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MODULATORY ROLE OF *AZIMA TETRACANTHA* LEAVES EXTRACT ON CARBON TETRACHLORIDE INDUCED OXIDATIVE STRESS IN RATS.

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ABSTRACT

Current hypothesis favour the concept that lowering oxidative stress can have a health benefits. In this study modulatory effect of ethanolic extract of *Azima tetracantha* was investigated using carbon tetrachloride (CCl_4)-induced oxidative stress as the experimental model. The oxidative stress rats were administered different doses (100, 200 and 400mg/ Kg BW) of *Azima tetracantha* leaves extract. Malondialdehyde (MDA) is a product of lipid peroxidation in CCl_4 – intoxicated rats was evidenced by a marked increment in the levels of thiobarbituric acid reactive substances (TBARS) and also a distinct diminution in glutathione (GSH) content in the liver. In CCl_4 + *Azima tetracantha* treated rats these biochemical parameters attained an almost normal as dose dependent manner. The decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidant, such as glutathione (GSH), vitamin C and vitamin E in CCl_4 intoxicated rats were observed. On treatment with different doses (100, 200 and 400mg/ Kg BW) of *Azima tetracantha* leaves extract increased in the activity as dose dependent manner. Among the various doses, 400mg/kg has potential activity than other doses. From these results, it was suggested that lipid peroxidation and oxidative stress elicited by CCl_4 intoxication had been nullified due to the effect of *Azima tetracantha*.

KEYWORDS: Antioxidant enzymes, Oxidative stress, Carbon tetrachloride, Lipid peroxidation, *Azima tetracantha.*

INTRODUCTION

Current hypothesis favour the concept that lowering oxidative stress can have a health benefit. Free radicals can be overproduced or the natural antioxidant system defenses weakened, first resulting in oxidative stress and then leading to oxidative injury and disease. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism (Tiwari, 2001). The most common Reactive oxygen species (ROS) include superoxide ($O_2^{\bullet-}$) anion, hydrogen peroxide (H_2O_2), peroxyl ($ROO^{\bullet}$) radicals, and the very reactive hydroxyl ($OH^{\bullet}$) radicals. The nitrogen-derived free radicals are nitric oxide ($NO^{\bullet}$) and peroxy nitrite anion ($ONOO^{\bullet}$). ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (Joyce, 1987).

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome P_{450} system in the endoplasmic reticulum to produce trichloromethyl free radical ($CCl_3^{\bullet}$). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical leads to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death (De Groot and Noll, 1986; Clawson, 1989; Reckengel *et al*., 1989). These result in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver damage (Recknagel 1983; Wolf *et al*., 1980; Azri *et al*., 1992).

The harmful effect of reactive oxygen species is neutralized by a broad class of protective agents called antioxidants, which prevents oxidative damage by reacting with free radicals before any other molecules can become a target. The non-enzymatic antioxidants (Vitamin E, C, reduced glutathione etc.) and antioxidant enzymes (SOD, CAT, GSHPx) play an important role in the protection of cells and tissues against free radical mediated tissue damage (Yu, 1994; Ray and Husain, 2002). Any compound, natural or synthetic, with antioxidant

properties might contribute towards the partial or total alleviation of this type of damage. As plants produce a lot of antioxidants to control the oxidative stress, they can represent a source of new compounds with antioxidant activity. A number of plants and plant isolates have been reported to protect free radical induced damage in various experimental models (Scartezzini and Sproni, 2000).

Natural antioxidants strengthen the endogenous antioxidant defences from ROS ravage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. The medicinal value of the chosen plant *Azima tetracantha* has been extensively worked out. However its therapeutic efficacy in the state of oxidative stress has not been evaluated. Previously reported that the presence of alkaloids, flavanoids, tannins, cardio glycosides, saponins and terpenoids like compounds in *Azima tetracantha* (Abirami et al., 2015; Janardhan et al., 2014). Hence in the present study an attempt has been made to create an animal model with oxidative stress using CCl_4 and the therapeutic efficacy of the extract of *Azima tetracantha* was evaluated.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately 3-4 months young rats (weighing approximately 140-160g) and 24-26 months old rats (weighing approximately 380-410g were used in this study. They were healthy animals procured from Sri Venkateswara enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water ad libitum. They were acclimatization to the environment for 1 week prior to experimental use. The experiment was carried out according to the guidelines of the Committee (Ethical No: SAC/IAEC/BC/2016/Ph.D-005) for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals*:* Carbon tetrachloride, Thiobarbituric acid, 2,4-Dinitro phenyl hydrazine and glutathione were purchased from sigma chemical, Mumbai. All other reagents and chemicals used in this study were of analytical grade with high purity.

Plant Material

The fresh leaves of *Azima tetracantha* were collected in the month of January 2015 at Melur, Thiruchirappalli District, Tamil Nadu, South India. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rabinat Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (EP001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preparation of Plant Extract

Fresh plant material was shade dried and powdered coarsely using electric blender. 250g of dried plant material was soaked in Ethanol for 48 hours. After 48 hrs of soaking the solvent was distilled off under reduced pressure at 50°C and dried in vacuum. The residue was dissolved in isotonic saline and used for the study.

