

HEPATOPROTECTIVE ACTIVITY OF SEEDCOAT AND COTYLEDON EXTRACT OF
CAJANUS CAJAN L. AGAINST CCL₄ INDUCED HEPATOTOXICITY IN MICEPratima H*[#], MD Liyakat Ahmad** and Pratima Mathad[#]

*Department of Botany, Karnataka State Women's university, Vijayapur, Karnataka, India


Department of Post-Graduate Studies and Research in Botany, Gulbarga University,
Kalaburgi- 585106, Karnataka, India

**Department of Pharmacognosy, Luqman Pharmacy College, Kalaburgi, Karnataka, India.

ABSTRACT: The ethanolic extract of *Cajanus cajan* seed coat and cotyledon (100 and 500mg/kg b.w) was screened for hepatoprotective and *in vivo* antioxidant activity in hepatotoxic Swiss albino mice induced via carbon tetrachloride. The phytochemical result shows that presence of alkaloids, steroids, phenols, flavonoids, tannins, lignins, glucosides whereas, saponin is absent. Cotyledon extract exhibited moderate protective effect by lowering the serum level of Serum Pyruvate Transaminase (SGPT), Serum Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), bilirubin and by increasing the level of protein. Whereas, highest activity was observed at higher concentration (500mg/kg) of seed coat extract and the reduction of serum level SGPT, SGOT and ALP is comparable with the standard hepatoprotective drug Liv-52 (100 mg/kg body weight) which served as positive control. These biochemical observations were in turn confirmed by histopathological examination of liver sections. The hepatic antioxidant statuses such as Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POX), Glutathione reductase (GR) activities were reduced and lipid peroxidation level increased in CCl₄ alone treated animals. Administration of ethanolic extracts to CCl₄ challenge restored the hepatic antioxidant status. These finding suggested that seed coat and cotyledon extract of *C. cajan* protects CCl₄ induced chronic hepatotoxicity in mice by restoring the liver antioxidant status.

Key words: Antioxidant, CCl₄, *Cajanus cajan*, hepatoprotective.

*Corresponding author: Pratima H, Department of Botany, Karnataka State Women's university, Vijayapur, Karnataka, India Email: pratimakalsanki@gmail.com

Copyright: ©2016 Pratima H. This is an open-access article distributed under the terms of the Creative Commons Attribution License , which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

INTRODUCTION

Liver is one of the vital organs endowed with several important homeostatic responsibilities. Liver diseases are the most serious ailment and are mainly caused by toxic chemicals like excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil etc. Due to excessive exposure to hazardous chemicals, the free radicals generated will be so high such that they overpower the natural defensive system leading to hepatic damage and cause cirrhosis, chronic hepatitis, fibrosis, hepatocellular carcinoma and fatty liver, which remain one of the serious health problems. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown (Gupta *et al.*, 2005). Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety.

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. Carbon tetrachloride (CCl₄) is a xenobiotic producing hepatotoxicity in human beings and animals (Brattin *et al.*, 1985 and Azer *et al.*, 1997).

Carbontetrachloride is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of $\text{CCl}_3\text{O}^\cdot$, a reactive free radical, which initiates lipid peroxidation (Zimmerman and Hayman 1976, Agarwal and Mehendale 1983). Antioxidants play a crucial role in hepatoprotective ability and hence the search for crude drugs of natural origin with this property has become a central focus of study of hepatoprotection today (Pradeep *et al.*, 2010).

Cajanus cajan L. is a perennial member of the family leguminosae, commonly known as pigeon pea or red gram in english, arhar in hindi and togari in kannad. About 90% of the World production of *C. cajan* is contributed by India where it is cultivated in semi-arid regions as kharif. The *C. cajan* seeds are extensively eaten as 'dhal' contains approximately 22% protein depending on cultivar's and location. Besides its high nutritional value, pigeon pea is also used as traditional folk medicine in India, China, Philippines and some other nations. Scorched seeds, when added to coffee alleviate headache and vertigo (Saxena *et al.*, 2010). The seeds are astringent, acrid, sweet, cooling, anthelmintic, resolvent, expectorant and constipation (Kirtiker and Basu, 2005; Naryan *et al.*, 2007). Fresh seeds are believed to help incontinence of urine in males, while immature seeds are recommended for treatment of kidney ailments (Duke 1981). Further, the extract from seeds showed hypolipidemic, antioxidant and antimicrobial activities (Oboh, 2006).

