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Research Article

HEPATOPROTECTIVE AND *IN VIVO* ANTIOXIDANT POTENTIAL OF AQUEOUS SEED EXTRACT OF *VIGNA UNGUICULATA* AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

To evaluate the hepatoprotective and *in vivo* antioxidant effect of aqueous seed extract of *Vigna unguiculata* (AVU) against Carbon tetrachloride (CCl_4) induced hepatic injury in rats. 30 male wistar rats were divided into five groups. Group 1 and 2 received plain groundnut oil (3ml/kg body weight p.o.) for 21 days and had free access to food and water. Group 3, 4 and 5 were pretreated with Silymarin (50 mg/kg body weight, p.o.) and AVU at a dose of 200 and 400 mg/kg body weight respectively for 21 days. Hepatotoxicity was induced by the intraperitoneal administration of a single dose of CCl_4 (50% v/v in groundnut oil at a dose of 3 ml/kg body weight) in all groups except group 1 on 21st day. Biochemical estimations were done on 22nd day followed by harvesting of liver for antioxidant and histopathological studies. Changes in serum hepatic enzymes, total bilirubin, total protein, total cholesterol, total triglycerides and oxidant parameters induced by CCl_4 administration were reversed by AVU treatment in a dose dependent manner and the same trend was observed with Silymarin treated group. The biochemical and antioxidant observations were paralleled by histopathological findings in rat liver both in the case of CCl_4 and treatment groups. Pretreatment with AVU had significant protection against hepatic damage induced by CCl_4 and hence can be used as a safe, cheap, and effective hepatoprotective agent a, supporting its folklore use.

Keywords: Liver damage, Cow pea, Oxidative stress, Carbon tetrachloride, Legumes, Hepatotoxins.

INTRODUCTION

Liver diseases have become a global health burden because of unhealthy dietary habits, environment pollution, and viral infections. The liver is the major site of endo and xenobiotic detoxification process; hence it is under constant threat from obnoxious insults and several risk factors such as chronic alcohol consumption, dietary alfotoxin exposure, Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections, genetic and immune factors. These factors may contribute to derangement of hepatic function, acute liver injury and if left untreated may progress to chronic injury and subsequent development of malignant tumors.[1]

The pathogenesis of hepatic injury is not fully clear, but it is closely linked to the oxidative stress and inflammatory reaction. The excessive oxidative stress caused by reactive oxygen species and reactive nitrogen species increase the risk for hepatic injury through oxidation of biomolecules including DNA, lipids, and proteins and release of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1.[2,3]

Hepatocytes are involved in array of metabolic events and therefore the establishment of liver protective therapeutic agents is of paramount importance in protecting liver from hepatotoxins. In the absence of reliable hepatoprotective drugs in modern medicine, a number of medicinal plants and their formulations of traditional medicine are being used to cure hepatic disorders.[4] Complementary and alternative therapies are under active research worldwide and a number of herbal drugs are showing promising results in treating liver diseases. Herbal medicines claim that they are safe because they are "natural" and hence may both prevent and treat diseases.[5] The previous studies have also shown that phytoextracts and phytocompounds rich in natural antioxidants act as potent hepatoprotective agents. Thus, many bioactive compounds and extracts from plants have been investigated for hepatoprotective and antioxidant effects against hepatotoxin-induced liver damage.[6,7]

Vigna unguiculata which is most commonly called as "cow pea" is an ancient, annual edible herbaceous legume of the family Fabaceae. Amongst the rural people in Indo-Pakistan sub-continent, fresh leaves and immature pods of *Vigna unguiculata* are commonly used as vegetables and seeds are also used in culinary dishes, after processing such as soaking, dry heating, followed by cooking along with cooked rice. Known to be an exceptional source of protein, cowpea is also rich in vitamins, minerals, and soluble and insoluble dietary fiber.[8]

The seeds of the plant are claimed to have astringent, laxative, diuretic, anthelmintic, antibacterial, appetizer, aphrodiasic and galactogogue properties. The seeds also help in relieving the conditions like anorexia, constipation, jaundice, hepatic troubles and general debility.[9] The seed oil was also reported to contain tocopherols, the most important lipophilic antioxidants and methanolic extract of seeds was also proved as potent antiradical, thus providing a scientific evidence of its role in the management and prevention of diseases associated with oxidative stress.[10]

Although the seeds of *Vigna unguiculata* are used in folklore medicine in the management of liver ailments, there is no *in vivo* scientific evidence to support the same. Hence, the present study was conducted to validate the hepatoprotective activity of aqueous seed extract of *Vigna unguiculata* (AVU) on hepatic damage induced in albino rats by Carbon tetrachloride (CCl_4), a widely used hepatotoxin.