Experimental protocol

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows. Group I – Normal Rats. Group II – Negative control - Animals were administrated orally with CCl_4 (0.5 ml/150g of bw-v/v in olive oil) on 1^{st}, 8^{th} and 16^{th} day. Group III – Animals were administrated orally with CCl_4 (0.5 ml/150 g of bw-v/v in olive oil on 1^{st}, 8^{th} and 16^{th} day) and treated with *Azima tetracantha* leaves extract (100mg/ Kg BW) orally for 21 days. Group IV - Animals were administrated orally with CCl_4 (0.5 ml/150 g of bw-v/v in olive oil on 1^{st}, 8^{th} and 16^{th} day) and treated with *Azima tetracantha* leaves extract (200mg/ Kg BW) orally for 21 days. Group V –Animals were administrated orally with CCl_4 (0.5 ml/150 g of bw-v/v in olive oil on 1^{st}, 8^{th} and 16^{th} day) and treated with *Azima tetracantha* leaves extract (400mg/ Kg BW) orally for 21 days. Group VI –Animals were administrated orally with CCl_4 (0.5 ml/150 g of bw-v/v in olive oil on 1^{st}, 8^{th} and 16^{th} day) and treated with Silymarin (20mg/ Kg BW) orally for 21 days.

Biochemical analysis

Lipid peroxide content was estimated by the method of Ohkawa, (1979). The activities of antioxidant enzymes SOD, Catalase and Glutathione peroxidase were determine by the method of Misra and Fridovich (1972), Chance and B, Maehly, (1955) and Rotruck *et al*. (1973) respectively. The levels of non-enzymatic antioxidants such as GSH, Vitamin C and Vitamin E were estimated by the method of Moron *et al*. (1979), Omaye *et al*. (1979) and

Baker *et al*. (1980) respectively. The protein content was estimated by the method of Lowry's *et al*. (1951).

Statistical analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Duncan's test for multiple comparisons. The results were considered statistically significant if the p-values were 0.05 or less ($p<0.05$).

RESULTS

Table 1 shows the effect of *Azima tetracantha* extract on LPO in control and experimental animals. The concentration of LPO was significantly ($p<0.05$) higher in liver of CCl_4 treated rats, as compared to normal and *Azima tetracantha* extract treated animals. On treatment with different doses (100, 200 and 400mg/ Kg BW) of *Azima tetracantha* leaves extract decreased in the content of LPO as dose dependent manner. The silymarin treated rats shows did not have significant changes.

Table 1 shows the effect of *Azima tetracantha* extract on antioxidant enzymes as SOD, CAT, GPx and GST in control and experimental animals. The activities of SOD, CAT and GPx recorded a significant ($p<0.05$) decline in CCl_4 – administered rats, when compared with normal controls. In CCl_4 + *Azima tetracantha* treated rats, the activities of these enzymes attained a near-normalcy. On treatment with different doses (100, 200 and 400mg/ Kg BW) of *Azima tetracantha* leaves extract increased in the activity of SOD, CAT, GPx and GST as dose dependent manner. The silymarin treated rats shows restored the activities of all the enzymes.

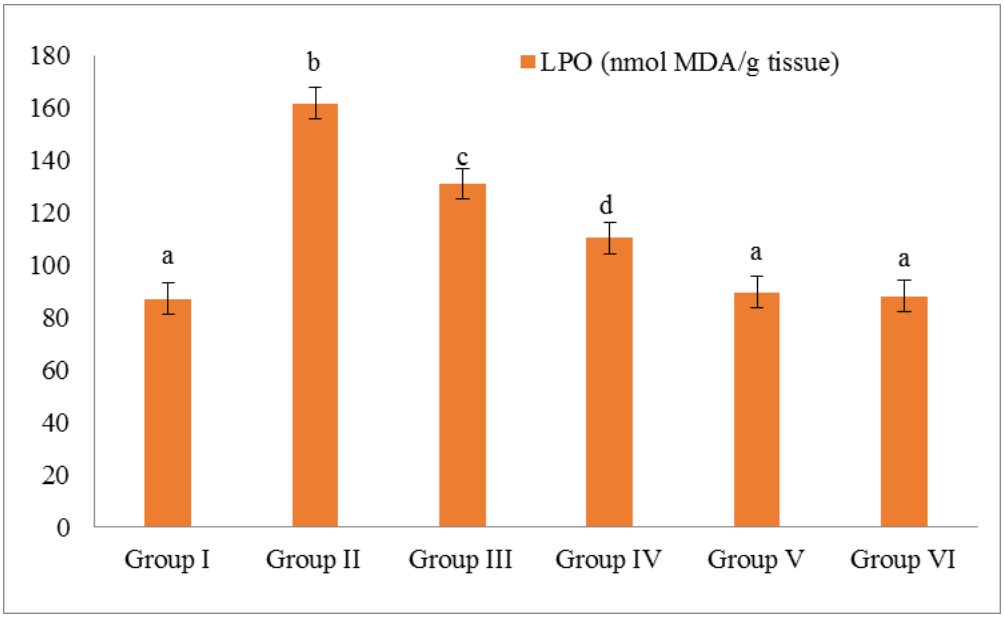
Table 1 shows the effect of *Azima tetracantha* extract on non antioxidant enzymes as GSH, Vitamin C and E in control and experimental animals. GSH, Vitamin C and E content in liver of Group II animals showed (Table 1) a significant decline ($p<0.05$) when compared with controls. On treatment with different doses (100, 200 and 400mg/ Kg BW) of *Azima tetracantha* leaves extract increased in the content of GSH, Vitamin C and E as dose dependent manner. There is no significant alterations were observed on treatment with silymarin.