The husk (seed coat) of pigeon pea is utilised mainly as cattle feed, recent reports find it to be an excellent material for treating waste waters containing low concentration of metal ions (Ahalya *et al.*, 2007) in manufacture of biscuits (Tiwari *et al.*, 2011) and also as an anti-microbial agent (Kanatt *et al.* 2011). Seed coat is a source of phenolic compounds (Bhattacharya and Chenchaiiah 2007) and as potent natural resource of anti-oxidant and anti-hyperglycaemic activity (Ashok kumar *et al.* 2013). The hepatoprotective activity of leaves of *C. cajan* L. were reported by many researchers (Siddharth singh, 2011; Rhitajit *et al.* 2013; Anjana Male *et al.* 2012; Oluseyi and Moshood, 2011; Suman Pattanayak *et al.* 2011; Ighodaro and Omole, 2010; Kasturi Sarkar *et al.* 2006), and hepatoprotective activity of seed have been reported by Iweala and Elekwa (2001) but there are no reports about the seed coat and cotyledon. Thus present study, a best effort is made to evaluate the hepatoprotective effect of seed coat and cotyledon of *C. cajan*, against CCl_4 induced hepatic damage and *in vivo* antioxidant activity in mice.

MATERIALS AND METHODS

Extract preparation: The seeds of *Cajanus cajan* var. Maruti (ICP-8863) were collected from Agriculture research station Gulbarga, Karnataka, India. Seeds were moistened for 1h and then dried in oven at 55°C overnight. The hull or seed coat was removed mechanically means by using hand grinder. The two fractions like seed coat and cotyledon were made into fine powder using grinder. Five hundred grams of the powdered seed coat and cotyledon were subjected to the soxhlet successive extraction method ($60\text{-}80^\circ\text{C}$) using 2.5 litter of ethanol solvent for a period of 18h. The extracts obtained were dried at 40°C .

Phytochemical screening: The extracts of the plant were screened for alkaloids, flavonoids, glycosids, saponins, tannins, phenols, lignins and steroids as described by Harborne (1998).

Tests for Phenols

Phenol test: 0.5 ml of FeCl_3 (w/v) solution was added to 2 ml of test solution, formation of an intense colour indicates the presence of phenols.

Ellagic acid test: The test solution was treated with few drops of 5% (v/v) glacial acetic acid and 5% (w/v) NaNO_2 solution. The solution turns muddy yellow, olive brown, Niger brown, deep chocolate colours depending on the amount of ellagic acid present.

Tests for Tannins

Gelatin test: The test solution was evaporated to dryness and the resulted residue was dissolved in 1% (w/v) liquefied gelatine. To this, 10% (w/v) NaCl solution was added. A white precipitate was obtained which indicate the presence of tannins.

Tests for Flavonoids

Pew's test: A pinch of zinc powder and about 5 drops of 5 N HCl were added to the test solution. It results deep purple red (dihydroquercetin) or cherry red (dihydrokaempferol) colours. Flavonones, dehydrochalcones and other flavonoids get at most pinkish or brownish colour.

Shinoda test: A pinch of magnesium powder and 5 N HCl were added to the test solution and a deep red or magenta colour formation indicates the presence of flavanone or dihydroflavanol. However, dihydrochalcones and other flavonoids did not react with this reagent.

NaOH test: 1 ml of 1 N NaOH solution was added to the 1 ml of test solution, formation of yellow colour indicates the presence of flavonoids.

Tests for Lignins

Labat test: Formation of olive green colour, when the gallic acid is added to the test solution, indicates the presence of lignin.

Lignin test: Formation of red colour, when 2% (w/v) furfuraldehyde is added to the test solution, indicates the presence of lignin.

Tests for Steroids

Liebermann-Burchard test: A green colour was formed, when the Liebermann-Burchard reagent is added to the solution, indicate the presence of steroids.

Salkowski's test: A wine red colour was developed when chloroform and Conc. H₂SO₄ were added to the test solution; indicate the presence of steroidal nucleus.

Tests for Alkaloids

Iodine test: 1 ml of KI in iodine solution was added to the 2 ml of test solution. A brown precipitate formation indicated the presence of alkaloids.

Dragendorff's reagent: 2 ml of Dragendorff's reagent and 2 ml of dilute HCl were added to the test solution. An orange-red coloured precipitate indicates the presence of alkaloids.

Wagner's test: 2 ml of Wagner's reagent was added to 2 ml of test solution. The formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for Glycosides

Conc. H₂SO₄ test: To the extract add Conc. H₂SO₄ and allowed to stand for few minutes, it turned into reddish colour.

Kellar Killiani test: The extract was dissolved in glacial acetic acid and after cooling 2 drops of ferric chloride solution was added to it. These content were transferred to a test tube containing 2 ml of Conc. H₂SO₄. A reddish brown ring was observed at the junction of two layers.

Tests for Saponins

Foam test: 0.01 g of crude extract was shaken vigorously in 2 ml of distilled water. Formation of honeycomb like froth persists for a few minutes indicate the presence of saponins.

Experimental animals: Swiss albino mice weighing 25-30 g of either sex were used. These animals were housed in standard metal cages and were provided food and water *ad libitum*. Animals were maintained according to the guidelines of institutional animal ethics committee.

Acute toxicity test: the acute toxicity test was determined according to the Organization for Economic CO-operation and Development (OECD 2001). Either sex of both mice (25-30g) was used for this study. After sighting study, starting dose of 1000mg/kg b.w test sample were given to various groups containing 6 animals in each group. The treated animals were monitored for 7 days for mortality and behaviour responses. No abnormal behaviour and death was observed till the end of 7 days. The test sample was found safe up to 1000mg/kg and from the results 100 and 500 mg/kg b.w (1/10th and 1/5th) of this dose were selected as the therapeutic dose for the evaluation.

