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Evaluation of Hepatoprotective Activity of Ethanol Seed Extract of Vigna unguiculata Against Paracetamol Induced Hepatotoxicity in Rats

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Abstract:
The present study was aimed to investigate hepatoprotective activity of ethanolic extract of Vigna ungiculata seed in paracetamol induced hepatotoxic rats. The seed of the plant was collected, dried and extracted with continuous soxhlet hot extraction. Qualitative analysis of various phytochemical constituents and physicochemical analysis were determined by the well-known test protocol available in the literature. In the present study, the ethanolic extract of Vigna unguiculata (L) seed was evaluated for its hepatoprotective effect on paracetamol induced acute liver damage on Wistar albino rats. The degree of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB) levels. Paracetamol induced group had enhanced levels of SGPT, SGOT, ALP and TB when compared with control group. Treatment with silymarin, 100mg/kg and 400mg/kg of Vigna unguiculata (L) seed extract had significantly brought down the elevated levels of SGPT, SGOT, ALP & TB. The results showed that ethanolic extract of Vigna unguiculata (L) seeds possesses significant hepatoprotective activity.

Keywords: Paracetamol, Vigna unguiculata (L) Hepatotoxicity, Wistar albino rat.

Introduction:

The liver is an important and largest organ of the human body. It starts developing in the human embryo between the 3rd and the 4th week of life, and it is the main site of hematopoiesis in the intrauterine period. The healthy human liver has weight around 1500 g, and it is located in the upper right corner of the abdomen and beneath the diaphragm[1].

The human liver consist of constitutes about 2.4% of total body weight four lobes have unequal in shape and connected with two hepatic blood vessels called as hepatic artery and hepatic portal vein look like reddish brown in color[2]. Two types of cells are present in heapatic lobes i.e. parenchymal and nonparenchymal cells.

Epidemiology

Indian population

Liver cirrhosis is newly diagnosed in India affected around 10 lakh people every year. As per the World

Health Organization, Liver diseases are the one of the tenth most common cause of death in India. According to the latest WHO data published in May 2014, caused by liver disease in India reached 2.44% of total deaths[3].

Worldwide affected population

In the European (EU) region approximately 29 million people affected from a chronic liver disease and approximately 30 million people are affected from the same condition in America. It was estimated that in 2013, liver cirrhosis resulted in 170,000 deaths in Europe. Alcoholic liver disease (ALD) causes liver cirrhosis as a result in 2010, 493,300 deaths was reported (156,900 female and 336,400 male). In context to Liver cancer many published reports revealed that it is the most common cause of cancer death in current scenario of life style all over the world. In, 2015 as per the report of W.H.O. there are 788,000 deaths occurred due to liver cancer globally. Out of more which more than 25,000 liver transplants were conducted globally in between 2014-2015 and more than 5000 liver transplants were performed each year in Europe only[4].

Hepatotoxicity

Hepatotoxicity is a type of liver dysfunction or damage liver of which is associated with improper uses of antibiotics and potent drugs. Those chemicals which cause liver injury are called hepatotoxins or hepatotoxic agents[5].

Drug induced hepatotoxicity

In the most essential sense, the reason for medicate digestion is to encourage discharge of a less polar medication through the arrangement of progressively polar metabolites.[6] The subsequent water solvent compound can be discharged from the body by the kidneys. Much of the time Phase I digestion through oxidation, decrease or hydrolysis by CYP450 catalysts delivers the polar metabolites that are then made water dissolvable and accessible for discharge by Phase II digestion by means of glucuronidation or sulphation[7]. Be that as it may, we can't disregard the way that exercises of hepatic medication processing catalysts might be influenced by articulation of qualities related with these proteins notwithstanding adjustments in blood stream to the liver in typical and pathogenic states.

According to Hamilton 2003, India has about 44% of flora, which is used medicinally. Although it is difficult to estimate the number of medicinal and aromatic plants present worldwide, the fact remains true that India with rich biodiversity ranks first in percent flora, which contains active medicinal ingredient. Over the past decade, interest in drugs derived from higher plants, especially the phytotherapeutic, has increased substantially and so its market is growing. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants[8].

Vigna unguiculata (L.) Walp. is a leguminous plant belongs to the family Papilionaceae. It is originated from Africa and is grown widely all over the world including Nigeria, India, Central America, China and Africa. It is an edible legume. The seeds and leaves are a major source of plant proteins and vitamins for man and feed for animals[9].