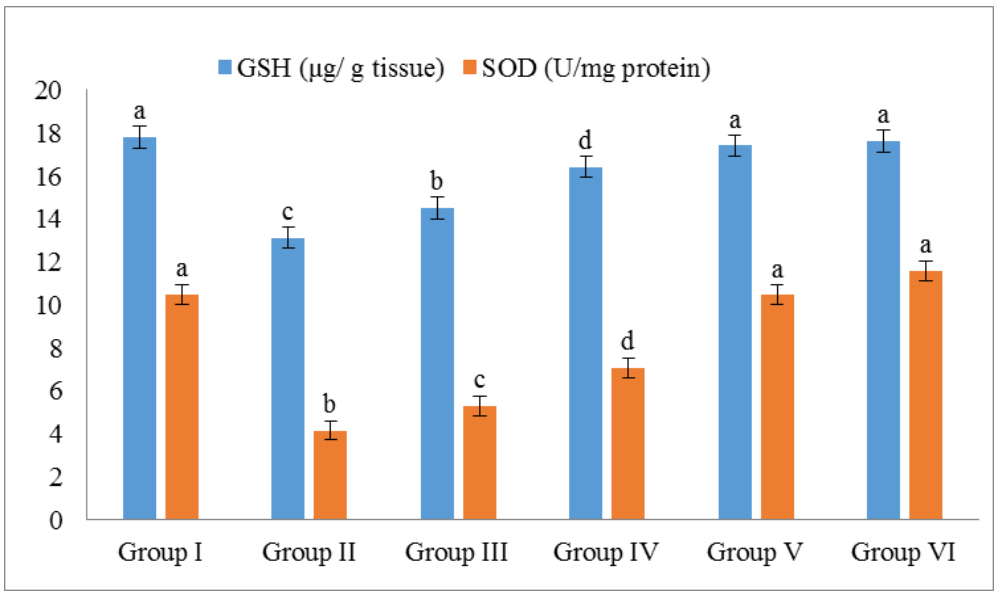
Table 1: Effect of plant extract on LPO, SOD, CAT, GPx, GST, GSH, Vitamin C and E of experimental animals.

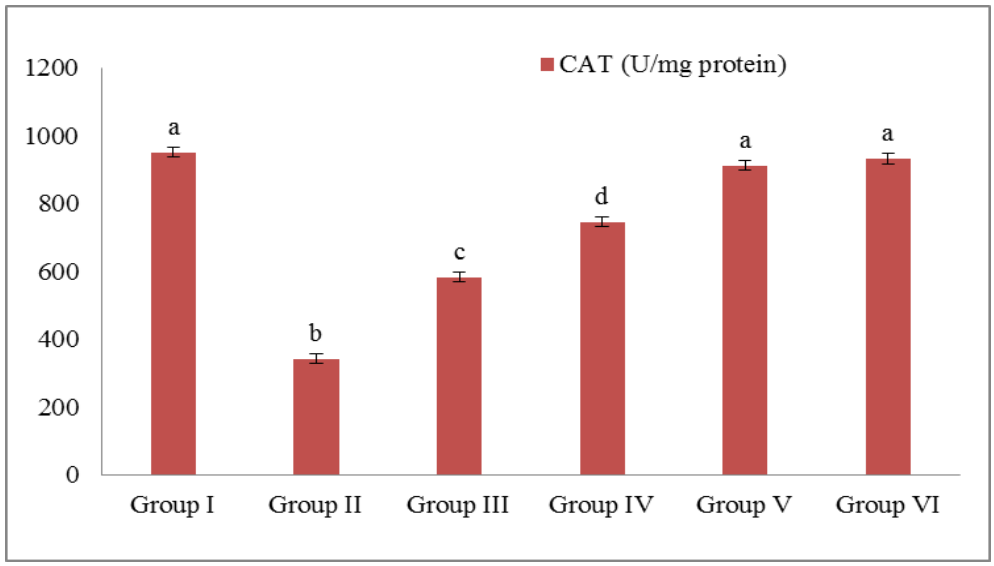
Groups	LPO (nmol MDA/g tissue)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/µg protein)	GST (µmoles of CDNB-GSH conjugate formed/min/mg protein,)	GSH (µg/ g tissue)	Vitamin E (µg/ g tissue)	Vitamin C (µg/ g tissue)
Group I	87.10±1.3[a]	10.47±0.90[a]	951.0±15.5[a]	9.51±0.15	333.55±2.01	17.8±0.1[a]	13.9±0.3[a]	0.063±0.002[a]
Group II	161.8±1.4[b]	4.13±0.34[b]	342.5±5.7[b]	3.43±0.06	173.65±1.82	13.1±0.1[b]	10.3±0.1[b]	0.045±0.001[b]
Group III	131.10±1.5[c]	5.26±0.35[c]	583.5±135.5[c]	5.84±1.35	209.70±2.09	14.5±0.2[c]	11.6±0.1[c]	0.052±0.001[c]
Group IV	110.4±1.3[d]	7.06±0.55[d]	746.5±5.4[d]	7.47±0.05	264.00±0.41	16.4±0.4[d]	12.9±0.1[d]	0.056±0.001[d]
Group V	89.50±1.8[a]	10.44±2.07[a]	912.5±9.23[a]	9.13±0.01	288.95±1.26	17.4±0.20[a]	13.40±0.10[a]	0.060±0.002[a]
Group VI	88.10±1.3[a]	11.57±0.43[a]	933.0±14.1[a]	9.33±0.14	298.75±2.46	17.6±0.10[a]	13.10±0.20[a]	0.061±0.002[a]