MATERIALS AND METHODS

Collection of Plant Materials

The dry seeds of the plant *Vigna unguiculata* were collected from in and around local markets of Tirupati. They were identified and authentified by Prof. B.Sitaram, Department of Dravyaguna, S.V. Ayurvedic College, Tirupati. (Reg. No. of the certificate PARC/2011/990).

Preparation of Aqueous Seed Extract

The aqueous seed extract of *Vigna unguiculata* (AVU) was prepared by the following method with slight modifications.[11] The dry seeds obtained were subjected to size reduction to obtain coarse powder using a grinding mill. 100gms of powder was mixed with 1000 ml of distilled water and mixture was kept for 48hrs in an orbital shaker at room temperature. Then it was filtered separately through five folds of muslin cloth and the filtrate was collected in screw glass vials. The supernatant was collected and filtered by using Whatmann's Filter No. 1 and this formed the stock solution of 99 mg/ml. The filtrate was kept in refrigerator at 4^0 C for further use.

Animals

Albino male Wistar rats (150 $\pm$ 20 g) purchased from Sri Raghavendra enterprises, Bangalore were used in the study. The animals were housed in cages, maintained under standard conditions (27±2°C, relative humidity 44 - 56% and 12 h light and dark cycles) and fed with standard rat diet and purified drinking water ad libitum. All experiments and protocols described in the study were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals (SPSP/CPCSEA/IAEC-1016/a/2014/001), Ministry of Social Justice and Empowerment, Government of India. The experiments were performed during day (08:00 a.m.-16:00 p.m.) and animals were transferred to the laboratory at least 1 h before the start of the experiment.

Chemicals

Silymarin was purchased from Sigma – Aldrich (Chennai), CCl_4 and all other reagents used were of analytical grade and were obtained from Merck – India Pvt. Ltd. (Bangalore). Diagnostic kits used in this study were procured from Span Diagnostics Ltd., India.

Instruments

Auto analyzer (Mispa Excel), UV-visible spectrophotometer (Analytical systems, model no: AUV 2060), Electronic balance (Shimadzu, model no: DS- 852 J), Colorimeter (Inco, model no: CL-157), Homogenizer (Ever shine, model no: 607), Cooling centrifuge (Remi, model no: KKLO- 9013).

Phytochemical Screening

A preliminary phytochemical screening of AVU was performed as described by Kokate.[12]

Acute Toxicity Studies

Acute oral toxicity studies were performed according to OECD-423 guidelines.[13] Female Wistar rats selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. AVU was administered orally at a dose of 2000 mg/kg initially and mortality if any was observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then lower (300, 50, 5 mg/kg) doses of AVU were employed for further toxicity studies.

Selection of Dose

For the assessment of hepatoprotective activity, dose levels were chosen in such a way that, doses were approximately one tenth (200mg/kg) and one fifth (400mg/kg) of the maximum dose employed in acute toxicity studies i.e. 2000mg/kg body weight.[14]

Carbon Tetrachloride (Ccl_4) Induced Hepatotoxicity

Male Wistar rats weighing 150-200 g were divided into five groups consisting of six animals in each group. Group 1 received plain groundnut oil (3ml/kg body weight p.o.) for 21 days. Group 2 (CCl_4 control) received CCl_4 50% v/v in groundnut oil at a dose of 3 ml/kg body weight p.o. on 21st day. Group 3 (Positive control) received Silymarin (50 mg/kg body weight, p.o.) for 21 days. Group 4 and 5 (Test groups) were pretreated with AVU 200 and 400 mg/kg body weight respectively for 21 days. Groups 3,4 & 5 received single dose of carbon tetrachloride (CCl_4) 50% v/v in groundnut oil at a dose of 3 ml/kg body weight orally on 21st day after one hour of silymarin and AVU treatment respectively.[15] After 24 hours of CCl_4 administration, the animals were anaesthetized by inhalation of ethyl ether. Blood samples were collected by retro orbital sinus and serum was separated by centrifugation at 2000 *g* for 5 minutes at 4 °C and used for biochemical analysis. The rats were then sacrificed by cervical dislocation and the liver was dissected, cleaned of extraneous tissue, washed by ice cold saline, blotted with filter paper and weighed immediately. Liver tissue from the left liver lobe was cut into two pieces, one was used for the preparation of liver tissue homogenate for anti-oxidant level estimations and the other was preserved in 10% formalin for histopathological examination.