Assessment of Hepatoprotective activity: A total at 42 animals were equally divided into seven groups (n=6 in each group). The treatment period was for 6 days. Group-I was maintained as normal control which was given distilled water only and Group-II received CCl₄ (2 ml/kg) diluted with liquid paraffin (1:1) given orally on third and sixth day. Group III received CCl₄ and standard drug Liv-52 (100 mg/kg p.o). Group IV and V received CCl₄ and cotyledon extract 100 and 500 mg/kg p.o. Similarly Group VI and VII received CCl₄ and cotyledon extract 100 and 500 mg/kg p.o. respectively, once simultaneously for 6 days. Food was withdrawn 12h before CCl₄ administration on the sixth day to enhance the acute liver damage in all the groups except Group- I animals. Mice were sacrificed on seventh day, 24h after administration of the last dose.

The animals were then anesthetized using anaesthetic ether, and blood collected by retro orbital puncture and biochemical parameters of blood serum was analysed for serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serume alkaline phosphatase (ALP) levels using enzopak reagent kits by the method proposed by Reitman and Frankel (1957), total proteins by Biuret method (Peters, 1968) and total bilirubins by Jenderassik and Grof (1938). The animals were sacrificed by overdose of ether and autopsied. Livers from all animals were removed, washed with ice-cold saline small piece of liver tissue collected and preserved in 10% formalin solution for histopathological studies.

In-vivo Antioxidant activity: Small piece of liver tissue (200mg) was homogenized with ice-chilled 10% KCL solution and centrifuged at 1200 rpm for 15min at 4°C. The supernatant was used for the assay of antioxidant parameters. The SOD activities in liver were estimated by Dhindsa *et al.*, (1981). The peroxidase activity was assayed according to the method of Malick and Singh (1980). Catalase activity was assayed by estimating residual hydrogen peroxide by titanium reagent (Teranishi *et al.*, 1974). The glutathione reductase was assayed by Smith *et al.*, (1988). Reduced glutathione (GSH) content was estimated as described by Dekok and Kuiper (1986). The level of lipid peroxidation was measured the method of Heath and Packer (1968).

Statistical analysis: The data of all measurements are means from three replications. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey-Kramers multiple comparison tests.

RESULTS

Phytochemicals

The results of the preliminary screening of ethanol extract of seed coat and cotyledon showed the presence of secondary metabolites such as phenols, flavonoids, tannins, lignins, terpenoids, alkaloids, glycosides and steroids whereas, saponins were absent. (Table 1).

Acute toxicity studies

The result of the acute toxicity test shows that the crude ethanolic extract of seed coat and cotyledon up to 1000mg/kg p.o did not produce any mortality so, it was non-toxic to mice and hence $1/10^{\text{th}}$ and $1/5^{\text{th}}$ of this dose i.e 100 and 500 mg/kg , p.o of crude ethanolic extract of *C.cajan* seed coat and cotyledon was used for *in- vivo* study.

Table 1. Phytochemical screening of ethanol extract of seed coat and cotyledon of *Cajanus cajan* L.

Secondary metabolites	Name of the test	Seed coat	Cotyledon
Phenols	Ellagic acid test	+	+
	Phenol test	+	+
Tannins	Gelatin test	+	+
Flavonoids	Pews test	+	+
	Shinoda test	+	+
	NaOH test	+	+
Lignins	Labat test	+	+
	Lignin test	+	+
Steroids	Libermann-burchard test	+	+
	Salkowski test	+	+
	Iodine test	+	+
Alkaloids	Dragendroff's test	+	+
	Wagner's test	+	+
	Kellar-kilani test	+	+
Glycosides	Conc. H ₂ SO ₄ test	+	+
	Foam test	-	-

‘+’ indicate Present, ‘-’ indicate absent

Effect on Serum biochemical's

The effect of ethanolic extract of seed coat and cotyledon of *C. cajan* on CCl₄ induced liver damage in mice with reference to the changes in the level of serum marker enzymes SGOT, SGPT, ALP and total protein and bilirubin were shown in Table 2 & 3. Results shows that administration of CCl₄ led to significant increased levels of serum enzymes SGOT (44.70±0.178 U/L), SGPT (54.32±0.4472 U/L), ALP (143.4±0.0268 U/L) and total bilirubin (0.95±0.0447mg/dl) and decreased the level of total protein (2.56±0.752mg/dl) was compared with the normal control group. Whereas, different concentration (100, 500mg/kg) of seed coat and cotyledon extract treated mice showed significant (P<0.001) reduction in the elevated levels of serum marker enzymes but, the maximum reduction of SGOT (25.01±1.366U/L), SGPT (38.50±0.4082 U/L), ALP (94.70±0.0447 U/L), bilirubin (0.55±0.0178 mg/dl) and increased protein (6.03±0.031 mg/dl) were observed at 500 mg/kg of seed coat extract treated group, which restored the altered level of enzymes indicating the recovery of hepatic cells against the damage. Liv-52 treated group showed significant decrease of serum marker enzymes (P<0.001) when compared to CCl₄ treated animals.