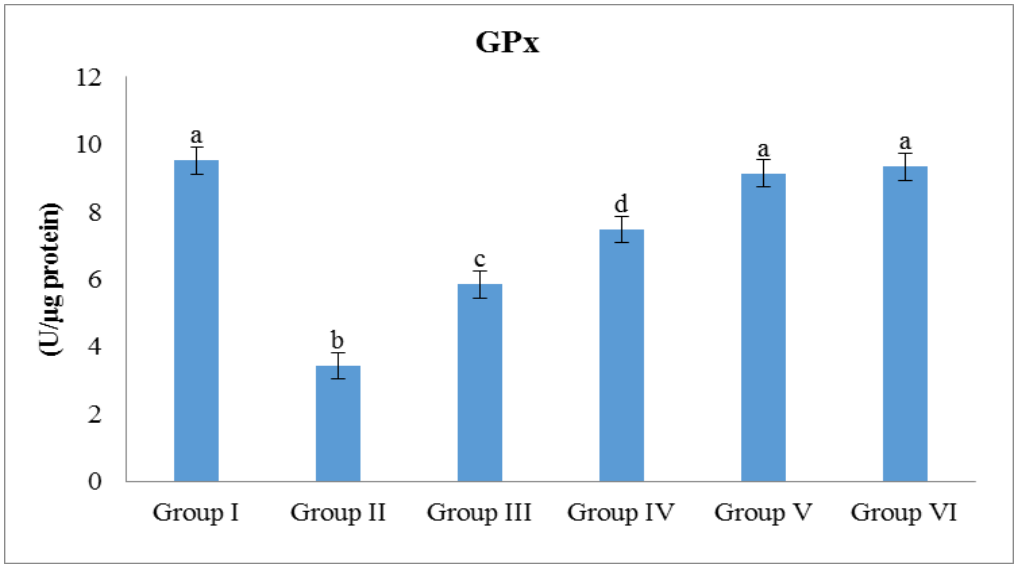
Results were expressed as Mean ± SD for six animals

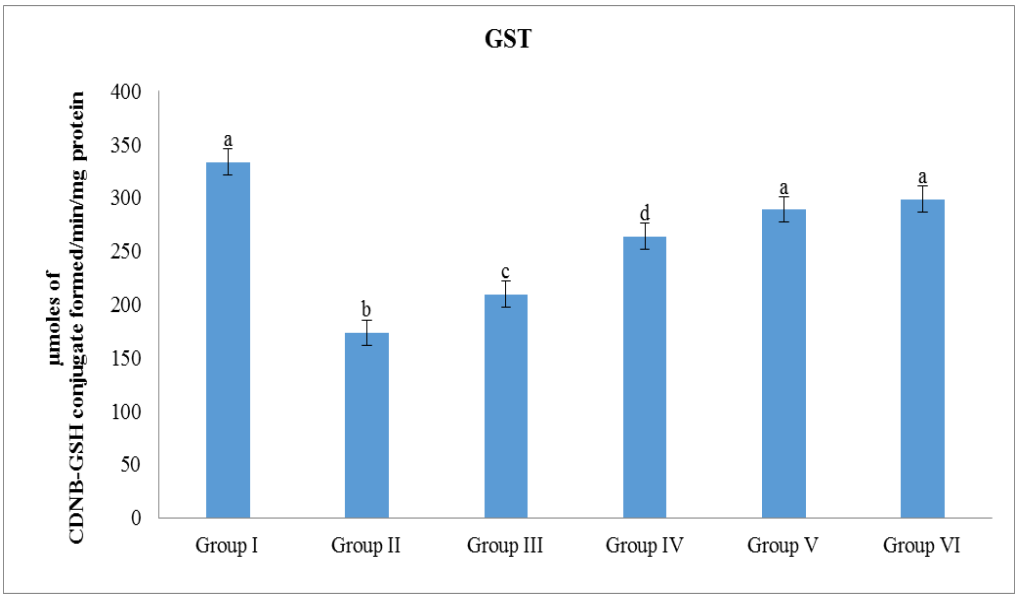
Mean values within the row followed by different letters (Superscript) are significant ($p < 0.05$) level different from each other and same letter are non-significant were comparison by Duncan's multiple range test (DMRT).