Fig 1: Vigna unguiculata seeds

Material & Methods:

Collection, Identification and Authentication of plant material: The fresh seeds of Vigna ungiculata were collected from the field of Varanasi district in the month of their natural habitat. All seeds were cleaned of extraneous matter. Plant and plant material was identified by comparing morphological features of plant. The herbarium of collected plant material was prepared & the taxonomical identification (authentication) of the plant is done by Botanical Survey of India, Pune, India. The collected seeds of Vigna ungiculata were dried in shade to avoid loss of phytoconstituents. After drying, seeds were coarsely powdered with grinding mill and kept in air tight container for use in the study. Plant material were powdered and passed through sieve #40, and subjected to standardization with different parameter[10]. Based upon the extensive literature survey, we decide to use the alcoholic solvent such as ethanol for extraction of phytoconstituent from powder of seeds of Vigna ungiculata for further study.

Extraction of plant materials:

The seeds were collected washed thoroughly in tap water; shade dried in an open air separately. Powder of seeds is obtained by grinding them mechanically. About 100gm of each dried powder of the plant seeds were soaked separately in 100ml of solvent like ethanol in conical flasks and then subjected to filtration, filtered with no.42 watsman filter paper separately. Conc. extracts were preserved in sterilized air light labelled and in refrigerator at 40°c until required for further use[11]. The extract was filtered under reduced pressure using rotary flash evaporator and subjected for further preliminary phytochemical test.

Figure 2: Continuous Hot Extraction of Vigna ungiculata Seed

Preliminary Phytochemical screening

The *Vigna ungiculata* seed extract was screened for the presence of alkaloids, saponins, tanins, carbohydrates, flavonoids, anthraquinones, cardiac glycosides, deoxy sugar, steroids and terpenes using standard methods as described by Harborne (1973), Sofowora (1993), Trease and Evans (1986), William, Trease, and Evans (1996).

Pharmacological Study

The study used a healthy adult 200–250 g female wistar albino rats of either sex were employedthat were received from animal house of SHEAT College of pharmacy, Varanasi. The rats were kept in rooms with standard room temperature (23 ± 1ºC) and relative humidity of 67%. They also received unrestricted access to water and standard rodent food pellets. One day before to the experiment and every day for at least one hour before the experiment, they were acclimated to the laboratory conditions. Every experiment was carried out between 09.00 and 17.00 hours[12]. All research was carried out in compliance with the guideline set forth by government of India committee for the purpose of control and supervision on experiments on animal (CCSEA), and prior approvals were obtained from IAEC 1committee of SHEAT College of Pharmacy, Varanasi.

Acute oral toxicity of Vigna ungiculata seed extract

Acute toxicity studies for methanolic leaf extract was conducted according to OECD guideline 420 using wistar albino rats. Before dosing, animals were fasted overnight and ethanolic seed extract was orally administered in a single dose. The volume given was not more than 2 ml/100 g body weight. Following the period of fasting, the fasted body weight of each animal was determined, and the dose was calculated according to the body weight. EVU was administered orally at a dose of 2000 mg/kg and after administration food was withheld for further 3-4 hrs. If no mortality was observed in the animals, then the extract was administered in another set of three animals to confirm the same. If test substance-related mortality is produced, further testing at the next lower level may need to be carried out[13].

Paracetamol induced hepatotoxicity model

The experiment will be perform according to the modified procedures described previously 36 rats were randomly divided into 6 groups and treated with normal saline, graded doses of ethanolic extract of Vigna ungiculata and silymarin for five days. Group A (Control group) received normal saline (NS) only (10 mL/kg, p.o.). Group B received Paracetamol (50 mg/kg, i.p.), Groups C, D and E received ethanolic extract of V. ungiculata 100 200 mg/kg p.o. and 400 mg/kg p.o[90]. Group F received Silymarin {standard drug}, (100 mg/kg, p.o.), respectively, plus Paracetamol (50 mg/kg, i.p.)[14].

Rats from various groups were sacrificed on day 6 of the treatment regimen after being sedated with light ether. This was done twenty-four (24) hours following the last treatment. The blood was drawn from the retro-orbital plexus and placed in a vial without anticoagulants. The separated serum sample was centrifuged at 5,000 rpm for 10 minutes, which was then used for biochemical parameters analysis using standard diagnostic kits. The liver was immediately taken out and washed with ice-cold saline. The blood and liver samples were assessed for their biochemical as well as histological observation[15].