Assessment of Hepatic Antioxidant Enzymes:

Liver homogenates (10%w/v) were prepared in ice-cold physiological saline, and the resulting suspension was centrifuged at 1000 rpm using a Teflon homogenizer for 10 min at 4^0C. The clear supernatant was used for the determination of superoxide dismutase (SOD), catalase (CAT), lipid peroxidase (LPO) and glutathione (GSH).

SOD activity was estimated using the method of Misra and Fridovichi L.[16] Catalase was assayed at 240 nm by monitoring the disappearance of H_2O_2 described by Aebi.[17] Hepatic level of reduced glutathione was determined by the method as described by Moron et al.[18] The lipid peroxidation level was assessed spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method, as illustrated by Slater and Sawyer.[19]

Statistical Analysis

All the data was expressed as Mean ± SEM. Statistical significance between more than two groups was tested using two-

way ANOVA followed by the Tukey test using computer based fitting program (prism graph pad version 5.04.). Statistical significance was set at $P < 0.05$.

RESULTS

Preliminary Phytochemical Screening of AVU: Preliminary phytochemical screening of the aqueous seed extract revealed the presence of alkaloids, flavonoids, proteins and the absence of resins, triterpenoids, carbohydrates, steroids, saponins and tannins [Table 1].

Acute Toxicity Study of AVU: In the acute toxicity study, it was observed that none of the doses (i.e. 5, 50 300, 2000 mg/kg) produced any lethality among the tested animals when administered as a single dose.

Effect of AVU on Serum Biochemical Parameters: There was a drastic and significant increase in the serum glutamate pyruvate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP), total protein, total bilirubin, total cholesterol and triglycerides levels in the CCl_4 group when compared to the normal group. Silymarin treated group showed a significant reduction in the above mentioned biochemical parameters when compared to CCl_4 group. The trend was same with that of test groups but the effect was augmented at a dose of 400mg/kg when compared to 200mg/kg. [Table 2].

Effect of AVU on Tissue and Functional Parameters: A marked reduction in the levels of SOD, catalase and reduced glutathione with a marked increase in LPO levels was observed in CCl_4 administered group when compared with normal group. The groups treated with the AVU and silymarin had shown significant increase in these antioxidant enzymes and decrease in LPO when compared to CCl_4. The effect was more at the dose of 400mg/kg in case of AVU. [Table 3].

Effect of AVU on Histopathology of Liver: The histopathological studies revealed an extensive hepatic damage in case of CCl_4 group when compared to the normal group. Silymarin and test groups showed a protective effect by decreasing the extent of centrilobular necrosis and steatosis when compared to CCl_4 group. The protective effect was intense at 400mg/kg dose of AVU [Figure 1].

Table 1: Preliminary phytochemical screening of AVU

CHEMICAL TEST	INFERENCE
Alkaloids	Alkaloids are present.
Mayer's Test	
Wagner'sTest	Alkaloids are present.
Hager's reagent	Alkaloids are present.
Carbohydrates	Carbohydrates are absent.
Molisch's test	
Phytosterols	Phytosterols are absent
1ml acetic anhydride + 2ml H_2SO_4	
1ml conc. H_2SO_4	
Glycosides	Saponins are absent
Shaken in graduated cylinder for15 min	
1ml NH_3+ 1ml lead acetate	Saponins are absent
Borntrager's test for anthraquinone glycosides	Glycosides are absent
Alkaline Reagent test for flavonoids	Presence of flavonoids.
Tannins and phenolic compounds	Tannins are absent
1ml 5% $FeCl_3$ solution	
1ml 10% lead acetate solution	Tannins are absent
Resins	Absence of resins.
Acetic anhydride and concentrated sulphuric acid	
Terpenoids	Absence of terpenoids.
Chloroform and 1 ml of acetic anhydride and 2 ml of conc. sulphuric acid	