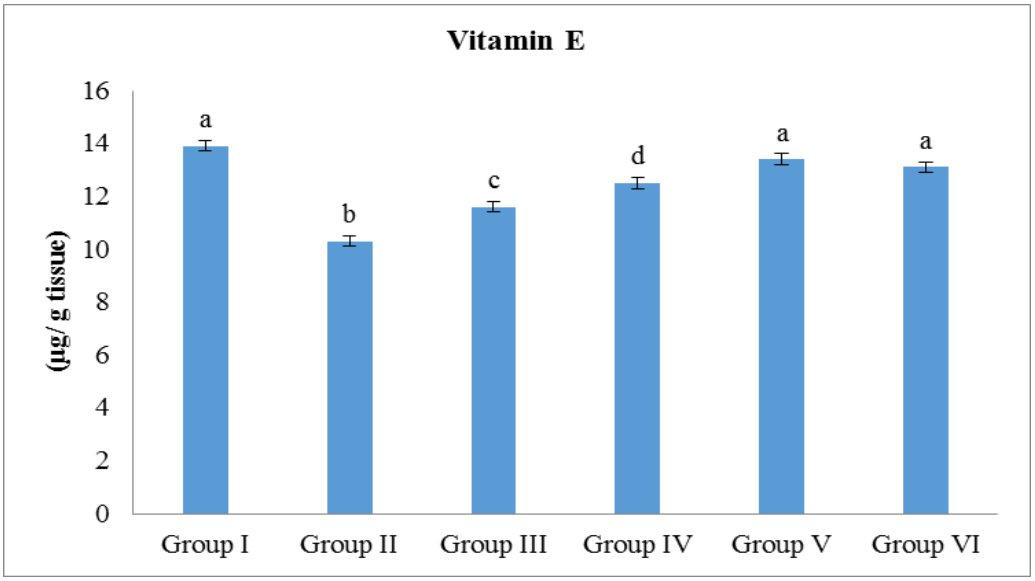

LPO (nmol MDA/g tissue)
180
160
140
120
100
80
60
40
20
0
a
b
c
d
a
a
Group I
Group II
Group III
Group IV
Group V
Group VI


GSH (µg/ g tissue)
SOD (U/mg protein)
20
18
16
14
12
10
8
6
4
2
0
a
a
c
b
b
c
d
d
a
a
a
a
Group I
Group II
Group III
Group IV
Group V
Group VI


CAT (U/mg protein)
1200
1000
800
600
400
200
0
a
b
c
d
a
a
Group I
Group II
Group III
Group IV
Group V
Group VI


GPx
(U/µg protein)
12
10
8
6
4
2
0
a
b
c
d
a
a
Group I
Group II
Group III
Group IV
Group V
Group VI


GST
µmoles of CDNB-GSH conjugate formed/min/mg protein
400
350
300
250
200
150
100
50
0
a
b
c
d
a
a
Group I
Group II
Group III
Group IV
Group V
Group VI


Vitamin E
(µg/ g tissue)
16
14
12
10
8
6
4
2
0
a
b
c
d
a
a
Group I
Group II
Group III
Group IV
Group V
Group VI