Histopathology:

The rats were sacrificed and their livers were removed thereafter. Following a thorough blotting to remove

blood and tissue fluids, this liver was then used for histological investigation. After the liver tissue had been fixated in 5% formalin for 48 hours, it was dehydrated using a series of ethyl alcohol-water solutions. After that, it was soaked in paraffin and finally xylene. Slices onto glass slides measuring 15 mm thick were made using a microtome. The liver sections were stained for three to five minutes with 10% haematoxylin, and then rinsed under running water to make the staining more visible. Subsequently, 10% eosin was applied to the sections and left to counterstain for 2 minutes. The chosen regions were shot after the slices were examined under a photomicroscope set at a magnification of 400.[16]

Statistical Analysis

The results were reported as Mean+SEM using Graph Pad Prism version 5.0 and subjected to a one-way ANOVA and the student's t-test were used to analyze the approach used to determine the difference in significance from a statistical perspective.

Results

The seeds of Vigna ungiculata was demonstrated the percentage yield as 45.02%.

Preliminary Phytochemical screening of extract

The phytochemical screening of cowpea seeds used in this study are presented in Table 1. The findings suggest that quantitative chemical analysis was effective for the preliminary phytochemical characterization of the species and could potentially aid in predicting the presence of bioactive compounds. From the Table 1, alkaloids, terpenoids, cardiacglycoside and flavonoids were maximum present in the cowpea seeds. Phenols and saponin were low presence in less quantity.

Fig. No. 3: Preliminary phytochemical investigation of Vigna uniculata seed extract

Table 1: Phytochemical screening of extract

Bioactive compound	Ethanolic extract
Flavonoids	+
Alkaloids	++
Steroids	-
Phenols	+

Saponins	-
Cardiac glycoside	+
Terpenoids	++

+ (Present in low quantity), ++ (Present in moderate quantity), +++ (Present in high quantity) and - (Absent)

Acute Oral Toxicity of V. ungiculata seed extract

The present study conducted as per the OECD guideline 423 revealed that the EVU did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 300, 600, 1200 & 2000 mg/kg body weight. Therefore, the approximate acute LD50 of V. unguiculata seed extract in female rats was estimated to be higher than 2000 mg/kg. No significant changes were observed in parameters used for evaluation of toxicity. So, the dose selected for further study was 100, 200 and 400 mg/ kg, p.o. for test extract.

Table No. 2: LD_{50} determination of V. ungiculata seed extract

Group	Species	Dose mg/kg	No. of Animals	Mortality	LD50 mg/kg
Group-I	Albino wistar rat	300	6	0/6	≥ 2000 mg/kg
Group-II		600	6	0/6	
Group-III		1200	6	0/6	
Group-IV		2000	6	0/6	

Effect of extracts of Vigna ungiculata seed on serum liver enzyme markers

The estimation of serum hepatic enzymes is a useful marker for determining extent and type of hepatocellular damage. Administration of paracetamol caused significant liver damage as revealed by elevated serum hepatic enzymes (AST, ALT and ALP). ACP group showed significant increase in AST ($p < 0.001$), ALT ($p < 0.001$), and ALP ($p < 0.001$) levels as compared to normal control group. Treatment with silymarin and V. ungiculata at a dose of 200 mg/kg and 400 mg/kg significantly reduced the elevated serum enzymes as compared to Paracetamol group. The enzyme levels in extract-100 mg/kg dose group were not significantly different from the ACP group, thereby revealing that 100 mg/kg dose of V. ungiculata was ineffective. The same inference was obtained when silymarin treatment group was compared with the V. ungiculata treatment groups. The comparison between various A. marmelos treatment groups showed dose dependent reductions in the enzyme levels.

Table No 3: Effect of Vigna ungiculata on PCM-induced alterations in serum ALT, AST, ALP and Bilurubin content.

Groups	ALT (U/l)	AST (U/l)	ALP (U/l)	Bilurubin (U/l)
Group-A (Control group)	92.27±1.482	47.18±3.18	167.07±5.40	2.51±0.105
Group-B	380.01±11.45	181.48±7.88	396.56±5.75	4.21±0.192
Group-C	340.08±10.64	146.02±08.37	347.81±17.11	3.85±0.162
Group-D	192.33±24.38	122.38±9.62	257.24±11.19	3.18±0.227

Group-E	150.90±16.96	96.92±5.84	204.59±10.05	2.83±0.207
Group-F	107.92±6.74	66.05±8.77	221.77±15.97	1.98±0.20

Each value is the mean ± S. E. M. of rats and *p <0.05compared with control group.