Effect on *In-vivo* antioxidant system

CCl₄ administration increased LPO concentration and decreased GSH level and POX, CAT, SOD, GR activity in liver compared with the normal control mice, suggesting that LPO was a result of CCl₄ poisoning (Table-4). The dose of 100mg/kg and 500 mg/kg treated group was found to significantly decreased (P<0.001) the level of LPO in liver homogenate when compared to CCl₄ treated group. The dose of 500mg/kg treated group of ethanolic extract of seed coat (176.±1.265nmol/mg) was found to be more effective compared to Live-52 (190.6±1.126nmol/mg) treated group. There was a marked decrease in the level of GSH and the activities of POX, SOD, GR and CAT in CCl₄ treated group when compared to normal control group. 100 mg/kg and 500 mg/kg treated group of both seed coat and cotyledon extracts significantly increased (p<0.001) the level of GSH and enzymatic antioxidants when compared with CCl₄ treated animals. However, the maximum level of GSH and enzymatic antioxidants were observed in seed coat extract treated group compared to cotyledon extract treated animals.

Histopathological studies

Histopathological studies revealed that, the maximum level of fatty changes, focal necrosis, congestion in central vein and congestion in sinusoidal spaces were found in hepatotoxin treated mice (Fig-1B). The liver sections of seed coat and cotyledon extract at 100mg/kg dose level plus CCl₄-treated mice showed mild prevention of CCl₄-induced degenerative changes with pyknotic nuclei and fatty vacuolization in cytoplasm (Fig-1D&F). The liver sections of seed coat and cotyledon extract at the dose level of 500mg/kg along with CCl₄-induction to mice (Fig-1 E&G) showed more or less normal lobular pattern with the devoid of degenerative changes and preserved cytoplasm with prominent nucleus without intracellular lipid accumulation almost comparable to the normal control and Liv-52 treated group (Fig-1A&C).

Table-2: Effect of ethanolic extract of seed coat and cotyledon of *C. cajan* on enzymes levels in CCl₄ induced hepatotoxic Albino mice

Group	Treatment	SGOT (U/L)	SGPT (U/L)	-ALP (U/L)
A	Control	23.35±0.0447	34.65±0.0447	85.7±0.0408
B	Toxicant CCl ₄	44.70±0.178	54.32±0.4472	143.4±0.0268
C	Standard Liv-52	29.70±0.044**	37.23±0.0268 ^{NS}	102.3±0.0178**
D	EESC(100mg)	36.34±0.0178**	46.61±0.01506**	99.56±0.0447**
E	EESC(500mg)	25.01±1.366**	38.50±0.4082**	94.70±0.0447**
F	EEC(100mg)	32.42±0.0178**	42.01±4.899**	85.40±0.0447**
G	EEC(500mg)	29.26±0.0268**	40.00±3.578**	103.0±0.0447**

Note: EESC-Ethanolic extract of seed coat, EEC-Ethanolic extract of cotyledon.

- The values were the average of triplicate samples (n = 6) ± S.E. Significance level based on Tukey-kramer's test: *p<0.01, **p<0.001 versus mice treated with CCl₄ alone and NS (non significant).

Table-3: Effect of ethanolic extract of seed coat and cotyledon of *C. cajan* on biochemical levels in CCl₄ induced hepatotoxic Albino mice

Group	Treatment	Total bilirubins (mg/dl)	Total proteins(mg/dl)
A	Control	0.490±0.0268	9.85±0.632
B	Toxicant CCl ₄	0.95±0.0447	2.56±0.752
C	Standard Liv-52	0.63±0.0089**	8.34±0.008**
D	EESC(100mg)	0.472±0.1623**	3.38±0.025*
E	EESC(500mg)	0.55±0.0178**	6.03±0.031 ^{NS}
F	EEC(100mg)	0.56±0.0447**	5.2±0.1265**
G	EEC(500mg)	0.66±0.447**	8.41±0.025**

Note: EESC-Ethanolic extract of seed coat, EEC-Ethanolic extract of cotyledon.

- The values were the average of triplicate samples (n = 6) ± S.E. Significance level based on Tukey-kramer's test: *p<0.01, **p<0.001 versus mice treated with CCl₄ alone and NS (non significant).