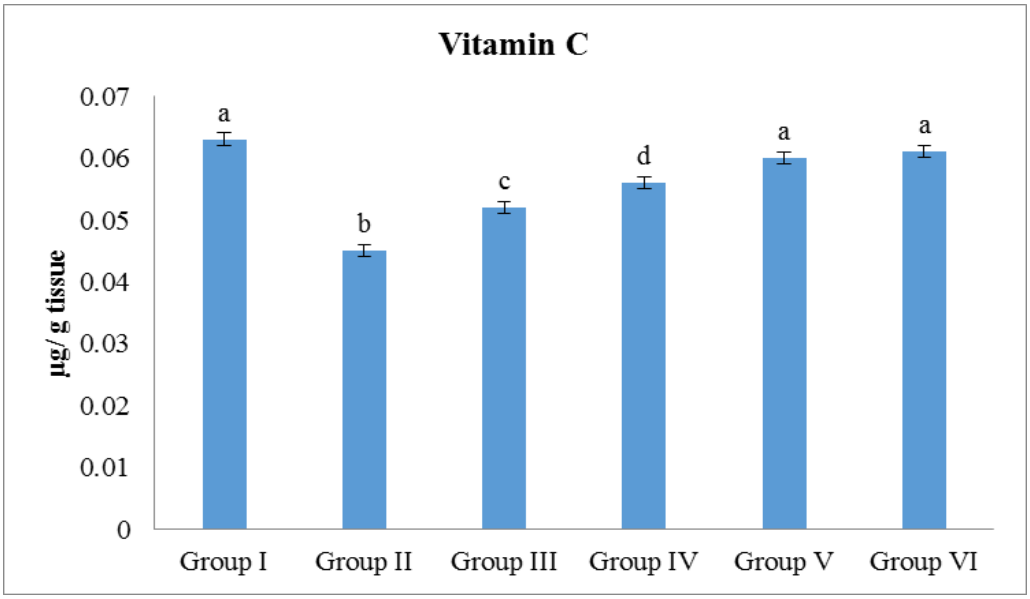


Figure 1: Effect of plant extract on LPO, SOD, CAT, GSH, Viamin C and Vitamin E of experimental animals.

DISCUSSION

Ample experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases (Tewari *et al*., 2000). It is now known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS may trigger a host of disorders in body resulting in tissue damage and necrosis in many instances (Prasad Varier *et al*., 1999). It has been hypothesized that one of the principal causes CCl_4- induced liver injury is LPO by free radical derivatives of CCl_4. Thus the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 –induced hepatopathy (Castro *et al*., 1974). CCl_4 mediated oxidative stress was taken here as the experimental model for hepatotoxicity and oxidative stress.

The study of lipid peroxidation is attracting much attention in recent years due to its role in disease processes. The peroxidation of polyunsaturated fatty acids as components of biological membranes is considered to be the starting point of many toxic processes (Plaa and Witschi, 1976). An intensively investigated and reasonably substantiated relationship exists between liver damage and lipid peroxidation produced by carbon tetrachloride (Recknagel, 1989; Recknagel and Glende, 1973; Kale and Situsawad 1990; Halliwell, 1991). In this context a marked increase (Table 1) in the concentration of LPO indicates oxidative stress in

CCl_4 intoxicated rats when compared to control rats. Administration of *Azima tetracantha* at a doses of at 100, 200 and 400mg/kg significantly ($p<0.05$) decreased in the level of LPO demonstrate the reduction of oxidative stress in *Azima tetracantha* and CCl_4 intoxicated rats. The LPO content at 400mg/kg is almost similar to control rats.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes such as SOD, CAT and GPx system (Proctor and McGinness, 1986). The SOD dismutases superoxide radicals O_2^- into H_2O_2 plus O_2, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. In this study, SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. In CCl_4 intoxicated rats, the activity of SOD decreased drastically compared to that of normal group. CCl_4 administered rats treated with ATLE at 100, 200 and 400mg/kg revealed that MDA and oxidative stress elicited by CCl_4 intoxication have been nullified due to the effect of *Azima tetracantha*. The SOD activity at 400mg/kg is almost similar to the activity shown by silymarin. This observation perfectly agrees with those of Lin *et al*. (1998) study.

CAT is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Caroline *et al*., 2008). In CCl_4 intoxicated rats, the activity of CAT decreased drastically compared to that of normal group. Administration of *Azima tetracantha* increases the activities of catalase in CCl_4 induced oxidative stress rats at 100, 200 and 400mg/kg of ATLE to prevent the accumulation of excessive free radicals and protects the liver from CCl_4 intoxication. The CAT activity at a dose of 400mg/kg is potential activity than other doses and similar to the activity shown by control rats. This observation agrees with those of Gupta *et al*. (2002) study.

GPx is a seleno-enzyme two third of which (in liver) is present in the cytosol and one-third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In CCl_4 intoxicated rats, the activity of GPx decreased drastically compared to that of normal group. The observed increase of GPx activity at 100, 200 and 400mg/kg of ATLE suggests that the

Azima tetracantha have efficient protective mechanism in response to ROS. And also, these findings indicate that *Azima tetracantha* may be associated with decreased oxidative stress and free radical-mediated tissue damage. This observation consisted with those of Venukumar and Latha (2002) study.

Drug-metabolizing enzymes, such as glutathione S-transferase (GSTs) function concertedly as the two major inducible defense systems against electrophiles and xenobiotic toxicity (Enomoto et al. 2001). GSTs may have significant roles in the detoxification processes of oxidatively stressed cells (Sagara et al. 1998). GSTs have endogenous substrates, such as lipid and nucleic acid hydroperoxides and alkenals, which result from the decomposition of lipid hydroperoxides (Coles and Ketterer 1990). In CCl_4 intoxicated rats, the activity of GST decreased drastically compared to that of normal group. The activity of GST recovered significantly ($p< 0.05$) at 100, 200 and 400mg/kg of *Azima tetracantha* extract compared to that of CCl_4 group. In contrast, the GST activity at 400mg/kg is almost similar to the activity shown by silymarin, a potent hepatoprotective agent. This observation agrees with those of Guntupalli *et al*. (2006) study.