Table 2: Effect of AVU on serum biochemical parameters on 22nd day

S.No	Group	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	TB (mg/dl)	TP (gm /dl)	TC (mg/dl)	TG (mg/dl)
1.	Normal	55.31± 0.3566	89.72± 5.139	151.2± 1.356	0.5435± 0.0306	0.8889± 0.0156	79.33± 0.4216	81.33± 0.3333
2.	CCl_4 treated	207.2± 3.611*	343.8± 0.821*	321.2± 8.763*	2.073± 0.0585*	0.5465± 0.0034*	145.3± 0.4216*	157.5± 0.3416*
3.	Silymarin (50 mg/kg b.w)	62.54± 1.044**	130.4± 0.6060**	189.7± 2.605**	0.7685± 0.0242**	0.8150± 0.0030**	71.83± 0.4773**	84.50± 0.2236**
4.	Test I (200 mg/kg PO)	85.99± 1.340**	161.2± 0.5843**	226.7± 3.874**	0.9305± 0.0246**	0.7595± 0.0009**	112.5± 0.7638**	98.50± 0.5627**
5	Test II (400 mg/kg PO)	74.55± 0.8879**	134.7± 0.2379**	206.0± 2.889**	0.8030± 0.0235**	0.7840± 0.0117**	83.83± 0.4773**	84.83± 0.4773**

All values are shown as Mean ± SEM and n=6., * indicate $p<0.05$, when compared to Normal, ** indicate $p<0.05$, when compared to CCl_4 treated.
SGPT = Serum glutamate pyruvate transaminase, SGOT = Serum glutamate oxaloacetate transferase, ALP = Alkaline phosphatase,
TB = Total bilirubin TP = Total protein, TC = Total Cholesterol, TG = Triglycerides

Table 3: Effect of AVU on tissue and functional parameters on 22nd day

S.No	Group	SOD (U/mg protein)	CAT (µM H_2O_2consumed/mg protein)	Reduced GSH (µg of GSH/mg protein)	LPO (nmol of MDA /mg protein)
1.	Normal Control	8.718± 0.06295	11.37 ± 0.06608	9.367± 0.1145	0.4433± 0.004944
2.	CCl_4 treated	4.585± 0.06888*	5.750± 0.05627*	6.400± 0.08563*	1.490± 0.01949*
3.	Silymarin (50 mg/kg b.w)	9.302± 0.1398**	10.30± 0.08976**	9.667± 0.08028**	0.4500± 0.4500**
4.	Test I (200 mg/kg PO)	6.522± 0.1650**	8.350± 0.1176**	8.367± 0.07149**	0.8150± 0.04372**
5.	Test II (400 mg/kg PO)	8.303± 0.06396**	9.950± 0.09822**	9.433± 0.05578**	0.5033± 0.004216**

All values are shown as Mean ± SEM and n=6, * indicate $p<0.05$, when compared to Normal, ** indicate $p<0.05$, when compared to CCl_4 treated.
SOD = Superoxidase dismutase, CAT = Catalase, GSH = Glutathione, LPO = Lipid peroxidase

