibition of lipid peroxidation. However, the protective and antioxidant qualities of *Vigna unguiculata* (L) Walp seed need to be confirmed by characterizing the active ingredient(s) of this seed as well as its mechanism(s) of action.

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