Table-4: Effect of ethanolic extract of seed coat and cotyledon of *C. cajan* on *in vivo* antioxidants in CCl₄ induced hepatotoxic Alino mice

Group	Treatment	Catalase (U/mg proteins)	Peroxidase (U/mg proteins)	Superoxide dismutase (U/mg proteins)	Glutathione reductase (U/mg proteins)	GSH (mg/100mg proteins)	LPO (nmol/mg proteins)
A	Control	72.4±0.0178	4.523±0.0178	14.2±0.0447	8.874±0.0163	34.6±1.2313	185.3±1.897
B	Toxicant CCl ₄	14.3±0.0447	2.814±0.0286	5.6±0.0447	2.132±0.0075	18.3±0.0316	383.7±0.376
C	Standard Liv-52	40.2±0.0408**	5.131±0.0089**	13.4±0.0376**	8.125±0.0163**	22.3±0.0314**	190.6±1.126**
D	EESC(100mg)	35.7±0.0163**	3.147±0.0225**	8.4±0.0347**	5.123±0.268**	18.6±0.0178**	250.7±0.311**
E	EESC(500mg)	50.5±0.0376**	6.245±0.0357**	10.3±0.0447**	8.024±0.0075**	23.2±0.0376*	78.7±1.265**
F	EEC(100mg)	29.3±0.0178**	3.456±0.0268**	6.8±0.0178**	5.106±0.0633**	15.2±0.0326**	221.6±0.0316*
G	EEC(500mg)	42.3±0.0408**	5.102±0.0447**	8.5±0.0247**	8.116±0.0225**	22.6±0.9663	198.5±1.265**

The values were the average of triplicate samples (n = 6) ± S.E. Significance level based on Tukey-kramer's test: *p<0.01, **p<0.001 versus mice treated with CCl₄ alone and NS (non significant).

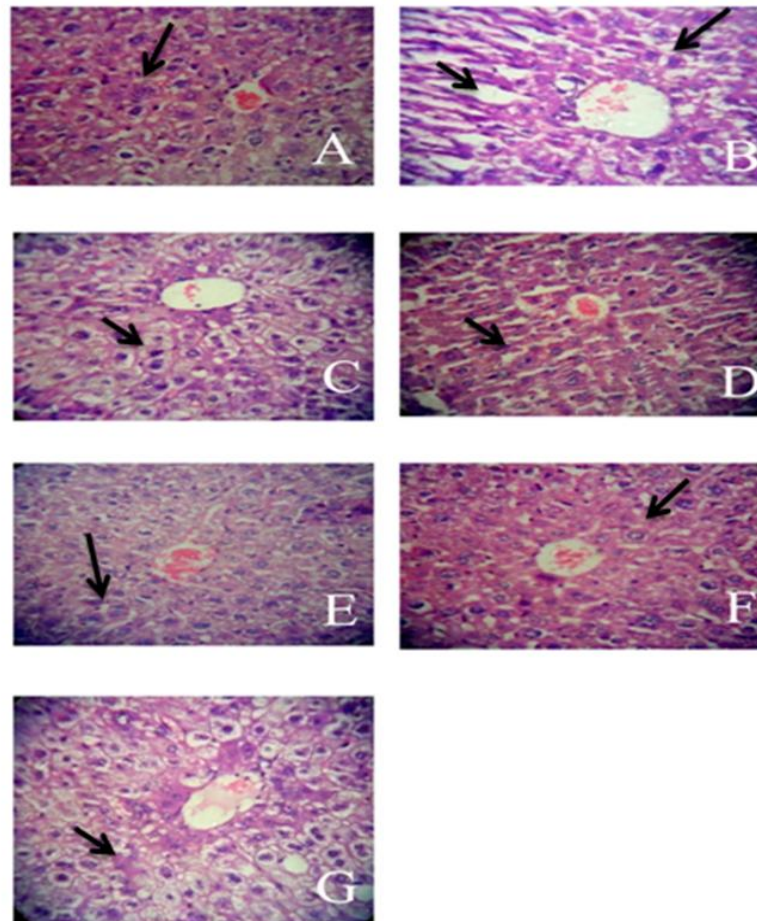


Fig-1: Histopathological changes in the Liver of mice caused by CCl₄ and preventive effect of ethanolic seed coat and cotyledon extract of *C. cajan* in different groups: (A) Control- a normal lobular architecture of the liver ; (B) CCl₄- showed liver cell necrosis, inflammation and degenerative hepatocytes ; (C) Liv-52- showed normal hepatocytes and their lobular architecture was normal; (D)- 100 mg/kg b.w EESC- showed minimal inflammation and shows mild focal rearrangement of cell ; (E) 500 mg/kg b.w. EESC- showed normal hepatocytes and absence of necrosis is a sign of protection. (F) 100mg/kg EEC- showed minimal inflammation; (G) 500mg/kg EEC- a normal lobular architecture of the liver.

DISCUSSION

The preliminary phytochemical screening of seed coat and cotyledon of *C.cajan* revealed the presence of phenols, flavonoids, tannins, lignins, alkaloids, glycosides, steroids. The presences of phenolic compounds are reported to have the free radical scavenging ability, which stabilizes lipid oxidation. Some studies suggest a correlation between phenolic content and hepatoprotective effect (Awaad *et al.*, 2012). Report also indicates that some steroids may be responsible for hepatoprotective effect (Afzal *et al.*, 2013). Suman Pattanayak *et al.* (2011) is reported the hepatoprotective activity of crude flavonoids extract of *Cajanus scarabaeoides*. Hence the presence of these metabolites may be responsible for the hepatoprotective and antioxidant activity of seed coat and cotyledon of *C.cajan*.

