

Amaranthus spinosus* ROOT EXTRACT PROTECTS THE CARBON TETRACHLORIDE INDUCED TOXICITY IN MALE ALBINO RATS*SURYAKANT KENGAR¹**

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ABSTRACT

Carbon tetrachloride is a highly toxic chemical agent that produce free radicals in tissues and cause cellular damage. The present study was aimed to investigate protective effect of *Amaranthus spinosus* roots in CCl₄ induced toxicity in male albino rat by biochemical estimations. In 15 days experimental schedule, ethanol extract of *A. spinosus* roots with varying concentrations (150, 300 and 450mg/kg body wt.) were given with CCl₄ intoxication simultaneously. The significant increase in all the studied biochemical parameters such as AST, ALT, ALP, bilirubin (conjugated, unconjugated, total) as well as urea and creatinine in CCl₄ induced group reflected the CCl₄ intoxication that produced cellular degeneration or destruction to the liver and kidney. Treatment of *A. spinosus* roots extracts with increasing doses restored the elevated levels of AST, ALT and ALP activities and bilirubin levels to the normal suggesting liver protective effect in induced oxidative metabolism. It also showed urea and creatinine clearance effect indicating renal protective activity and able to maintain renal functions. The results of present study indicates the dose dependent protective efficacy of *A. spinosus* roots in CCl₄ intoxicated rat, and hence suggests its use as a potential therapeutic agent in diseases caused by oxidative and altered metabolisms.

KEYWORDS: *Amaranthus spinosus*, CCl₄ Intoxication, Oxidative Metabolism, Protective Efficacy.

Numerous toxic substances causes toxicological alterations by inducing oxidative stress in cells and tissues (Brook et al;2004.,Sumida et al ; 2013). It is caused by generation of free radicals by cellular metabolism (Bhattacharya et al ;2014.,Trnkova et al;2015). Carbon tetrachloride (CCl₄) is a highly toxic chemical agent and is commonly used as a hepatotoxin that produce free radicals in tissues and cause cellular damage (Ozturk et al; 2003., Bahcecioglu et al; 1999., Recknagel et al; 1989., Sundari et al; 1997., Mehendale et al; 1985., Roullieret al 1963). It is known to be hepatotoxic and nephrotoxic (Sanzgiri et al; 1997., Abrahamet al; 1999., Sener et al; 2002., Teli et al; 2014).

In traditional system of medicine numerous plants and drugs prepared from them are suggested to protect oxidative stress induced diseases. *Amaranthus spinosus* Linn. (Family Amaranthaceae), commonly known as “pig weed” is known for its wide spectrum of medicinal properties. Various studies have reported that *A. spinosus* possesses several pharmacological activities including anti-inflammatory, anti-malarial, anti-diuretic, antiviral and hepatic disorders, anti-nephritic, anti-diabetic, antitumor, analgesic, antimicrobial, spasmolytic, bronchodilator, hepatoprotective, spermatogenic, anti-fertility, anti-malarial, antioxidant properties, etc (Samy et al ; 1999., Srivastava et al; 1998., Harsha et al; 2011., Berghoferet al; 2002., Zeashan et al; 2008., Zeashan et al; 2009a., Zeashan et al; 2009b).

In the present study ethanol extracts of *A. spinosus* roots were tested for its protective effect

against CCl₄ induced toxicity in 15 days experimental schedule in male albino rat.

MATERIALS AND METHODS**Plant Material and Preparation of Extracts**

The fresh root of *A. spinosus* were collected from the rural area of the Karad (Dist. Satara, MH, India) and were properly identified. The roots were washed, shed dried and powdered mechanically with standard method. 50gm of root powder was weighed and extracted with 250 ml ethanol (1:5). The dissolved extracts were filtered through Whatman filter paper (No.1). Supernatants of each extracts were evaporated at 60° C to dryness. After evaporation extracts were collected and stored at 4°C.

To study the protective effects of ethanolic root extracts of *A. spinosus* against CCl₄ induced toxicity different doses viz; 150, 300 and 450 mg/kg body wt were used.