GSH is a major non- protein thiol in living organism, which plays a central role of co-ordinating the body's antioxidant defense process. It is implicated in the cellular defense against xenobiotics and naturally occurring deleterious compounds such as free radicals. Glutathione status is a highly sensitive indicator of cell functionality and viability. Perturbation of GSH status of a biological system has been reported to lead to serious consequences (Pastore, 2003). In CCl_4 intoxicated rats, the content of GSH improved significantly ($p< 0.05$) at 100, 200 and 400mg/kg of *Azima tetracantha* extract compared to control rats and its subsequent return towards near normalcy in CCl_4 and *Azima tetracantha* treated rats reveal antioxidant effect of *Azima tetracantha*. Explanations of the possible mechanism underlying the hepatoprotective properties of drugs include the prevention of GSH depletion and destruction of free radicals (Fraga *et al*., 1987).

Ascorbate (Vitamin C) plays an important role with the lipophilic antioxidant α – tocopherol in protecting the membrane from oxidative stress. Recycling of ascorbic acid requires GSH, which reduces dehydroascorbate to ascorbate (Winkler, 1992). Ascorbte in turn is essential for the recycling of tocopherol radical to tocopherol (Packer *et al.,* 1997). In the present study, significantly decreased level of vitamin C and α- tocopherol in CCl_4 intoxicated rats, demonstrating the increased free radical accumulation in CCl_4 administered rats. The

observed decline in glutathione level may contribute to the decrease in ascorbate as well tocopherol concentration in CCl_4 intoxicated rats. Supplementation of *Azima tetracantha* to CCl_4 intoxicated rats improved vitamin C and α- tocopherol level as compared to control rats (Table 2), which may be due to increase the GSH in *Azima tetracantha* treated rats improve the recycling of vitamin C and α- tocopherol. In CCl_4 intoxicated rats, the content of GSH, vitamin C and E improved significantly ($p< 0.05$) at 100, 200 and 400mg/kg of *Azima tetracantha* extract compared to control rats The GSH, vitamin C and α- tocopherol content were higher at a dose of 400mg/kg of *Azima tetracantha* treated and similar to control rats.

In conclusion, the entire variable tested i.e., SOD. CAT, GPx, reduced glutathione, vitamin C and vitamin E recorded a significant decline on CCl_4 treatment. However, treatment with different doses of *Azima tetracantha* extract (100, 200 and 400mg/kg) significantly increased in the antioxidant activity as compared to CCl_4 intoxicated, suggesting the therapeutic effect of *Azima tetracantha* to counter the oxidative stress. Among the various doses, 400mg/kg has potential activity than other doses. It can be suggested said that ethanolic extract of *Azima tetracantha* exhibit against CCl_4 induced oxidative stress and possessed anti-lipid peroxidative and antioxidant activities. This indicates that the lipid peroxidation and oxidative stress elicited by CCl_4 intoxication had been nullified due to the effect of phytochemicals present in *Azima tetracantha*.

REFERENCES

1. Abirami H., M. H. Muhammad Ilyas, K. Prem Kumar and T. Nargis Begum. Chemical composition of the ethanolic extract of leaves of *Azima tetracantha* (Lam.) Asian Journal of Plant Science and Research, 2015; 5(3): 1-5.
2. Azri, S., Mata, H.P., Reid, L., Gandlofi, A.J., Brendel, H.P., 1992. Further examination of selective toxicity of CCl_4 rat liver slices. Toxicology and Applied pharmacology, 112: 81-86.
3. Baker H, Frank O, De Angeles B, Feinglod S. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr Repor Inter, 1980; 21: 531.
4. Beers R, Sizer I, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem, 1952; 195: 133.
5. Beuge JA, Aust SD. The thiobarbituric acid assay. Method Enzymol, 1978; 52: 306-307.
6. Caroline D. Matheus A.B. P., 2 Marcos R. O., Fernanda M. U., Mirian S., Joao A.P. H., and Jose C.F.M. (2008) Protective Effects of Purple Grape Juice on Carbon

Tetrachloride-Induced Oxidative Stress in Brains of Adult Wistar Rats. J Med Food, 11(1): 55–61.