The ethanolic extract of *C. cajan* seed coat and cotyledon showed good hepatoprotective activity when administration at doses of 100 and 500mg/kg b.w. Mice treated with CCl₄ developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes when compared to normal control. SGPT, SGOT and ALP are a cytosolic enzyme primarily present in the liver. These enzyme levels in serum increases due to leakage of this cellular enzyme into plasma by toxicants induced hepatic injury (Chenoweth and Hake, 1962). In the current study treatment of animals with seed coat and cotyledon extract (500mg/kg) significantly decreased the levels of SGPT, SGOT and ALP in serum which is an indicative of hepatoprotective. Similar results are observed by Sidharth Singh *et al.* (2011) in hydroalcoholic extract of leaf of *C. cajan* against CCl₄ induced liver damage. Stabilization of serum total bilirubin level by the administration of seed coat and cotyledon extracts of *C.cajan* is further a clear signal of the improvement of the functional status of the hepatic cells (Dharmendra Singh *et al* 2014). The total protein estimation is useful in hepatoprotective study as its decreased level indicates severe non viral liver cell damage (Shenoy *et al.*, 2001). After CCl₄ administration considerably reduced serum total protein levels which was significantly elevated on treatment with *C. cajan* seed coat and cotyledon extract, indicating its protective role against liver cell damage.

Carbon tetrachloride (CCl₄) intoxication leads to formation of lipid peroxides, which in turn produce MDA that cause damage to cell membranes (Siddhartha *et al.*, 2011). In our present study, the measurement of LPO in the liver tissue is a convenient method to monitor oxidative cell damage. Inhibition of elevated LPO has been observed in both seed coat and cotyledon extract and standard Liv-52 treated groups due to its antioxidant and free radical scavenging activities through re-establishment of biomembranes of hepatic parenchyma cells (Singh *et al.*, 2009). Glutathione (GSH) is one of the most abundant naturally occurring tripeptide, non-enzymatic biological antioxidant present in liver (Gul *et al.*, 2000). It plays a major role in the elimination and detoxification of many exogenous toxicants, also important in quenching the reactive intermediates and radical species generated during oxidative stress so coordinating the body's antioxidant defence processes (Lee *et al.*, 2000; Mohammad *et al.*, 2011). In the present study, the decreased level of GSH has been associated with an enhanced level of lipid peroxidation in CCl₄ intoxicated groups of mice. The seed coat extract showed higher increase in GSH content (9.43±0.19 µMol/mg) at 500mg/kg b.w. Similarly, Harraz *et al.* (2015) have been reported in ethanolic extract of aerial part of *Tribulus terrestris*. A significant depletion in the activities of antioxidant enzymes like SOD, CAT, POX and GR during CCl₄ intoxicated animals might be due to the enhanced free radical formation leading to oxidative stress in the tissue. Pre-treatment with seed coat and cotyledon extract showed good protection against CCl₄ induced hepatotoxicity by acting as a strong free radical quencher and protecting the hepatic cells. Therefore SOD, CAT, POX, and GR are essential for the endogenous antioxidative defence system to scavenging reactive oxygen species and maintain the cellular redox balance (Molina *et al.*, 2003; Parimoo *et al.*, 2014). The attenuation, suppression or inhibition of free radicals or reactive oxygen species activity by antioxidant is important in providing protection against liver damage (Kidd 1997).

CONCLUSION

Our result demonstrates a very good protective effect at 500mg/kg b.w. of ethanolic extract of *C. cajan* seed coat compared to cotyledon against CCl₄ induced oxidative stress and hepatic injury. The activity may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defence contributing to the protection against oxidative damage in CCl₄-induced hepatotoxicity. *C. cajan* seed coat possess potent anti-oxidant and hepatoprotective activity. It may become an economical natural organic resource for development of functional food/nutraceuticals. Further studies are underway to isolate and elucidate the active principle responsible for the hepatoprotective and antioxidant effects.

ACKNOWLEDGEMENT

The authors are thankful to the Chairman, Department of Botany, Gulbarga University Gulbarga and also thankful to Principle, Department of pharmacology, Luqman pharmacy college, Gulbarga for providing the laboratory facilities for animal experiments.