Experimental Design for Hepatoprotective Activity

Male albino rats weighing 180-220g were used for the experiments. The rats were grouped and maintained under standard laboratory condition and were fed with standard pellet diet (Amrit feeds, Sangli, Maharashtra, India). Animals were randomly allocated into six groups of six each as per various treatments given as follows:

Group I: Normal rats without any treatment.

Group II: Acute hepatotoxicity induced by administration of 3.0 ml CCl₄ /kg body wt/day for 15 days *subcutaneously*.

Group III: 3.0 ml CCl₄ /kg body wt/day for 15 days (sc) + 50 mg dose of *A. spinosus* root extract/kg body wt. for 15 days simultaneously.

Group IV: 3.0 ml CCl₄ /kg body wt/day for 15 days (sc) + 150 mg dose of *A. spinosus* root extract/kg body wt. for 15 days simultaneously.

Group V: 3.0 ml CCl₄ /kg body wt/day for 15 days (sc) + 300 mg dose of *A. spinosus* root extract/kg body wt. for 15 days simultaneously.

Group VI: 3.0 ml CCl₄ /kg body wt/day for 15 days (sc) + 450 mg dose of *A. spinosus* root extract/kg body wt. for 15 days simultaneously.

At the end of experimental period, all the animals were sacrificed and blood sample was taken for biochemical studies.

Collection of Serum

After completion of the experimental schedule, animals were killed by giving deep ether anaesthesia. Blood was collected and was allowed to clot at room temperature by leaving it for 20-30 minutes undisturbed. Serum samples were obtained by centrifugation of clots at 12000rpm for 10 min.

Biochemical Estimations

To test the protective efficacy of ethanol extracts of *A. spinosus* roots, liver and kidney functional tests were determined by estimation of biochemical parameters of marker enzymes and antioxidant enzymes. Diagnosis of liver function is performed by measuring serum AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), Alkaline phosphatase (ALP) and bilirubin (conjugated and unconjugated). Kidney functions were determined by measuring serum urea and creatinin. All these biochemical tests were performed using Autoanalyser.

Statistical Analysis

All the results were analyzed statistically by student 't' test. The values of p<0.05, p<0.01, p<0.001 were considered as significant.

RESULTS

Effect of *A. spinosus* root extracts on serum AST, ALT and ALP levels in CCl₄ intoxicated male albino rats

For the assessment of liver functioning serum AST, ALT and ALP activities were determined. The alterations are given in (Table 1).

Table 1: Effect of *A. spinosus* in CCl₄ induced alterations of serum AST, ALT and ALP levels. (Values expressed as units/ml serum)

Sr. No.	Group	AST	ALT	ALP
1	Normal	24.48 ± 1.98	16.58 ± 0.99	26.38 ± 1.11
2	CCl ₄ (3.0 ml/kg body wt) sc	44.21 ± 2.09 ^c	24.69 ± 1.54 ^b	37.99 ± 1.99 ^c
3	CCl ₄ + 150 mg extracts /kg body wt) <i>po</i>	42.54 ± 1.57 ^c	23.36 ± 2.01 ^a	36.09 ± 0.68 ^c
4	CCl ₄ + 300 mg extracts /kg body wt) <i>po</i>	39.87 ± 3.08 ^b	20.87 ± 1.08 ^a	34.12 ± 1.14 ^b
5	CCl ₄ + 450 mg extracts /kg body wt) <i>po</i>	27.58 ± 1.54 ^z	18.57 ± 1.34 ^x	27.89 ± 1.03 ^x

Values are mean ± SE of 6 animals.

p-values – a<0.05, b<0.01 and c<0.001 vs. normal rat

x<0.05, y<0.01 and z<0.001 vs. CCl₄ treated rat

AST, ALT and ALP are reported to be sensitive indicators of liver injury. The biochemical estimation for AST, ALT and ALP activity in normal rat serum exhibited 24.48, 16.58 and 26.38 units activity/ml serum respectively; which were significantly increased by 1.80 fold (p<0.001), 1.48 fold (<0.01) and 1.44 fold (<0.001) respectively after CCl₄ administration. Treatments of 150, 300 and 450 mg doses of *A. spinosus* root extracts per day for 15 days; exhibited increased AST activities by 1.73, 1.62 and 1.12 folds respectively as compared with normal rat. However it was reduced by 3.77, 9.81 and 37.61%