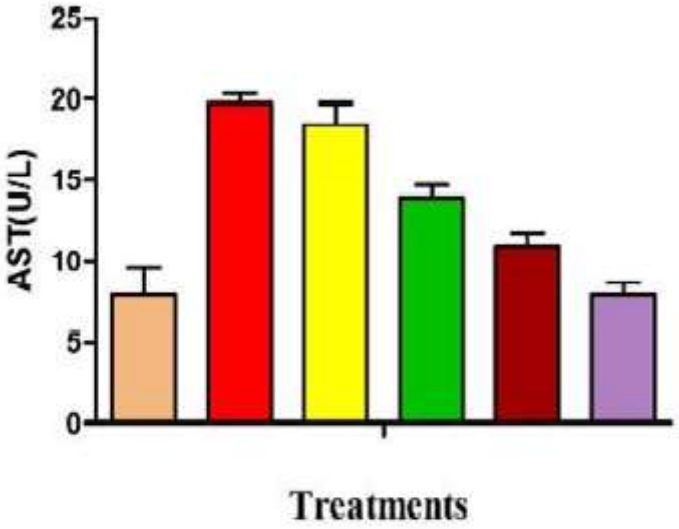
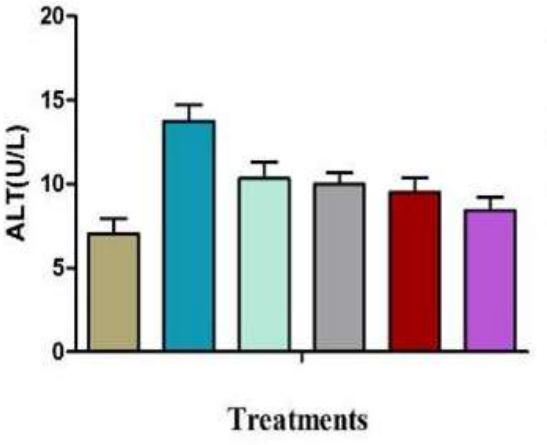


Fig. 4: Effect of extract on AST levels

	Control
	PCM control (50 mg/kg)
	PCM+VUE (100 mg/kg)
	PCM+VUE (200 mg/kg)
	PCM+VUE (400 mg/kg)
	PCM+SLM (100 mg/kg)