REFERENCES

- Afzal M., Khan R., Kazmi I., Anwar F. (2013). Hepatoprotective potential of new steroid against carbon tetrachloride-induced hepatic injury. *Mol Cell Biochem.* 378, 275-81.
- Agarwal A.K. and Mehendale J.K. (1983). Potentiation of carbon tetrachloride hepato toxicity and lethality by chlordecone in female rats. *Toxicology.* 26, 231-42.
- Ahalya N., Kanamadi R.D., Ramachandra T.V., (2007). Cr (VI) and Fe (III) removal using *Cajanus cajan* husk. *J Environ Biol.* 28, 765-9.
- Anjana Male, Naga Bhavani, M., Renuka, K. and Kalyani, M. (2012). Hepatoprotective activity of leaf extracts of *Cajanus cajan* L. *International Journal of Universal Pharmacy and Life Sciences.* 2(1), 228-232.
- Ashok Kumar Tiwari, Bacha Abhinay, Katragadda suresh Babu, Domati Anand Kumar, Amtul Zehra, Kuncha Madhusudhana (2013). Pigeon pea seed husks as potent natural resource of anti-oxidant and anti-hyperglycaemic activity. *International Journal of Green Pharmacy.* 7(3), 252-257.
- Awaad A.S., Soliman G.A., El-Sayed D.F., El-Gindi O.D., Alqasoumi S.I. (2012). Hepatoprotective activity of *Cyperus alternifolius* on carbon tetrachloride-induced hepatotoxicity in rats. *Pharm Biol.* 50,155-61.
- Azer, S.A., Mc Caughan, G.M. and Stacey, N.H. (1997). *Liver and environmental xenobiotic*; Narosa Publishing House, New Delhi, P. 178.
- Bhattacharya, A.K. and Chenchiah, K. C. (2007). Seed coat phenolic compounds of *Cajanus cajan* as chemical barrier in formulation of artificial diet of *Spodoptera litura* (F.) *Annals of Plant Protection Sciences.* 15(1), 92-96.
- Brattin, W.J., Glende, E.A. and Recknagel, R.O. (1985). Pathological mechanism in carbon tetrachloride hepatotoxicity. *J Free Radic. Biol. Med.* (1), 27-38.
- Chenoweth M.B, Hake C L. (1962). The smaller halogenated aliphatic hydrocarbons. *Ann Rev. Pharmacol.* 2, 363-98.
- Dekok, L.J. and Kuiper, P.J.C. (1986). Effect of short term inoculation with sulphate chloride and selenate on the glutathione content of spinach leaf discs. *Physiol. Plant.* 68, 477-482.
- Dharmendra Singh, Priya Vrat Arya, Ved Prakash Aggarwal and Radhey Shyam Gupta. (2014). Evaluation of Antioxidant and Hepatoprotective Activities of *Moringa oleifera* Lam. Leaves in Carbon Tetrachloride-Intoxicated Rats. *J. Antioxidants.* 3, 569-591.
- Dhindsa, R.S., Plumb-Dhindsa, P. and Thrope, P.A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Expt. Bot.* 32, 93-101.
- Duke J.A. (1981). *Hand book of legumes of world economic importance.* New York: Plenum Press.
- Gul M., Kutay F. Z, Temocin S., Hanninen O(2000). Cellular and clinical implications of glutathione. *Indian J Exp Biol.* 38, 625.
- Gupta M.P, Solis P.N, Calderon A.I, Guionneau-Sinclair F, Correa M, Galdames C, Guerra C, Espinosa A, Alvenda GI, Robles G, Ocampo R (2005). Medical ethnobotany of the Teribes of Bocas del Toro, Panama. *J.Ethnopharmacol.* 96, 389-401.
- Harraz F. M., Ghazy N. M., Hammada H. M., Nafeaa A. A. and Abdallah I. I.(2015). Hepatoprotective and Antioxidant Activities of *Tribulus Terrestris*. *J Phys Pharm Adv.* 5(11), 787-794.
- Harborne, J.B. (1998). *Phytochemical methods, A guide to modern techniques of plant analysis* 3rd Edn. Chapman and Hall, Madras. P. 302.
- Heath, R. L. and Packer, K. (1968). Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* 32, 93-101.
- Ighodaro, O.M. and Omole, J.O. (2010) have studied the effect of *Cajanus cajan* aqueous leaf extract on serum amino transferase, alkaline phosphatase and electrolytes concentrations of normal wistar rats. *Animal Research International.* 7(3), 1304 – 1308.
- Iweala Emeka, E.J. and Elekwa Ify (2001). Hepatic status and lipid peroxidation in rats fed Bambara groundnut (*Voanzela subterranea*) and pigeon pea (*Cajanus cajan*). *Journal of Health and Visual Sciences.* 3 (3), 147-154.
- Jendrassik, L. and Grof, P. (1938). Quantitative determination of total and direct bilirubin in serum and plasma. *Biochem.* 297, 81-90.
- Kanatt S.R., Arjun K., Sharma A.(2011) Antioxidant and antimicrobial activity of legume hulls. *Food Res Int.* 44, 3182-7.
- Kasturi Sarkar, Ghosh, A., Kinter, M., Mazumdor, B., Silp, C. (2006). Purification and characterization of a 43kD hepatoprotective protein from the herb *Cajanus indicus* L. *J.Protein.* 25(6),411-21.
- Kidd P.M.(1997) Glutathione systemic protestant against oxidative and free radical damage. *Altern Med Rev.* 2, 155-176.