Effect of *A. spinosus* root extracts on serum bilirubin levels in CCl₄ intoxicated male albino rats

(<0.001) respectively. The treatments of 150, 300 and 450 mg doses of extracts showed 1.40, 1.25 and 1.12 folds increase in ALT activities respectively, when compared with normal value. In contrast, activities showed 5.38, 15.47 and 24.78% decline after comparison with CCl₄ treated rat enzyme activity. Treatments of similar doses of *A. spinosus* root extracts showed the exact trend to the ALP activities also; where it showed 1.36, 1.29 and 1.05 fold increased the ALP activities as compared to the normal rat. When compared with CCl₄ treated rat, ALP activities was reduced by 5.0, 10.18 and 26.58 % respectively.

For the assessment of liver functioning serum conjugated, unconjugated and total bilirubin content were estimated. Alterations are given in (Table 2).

Table 2: Effect of *A. spinosus* in CCl₄ induced alterations of serum bilirubin levels (Values are expressed as mg/dl serum).

Sr. No.	Group	Conjugated	Unconjugated	Total
1	Normal	0.17 ± 0.011	0.05 ± 0.003	0.22 ± 0.02
2	CCl ₄ (3.0 ml/kg body wt) sc	0.29 ± 0.012 ^c	0.08 ± 0.002 ^c	0.37 ± 0.02 ^c
3	CCl ₄ + 150 mg extracts /kg body wt) po	0.27 ± 0.033 ^a	0.06 ± 0.002 ^{az}	0.33 ± 0.01 ^c
4	CCl ₄ + 300 mg extracts /kg body wt) po	0.24 ± 0.012 ^{b,x}	0.06 ± 0.004 ^x	0.30 ± 0.03 ^a
5	CCl ₄ + 450 mg extracts /kg body wt) po	0.17 ± 0.011 ^z	0.05 ± 0.001 ^z	0.21 ± 0.02 ^z

Values are mean ± SE of 6 animals.

p-values – a<0.05, b<0.01 and c<0.001 vs. normal rat

x<0.05, y<0.01 and z<0.001 vs.CCl₄ treated rat

Normal rat exhibited 0.17 mg/dl conjugated bilirubin content. After CCl₄ administration it was increased by 1.70 fold. Treatments of 150 and 300mg doses of *A. spinosus* extracts showed 1.58 and 1.41 folds increase in conjugated bilirubin, but the content remained unaltered after 450 mg dose treatment. It was progressively reduced to normal by 6.89, 17.24 and 41.37% respectively to the 150, 300 and 450 doses treatments. Similarly 0.05 mg/dl serum unconjugated bilirubin in normal rat was significantly increased by 1.6 folds (<0.001) after CCl₄ intoxication. This elevated level was reduced to normal by the treatment of *A. spinosus* root extracts. Total bilirubin content of normal rat was 0.22 mg/dl serum which was increased by 1.68 fold after CCl₄ intoxication. Increases of 1.5 and 1.36

folds were noted after 150 and 300 mg *A. spinosus* extract treatments, but it remained same after 450 mg dose on comparison with that of normal rat. Reduction of 10.81, 18.91 and 43.24% were observed after 150, 300 and 450mg doses treatments respectively, when compared with CCl₄ treated rat content.

Effect of *A. spinosus* root extracts on serum urea and creatinine contents in CCl₄ intoxicated male albino rats

For the assessment of kidney functioning serum urea and creatinine content were estimated. Alterations in serum urea and creatinine contents are given in (Table 3).