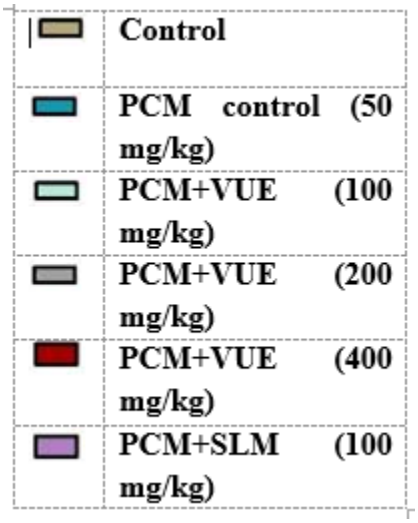


Fig. 5: Effect of extract on ALT levels

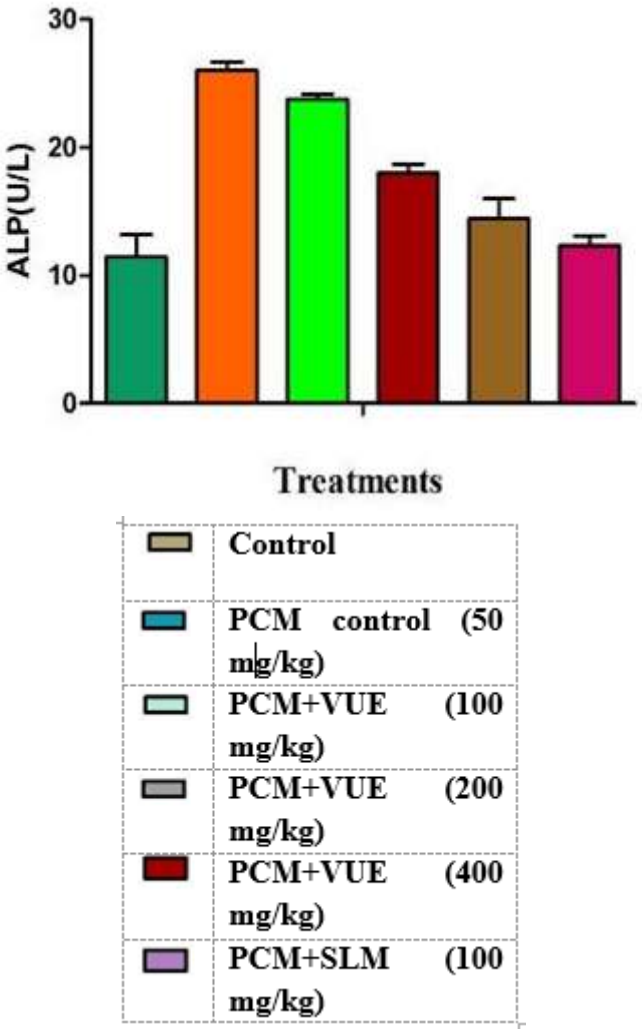


Fig. 6: Effect of extract on ALP levels

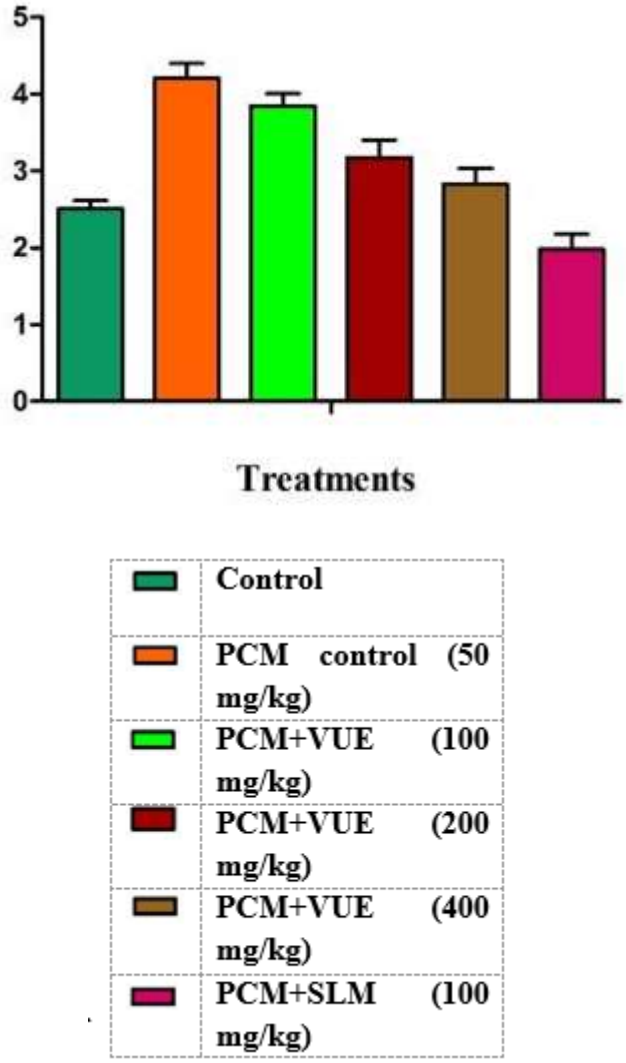


Fig. 7: Effect of extract on Bilurubin levels

Histopathological Examination:

Histological analysis revealed that PCM caused marked hepatotoxicity as evident by shrinkage of central veins, hepatocellular hypertrophy and necrosis. Fig. 8 shows normal architecture of hepatocytes and liver parenchyma, distinct hepatic cords and central vein in normal control group. Treatment with A. ungiculata (400 mg/kg) reduced severity of hepatic damage as compared to PCM group. Moreover, vascular distortion and lymphocyte infiltration were also reduced in extract-100 mg/kg confirms its hepatoprotective effect.