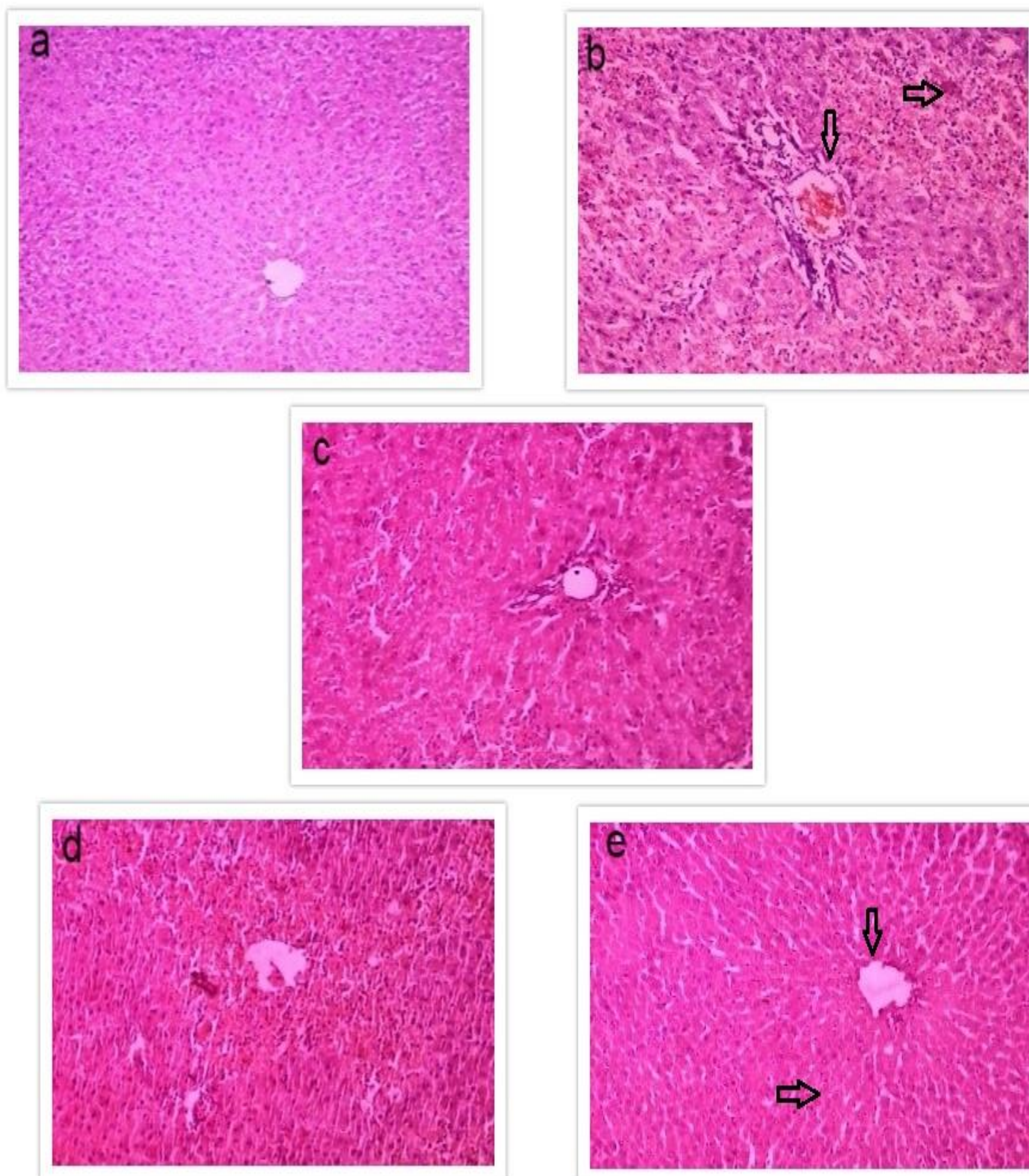

Figure 1: a) Normal group rats show normal hepatic lobular structure with central vein (10X). b) CCl_4 treated group rats show steatosis, centrilobular necrosis and fibrosis (10X). c) Silymarin pretreated group (10X). d) AVU pretreated group – 200mg/kg (10X). e) AVU pretreated group – 400mg/kg show marked protection against free radical damage with near to normal hepatological structure (10X)

DISCUSSION & CONCLUSION

Liver is the major vital organ in the body that is employed in metabolism and excretion of xenobiotics. Hepatic injury or hepatic dysfunction has become worldwide health burden imposing challenges not only to healthcare professionals but also the drug development agencies. Hepatocytes are involved in a variety of metabolic events; therefore, the establishment of liver shielding agents is of supreme importance. Although some synthetic medicines were recommended for liver therapy in the last years, most of them are immunosuppressive and to treat liver in this condition with these medicines leads to further hepatic damage. Hence, herbal drugs which have been used in the treatment of liver diseases from time immemorial are becoming increasingly popular and promising.[20] However, most of these herbal medicines lack scientific pharmacological validation. Hence, preclinical evaluation of safety and efficacy of these medicines followed by clinical evaluation is getting paramount attention in recent years.[21,22,23]

In this study CCl_4 was selected as a hepatotoxicant to induce liver damage and primary objective of this study was to assess the hepatoprotective activity of aqueous seed extract of *Vigna unguiculata* at doses of 200 mg/kg and 400 mg/kg body weight against CCl_4 induced liver damage.