Table 3: Effect of *A. spinosus* in CCl₄ induced alterations of serum Urea and Creatinin content (Values expressed as mg/dl serum)

Sr. No.	Group	Urea	Creatinin
1	Normal	20.34 ± 1.63	3.09 ± 0.29
2	CCl ₄ (3.0 ml/kg body wt) sc	31.87 ± 1.74 ^e	5.17 ± 0.11 ^c
3	CCl ₄ + 150 mg extracts /kg body wt) po	30.68 ± 1.45 ^c	5.10 ± 0.24 ^c
4	CCl ₄ + 300 mg extracts /kg body wt) po	28.74 ± 1.87 ^b	4.57 ± 0.18 ^b
5	CCl ₄ + 450 mg extracts /kg body wt) po	22.54 ± 1.37 ^y	3.97 ± 0.29 ^x

Values are mean ± SE of 6 animals.

p-values – a<0.05, b<0.01 and c<0.001 vs. normal rat

x<0.05, y<0.01 and z<0.001 vs.CCl₄ treated rat

Normal rat showed 20.34 mg/dl serum urea content. It was increased by 1.56 fold after CCl₄ administration ethanol extracts of *A. spinosus* treatments resulted in 1.50, 1.41 and 1.10 folds increases after 150, 300 and 450 mg doses respectively when compared with normal rat urea content. But it was dose dependently reduced by 3.73, 9.82 and 29.27% respectively after similar doses treatments. The creatinine content in normal rat (3.09mg/dl) was significantly increased after CCl₄ intoxication (<0.001); which was also dose dependently reduced to normal.

DISCUSSION

The present study was undertaken to investigate possible protective efficacy of *A. spinosus* root extracts in CCl₄ intoxicated male albino rats. It was determined by estimation of biochemical parameters that reflecting liver and kidney functions.

The results of biochemical parameters revealed the significant elevation of enzyme levels in CCl₄ treated group indicating that CCl₄ induces oxidative damage to the liver by cellular degeneration resulting

into release of enzymes from the cytoplasm into the blood circulation. It is mainly attributed to the toxic metabolites that may alter plasma membrane, intracellular ion homeostasis or degenerative enzyme activity resulting cell necrosis and consequent cell death (Recknagel et al; 1989., Sallie et al; 1991., Slater; 1984).

The significant increase in all the studied biochemical parameters such as AST, ALT and ALP, bilirubin (conjugated, unconjugated, total) as well as urea and creatinine in CCl₄ induced group is in agreement with several researchers (Teli et al; 2013., Teli et al; 2014., Reyes et al; 2007). Who recorded significant increase in liver enzymes and kidney function in CCl₄ intoxication of rats. After simultaneous treatment of graded doses of ethanol extracts of *A. spinosus* roots, in 15 days protective experimental schedule normalized the elevated levels of AST, ALT and ALP indicating protective effect of *A. spinosus* extract during oxidative metabolism especially by 450 mg dose of extract. It has been attributed to antioxidant and membrane stabilizing activities. The depleted CCl₄ influenced biochemical parameters towards normal level may be due to its membrane stabilizing activity as well as repair of hepatic tissue damages caused by CCl₄.

Administration of CCl₄ induce significant increase in urea and creatinine contents. Treatment of graded doses of *A. spinosus* roots reduced this elevated level indicating renal protective activity of this plant and able to maintain renal functions. The effective dose was 450mg. CCl₄ influenced elevated contents of conjugated, unconjugated and total bilirubin was reduced by 150 and 300 mg doses of extracts but the levels were not normalized indicating that low concentration are not able to clear the arrested bilirubin in serum and remained high over the normal. But treatment of 450 mg dose of extract exhibited bilirubin clearance efficiency that may be helping for the stability of biliary dysfunction and improvement in liver function tests.

Several studies have reported that the substances having with antioxidative and free radical scavenging properties playing a promising role in protecting liver and kidney toxicity (Nikolova et al; 2011., Kumar et al; 2012., Ozturk et al; 2009., Ogeturk et al; 2005). *A. spinosus* revealed the presence of alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponin, betalain, β -sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins and carotenoids (Harsha et al; 2011., Azhar-ul-Haq et al; 2006., Blunden et al; 1999).

The results of present study indicated that ethanol extracts of *A. spinosus* roots exert positive effects on antioxidant status and protect the toxicity of liver and kidney by normalizing the metabolism induced by CCl₄ with significant protective effect at higher doses in male albino rat. It may be attributed to its several bioactive constituents like flavonoids, phenolic compounds, glycosides, β -sitosterol. However, further studies are required to elucidate mechanism of potential therapeutic agent for its application in diseases caused by oxidative and altered metabolisms.

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