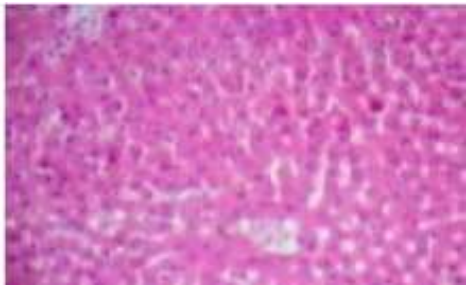


Fig. A Vehicle control

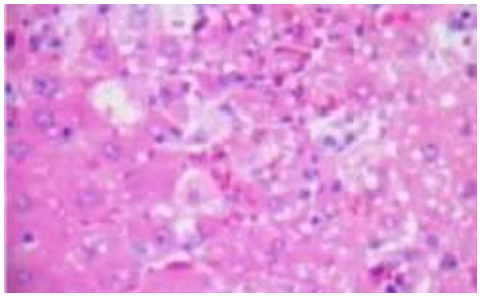


Fig. B Toxic control

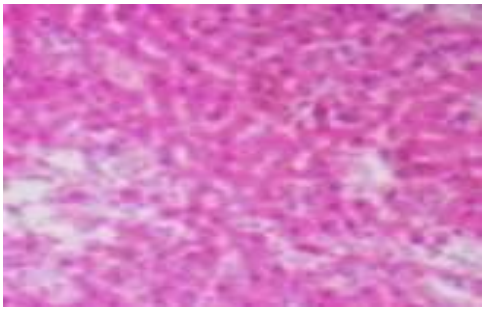


Fig. C Vigna ungiculata (100 mg/kg)

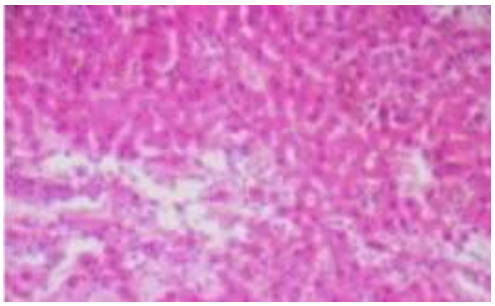


Fig. D Vigna ungiculata (200 mg/kg)

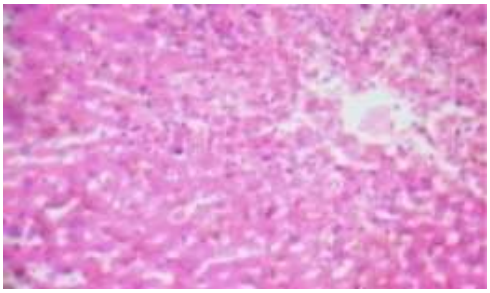


Fig. E Vigna ungiculata (400 mg/kg)

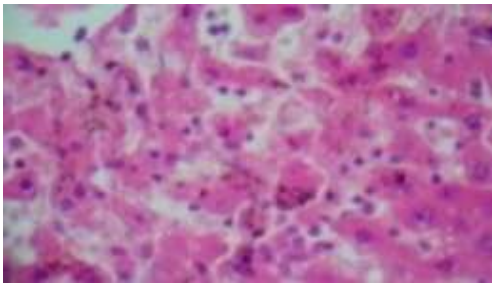


Fig. F Standard Silymarin (100 mg/kg)

Figure 8: Histopathological examination

Discussion

In present study administration of paracetamol for 21 days increased serum enzymes like SGPT, SGOT, ALP and total bilirubin which indicated the ability of paracetamol to produce hepatic damage. Administration of Vigna unguiculata (L) seed extract with paracetamol (Group III & V) reduced the elevated levels of SGPT, SGOT, ALP and total bilirubin levels indicated their hepatoprotective effect against paracetamol induced liver cell damage[79]. There is an innate antioxidant defense mechanism present in the liver.

The preliminary phytochemical studies confirmed the presence of Carbohydrates, Alkaloids, Glycosides, Polyohenols, tannins, flavonoids, saponins and proteins. Phytoconstituents like the flavonoids, triterpenoids, saponins and alkaloids are known to possess hepatoprotective activity[80, 81]. The presence of flavanoids in our extract may be responsible for its antioxidant and thus hepatoprotective activity[17, 18]. Numerous studies have suggested that flavonoids commonly function as antioxidants and may protect plants against oxidative stress caused by suboptimal environmental conditions. 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. It was also supported by the histopathological examination carried out on isolated liver[19].

Conclusion

In conclusion, results of our study reported that ethanol extract of Vigna unguiculata (L) seed was effective in the treatment of hepatotoxicity induced by paracetamol. The degree of protection was measured by using biochemical parameters like SGPT, SGOT, ALP and Total bilirubin. Our results shows that the hepatoprotective effects of Vigna unguiculata (L) seeds extract may be due to both an increase in the

activity of the antioxidant-defense system and an inhibition of lipid peroxidation. The aqueous extracts showed the significant hepatoprotective activity comparable with standard drug silymarin. However, the protective and antioxidant qualities of Vigna unguiculata (L) seed need to be confirmed by characterizing the active ingredient(s) of this seed as well as its mechanism(s) of action. These findings support the need for more research on extracts from this species. More research is required to clarify the precise mechanism or mechanisms of action. Further studies, including molecular-level investigations and clinical validation, are recommended to explore their therapeutic applications.

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