The purpose of using male albino rats as an experimental animal in the present hepatoprotective study is fundamentally because of the structural homology of rat CYP 450 enzymes with that of humans[24] and moreover female rats are less susceptible to CCl_4-induced liver damage, especially hydroxyproline accumulation.[25]

The acute toxicity study revealed the absence of lethality among the tested animals when administered with AVU PO as a single dose (5, 50, 300 and 2000 mg/kg). There were no signs of any gross behavioral changes except for an increase in urination indicating the safe usage of the extract at doses of 200 mg/kg and 400 mg/kg body weight.

Carbon tetrachloride has been widely used to examine chemical toxin-induced hepatic damage in animal models. Fatty liver, cirrhosis, and necrosis are the remarkable pathological characteristics of CCl_4-induced hepatotoxicity. After single dose of CCl_4, necrosis and steatosis develops within a short period of time followed by an injury towards the endoplasmic reticulum, leading to functional defects in hepatocytes and numerous biochemical manifestations of hepatic injury. Mechanism of coupling of triglycerides to the appropriate apoprotein to form the lipoprotein carrier molecule is disrupted, leading to blockade of fat movement out of hepatocytes causing steatosis. During hepatic damage, enzymes like SGOT, SGPT and ALP present in the hepatocytes leak into the blood, resulting in enhanced concentration.[26]

Amino transferases are a group of liver specific enzymes that catalyze the interconversion of amino acids and α-keto acids by the transfer of amino groups. These are very sensitive and dependable biomarkers for necessary hepatotoxic as well as hepatoprotective or curative effect of various compounds.[27] Both SGOT and SGPT levels increase due to toxic compounds that affect the integrity of hepatocytes.[28] Attenuation in the levels of transaminases specify stabilization of plasma membrane and protection of liver cells against damage caused by hepatotoxins. It was demonstrated that oral administration of AVU at 200 and 400 mg/kg significantly and dose-dependently inhibited the elevation of serum levels of SGOT and SGPT when compared with the disease control group ($P < 0.05$). AVU at 400 mg/kg showed comparable potency of hepatoprotective effect to the standard control Silymarin. This is in covenant with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and hepatocytic regeneration.[29]

ALP is a membrane bound glycoprotein marker enzyme for plasma membrane and endoplasmic reticulum, located predominantly in the microvilli of bile canaliculi. Along with liver, it is produced by many other organs and is finally excreted into the bile. During hepatic damage or biliary obstruction, there will be an increase in plasma phosphatase levels due to inability of the liver to excrete the enzyme. In this condition, rate of bile secretion by the hepatocytes is also reduced leading to surge in plasma bilirubin levels.[30, 31] Pretreatment with AVU and Silymarin decreased raised ALP and bilirubin levels but the reduction was near to that of Silymarin in case of AVU given at a dose of 400 mg/kg. Reduction of ALP levels with concurrent depletion of raised bilirubin levels suggests stabilization of hepatic plasma membrane and biliary function with AVU during injury with CCl_4.

Liver plays a key role in the synthesis of plasma proteins like albumin, globulins (α, β, γ) and fibrinogen. During liver injury, metabolic biotransformation of amino acids may be impaired leading to reduction in serum total protein (TP) levels. This attenuation is credited to the early damage produced and limited to the endoplasmic reticulum which results in the loss of CYP 450 enzymes leading to its functional failure with a decline in protein synthesis.[32] The test groups at both doses considerably enhanced the synthesis of TP which may be by accelerating the regeneration process and protecting the liver cells but the rise was higher with AVU at the dose of 400 mg/kg. The increased levels of total protein in serum are indicative of the hepatoprotective activity.