- Kirtikar, K.R. and Basu, B.D. (1998). Indian medicinal plants. 2 eds. Vol. 2. International Book Distributor Dehradun, India, pp. 340.
- Lee M. K., Yeo H., Kim J., Kim Y.C. (2000). Protection of rat hepatocytes exposed to CCl₄ in-vitro by cynandione A, a biacetophenone from *Cynanchum wilfordii*. J. Pharm. Pharmacol., 52, 341-5.
- Malick, C.P. and Singh, M.B. (1980). In plant enzymology and histoenzymology. Kalyani Publishers, New Delhi, P. 286.
- Mohammad R, Daryoush M, Ali R, Yousef D, Mehrdad N (2011). Attenuation of oxidative stress of hepatic tissue by ethanolic extract of saffron (dried stigmas of *Crocus sativus* L.) in streptozotocin (STZ)-induced diabetic rats. Afr. J. Pharm. Pharmacol. 5, 2166-73.
- Molina, M.F.; Sanchez-Reus, I.; Iglesias, I.; Benedi, J. (2003). Quercetin, a flavonoid antioxidant, prevents and protects against ethanol induced oxidative stress in mouse liver. Biol. Pharm. Bull. 26, 1398-1402.
- Narayan, B.S.V., Gatzoulis, M.A., Kilner, P.J. (2007). Non-invasive imaging in adult congenital heart disease using cardiovascular magnetic resonance. J. Cardiovascular Medicine. 1(8), 23-29.
- Parimoo, H.A.; Sharma, R.; Patil, R.D.; Sharma, O.P.; Kumar, P.; Kumar, N.(2014). Hepatoprotective effect of *Ginkgo biloba* leaf extract on lantadenes-induced hepatotoxicity in guinea pigs. Toxicol. 81, 1-12.
- Peters, T. (1968). Proposals for standardisation of total protein assays. Clin. Chem. 14, 1147-59.
- Pradeep, K., Mohan, C.V.R., Gobianand, K. and Karthikeyan, S. (2010). Protective effect of *Cassia fistula* L. on diethylnitrosamine induced hepatocellular damage and oxidative stress in ethanol pretreated rats. Biol. Res. 43, 113-125.
- Oboh G. (2006) antioxidant properties of some commonly consumed and underutilized tropical legumes. European Food Research and Technology. 224 (1), 61-65.
- OECD (2001). *Guidelines for testing chemicals*, Acute Oral toxicity, Environmental health and safety monograph series on testing and adjustment no 420.
- Oluseyi Adeboye Akinloye and Moshood Olajire Olaniyi (2011). Hepatoprotective effect of *Cajanus cajan* on tissue defense system in D-galactosamine-induced hepatitis in rats. Turkish Journal of Biochemistry. 36(3), 237-241.
- Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 28, 56-63.
- Rhitajit Sarkar, Bibhabasu Hazra and Nripendranath Mandal (2013). Antioxidative protection against iron overload-induced liver damage in mice by *Cajanus cajan* (L.) Millsp leaf extract. Indian J. Experiment Biology. 51, 165-173.
- Saxena K.B., Vijaya K.R., Sultana R. (2010) . Quality nutrition through pigeon pea: A review. J.Health 2, 1335-44.
- Shenoy K. A., Somayaji S.N., Bairy K.L. (2001). Hepatoprotective Effects of *Ginkgo biloba* Against Carbon tetrachloride Induced Hepatic Injury in Rats. Indian Journal of Pharmacology. 33, 260- 266.
- Siddhartha Singh, Archana Mehta and Pradeep Mehta (2011). Hepatoprotective activity of *Cajanus cajan* against carbon tetrachloride induced Liver damage. International J. Pharmacy and Pharmaceutical Sciences. 3(2), 146-147.
- Singh, D.; Singh, R.; Singh, P. Gupta, R.S. (2009). Effects of embelin on lipid peroxidation and Free radical scavenging activity against liver damage in rats. Basic Clin. Pharmacol. Toxicol. 105, 243-248.
- Smith, I.K., Vierheller, T.L. and Thorne, C.A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis (2-nitrobenzoic acid). Ann. Biochem. 175, 408-413.
- Suman Pattanayak, Siva Sankar Nayak, Durga Prasad Panda, Suhas Chandra Dinda, Vikas Shende, Amol Jadav. (2011). Hepatoprotective activity of crude flavonoids extract of *C. scarbaeoides* (L.) in paracetamol intoxicated Albino rats. Asian J. Pharm. Biol. Res. 22-27.
- Teranishi, Y., Tanaka, A., Osumi, M. and Fukui, S. (1974). Catalase activity of hydrocarbon utilizing *Candida* yeast. Agric. Biol. Chem. 38, 1213-1216.
- Tiwari B.K., Brennan C.S., Jaganmohan R., Surabi A., Alagusundaram K. (2011). Utilisation of pigeon pea (*Cajanus cajan* L.) by products in biscuit manufacture. Food Sci Technol Int. 44, 1533-7.
- Zimmerman M.D. and Hayman J. (1976). Function and integrity of the liver, In: Clinical diagnosis and management by laboratory methods In; Clinical diagnosis and management by laboratory methods. 17th Ed. 217-50.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY



Email : ijabpt@gmail.com

Website: www.ijabpt.com