Lipids such as cholesterol and triglycerides are increased in hepatopathy. The lipid content of hepatocytes is regulated by the integrated activities of cellular enzymes that catalyze lipid uptake, synthesis, oxidation and export. Fat accumulates within the hepatocytes when the input of fatty acids to hepatocytes exceeds their output. Many extrahepatic and intrahepatic factors, can impair both regulator mechanisms and, therefore, promote cholesterol and triglyceride accumulation in the liver.[33] The test groups at both doses considerably reduced the levels of triglycerides and cholesterol enhanced due to CCl_4 administration and prevented their accumulation in the liver cells but the reduction was higher with AVU at the dose of 400 mg/kg.

Reactive Oxygen Species (ROS) may play an important role in many retrogressive diseases, such as liver injury and chronic fatigue syndrome.

Biological systems are especially sensitive to ROS, the reactive forms of oxygen which arise as either by-products of oxidative phosphorylation in the mitochondria, or as the result of exposure to environmental chemicals and toxins.[34]

The acute toxic effects of CCl_4, a well-known hepatotoxin are known to be mediated through free radicals *in vivo.* Thus inhibition of free radicals by *in vivo* antioxidant systems like SOD, CAT and GSH could play a vital role protection of liver against CCl_4-induced free radical damage. Superoxide dismutase is an enzyme that alternately catalyzes the dismutation of the superoxide (O_2^-) radical into either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2).[35] Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index of hepatocellular damage and is the most sensitive enzymatic index in liver injury. Catalase (CAT) is an enzymatic antioxidant extensively distributed in all tissues with its highest

activity found in the red blood cells and liver. It decomposes H_2O_2 and inhibits the production of highly reactive hydroxyl radicals.[36] Therefore, reduction in the activity of CAT may result in a number of harmful effects. Glutathione (GSH) is one of the most plentiful, non-enzymatic biological antioxidant present in the liver, involved in the removal of free radical species, maintenance of membrane protein thiols and also acting as a substrate for glutathione peroxidase (GPx). Decreased levels of GSH are associated with an enhanced lipid peroxidation in CCl_4 treated rats, which in turn gives rise to other products like malondialdehyde that extensively causes membrane damage.[37] The present study demonstrated that both the AVU and Silymarin pretreated groups significantly reduced the oxidative stress by recovering the decreased activities of SOD, CAT, GSH and reducing the levels of malondialdehyde in CCl_4-induced acute liver injury. The anti-oxidant activity was higher with test group administered AVU at a dose of 400mg/kg body weight when compared to 200 mg/kg body weight. This indicates the antioxidative and free radical scavenging properties of AVU.

Histopathological studies reveal the extent of hepatic damage in CCl_4 treated animals when compared with that of normal group animals. The rats in the CCl_4 group showed severe hepatotoxicity evidenced by profound steatosis, centrilobular necrosis and fibrosis as compared to the normal hepatic architecture of the normal group animals. Both the test groups prevented the action of free radicals on parenchyma but the outcome test group treated with AVU at a dose of 400 mg/kg was comparable to that of the standard group treated with Silymarin.

Preliminary phytochemical screening of aqueous extract of *Vigna unguiculata (*AVU) seeds revealed the presence of alkaloids, flavonoids and proteins in the aqueous extract of *Vigna unguiculata.* Ironically the activity of any herb is due to the chemical constituents present in it. The AVU extract may be protecting the liver by free radical scavenging activity and thus preventing peroxidation of lipids of the endoplasmic reticulum and this may be due to the presence of flavonoids like quercetin, kaempferol, and isorhamnetin and alkaloidal pigments in the extract. Phytoconstituents like the flavonoids and alkaloids are also known to possess hepatoprotective activity which are components of AVU.[38,39] Thus the hepatoprotective action of AVU may be due to the presence of the above mentioned phytochemical constituents. In summary, this study suggests that the oral administration of AVU significantly ameliorates CCl_4 induced hepatotoxicity in rats at a dose of 400 mg/kg when compared to 200 mg/kg. Thus, seeds of *Vigna unguiculata,* may act as potential anti-hepatotoxic agent.

From all these findings, we can conclude that the seeds of *Vigna unguiculata,* most commonly consumed culinary dish has significant hepatoprotective activity and the potential usefulness of the AVU in clinical conditions associated with liver damage is still to be demonstrated. Further work should be done for the isolation and characterization of active principles responsible for the hepatoprotective activity and to establish the effectiveness and pharmacological rationale for the use of *Vigna unguiculata* as hepatoprotective agent.

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