7. Castro JA Ferrya GC Castro CR Sasame H Fenos OM and Gillette JR. 1974. Prevention of carbon tetrachloride-induced necrosis by inhibitors of drug metabolism. Further studies on the mechanism of their action. Biochem. Pharmacol. 23: 295-302.
8. Chance B, Maehly AC, 1955. Assay of catalase and peroxidase. Meth. Enzymol, 2: 764-775.
9. Clawson, G.A., 1989. Mechanism of carbon tetrachloride hepatotoxicity. Pathology and immunology research, 8: 104–112.
10. De Groot, H., Noll., 1986 . The crucial role of low steady state oxygen partial pressure in halo alkane free radical mediated lipid peroxidation. Biochemical pharamacology, 35: 15-19.
11. Fraga C, Leibovitz B and Tappel A. 1987. Halogenated compounds as induced of lipid peroxidation in tissue slices. Free Rad. Biol. Med. 3: 119-123.
12. Gupta M, Mazumder UK, Sivakumar T, Gomathi P and Sampath Kumar R. 2004. Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetrachloride induced liver damage in rats. Iranian journal of Pharmacology and Therapeutics. 3: 12-20.
13. Guntupalli M. Mohana Rao, Chandana V. Rao Palpu Pushpangadan, Annie Shirwaikar (2006) Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. Journal of Ethnopharmacology, 2006; 103: 484–490.
14. Halliwell B. 1991. Reactive oxygen species in living systems; source, biochemistry and role in human disease. Am. J. Med. 91: 14s.
15. Harvey J, Paige SM. The Instat Guide to choosing and interpreting statistical tests: A manual for Graph pad Instat, Version 3. San Diego, CA USA. 1998.
16. Janardhan L, Vineetha M Shrikanth, Kiran K Mirajkar and Sunil S More. 2014 In vitro screening and evaluation of antivenom phytochemicals from Azima tetracantha Lam. leaves against Bungarus caeruleus and Vipera russelli. Journal of Venomous Animals and Toxins including Tropical Diseases, 2014; 20: 12.
17. Joyce DA. (1987) Oxygen radicals in disease. Adverse Drug Reaction Bulletin. 127: 476-479.
18. Kadiiska MB, Gladen BC, Baird DD, Dikalova AE, Sohal RS, Hatch GE, Jones DP, Mason RP and Barrett JC. 2000. Biomarkers of oxidative stress study: Are plasma

antioxidants markers of CCl_4 poisoning? Free Radical Biology & Medicine, 28(6): 838–845.

19. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of SOD. Ind J Biochem Biophy, 1984; 21: 130-132.
20. Kale, R.K., & Sitasawad, S.L., Radiation–induced lipid peroxidation in liposomes Radiat phys chem., 1990; 36: 361.
21. Lin CC, Yen MH, Lo TS and Lin JM. 1998. Evaluation of the hepatoprotective and antioxidant activity of *Boehmeria* nivea var. nivea and B. nivea var. tenacissma. J. Ethnopharmacol. 60: 9-17.
22. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin's reagent. J Biol Chem. 1951; 193: 265-276.
23. Misra, H. P., and I. Fridovich (1972). The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170-3175.
24. Moron MS, DsePierre JW, Manerwik KB. Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochim Biophy Acta, 1979; 582: 67-68.
25. Omaye ST, Tumball JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Method Enzymol, 1979; 62: 1-11.
26. Packer L., Tritschler H.J and Wessel K. (1997) Neuroprotection by the metabolic antioxidant alpha-lipoic acid. Free Radical Biology and Medicine. 22: 359- 378.
27. Prasad Varier S, Venkatachalam SR, Ramesh Chander R and Paul Thomas R. 1999. Dietary antioxdant natural defence against disease. Aryavaidyan. 12(3): 149-158.
28. Proctor PH and McGinness JE. 1986. The function of melanin. Arch Dermatol. 122: 507-508.
29. Ray G and Husain SH. 2002. Oxidants antioxidants and carcinogenesis. Indian J Exp Biol. 40: 1213-1232.
30. Recknagel, R.O and Glende, EA. (1973). Carbon tetrachloride hepatotoxicity: An example of lethal cleavage. Crit. Rev. ToxicoL, 2: 263-297.
31. Recknagel, R.O., Glende, E.A., Jr, Dolak, J.A., Waller, R.L., 1989. Mechanisms of carbon tetrachloride toxicity. pharmacol. Ther. 43: 139–154.
32. Recknagel, RO. 1983 A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci. 33: 401-408.
33. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvate transaminases. Am J Clin Pathol. 1957; 28: 56-63.

34. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical roles as component of glutathione peroxidase. Sci., 1973; 179: 588-590.
35. Scartezzini P and Speroni E. 2000. Review on some plants on Indian traditional medicine with antioxdant activity. J. Ethanopharmacol. 71: 23-43.
36. Tewari S, Gupta V and Bhattacharya S. 2000. Comparative study of antioxidant potential of tea with without additives. Indian J Physiol. Pharmacol. 44(2): 215-219.
37. Tiwari A. (2001) Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidants therapy. Current science, 81: 1179-1187.
38. Venukumar MR and Latha MS. 2002. Antioxidant activity of *Curculigo orchioides* in carbon tetrachloride induced hepatopathy in rats. Ind. J. Clin. Biochem. 17(2): 80-87.
39. Winkler BS. (1992) Unequival evidence in support of the non-enzymatic redox coupling between glutathione/glutathione disulfide and ascorbic acid/dehydroascorbic acid. Biochimca Biophysics Acta. 1117: 287-290.
40. Wolf, C.R., Harrelson Jr., W.G., Nastainezyk, W.M., Philpot, R.M., Kalayanaraman, B., Mason, R.P., 1980. Metabolism of carbontetrachloride in hepatic microsome and reconstitution monoxygenase systems and its relationship to lipid peroxidation. Molecular pharmacology, 18: 558–558.
41. Yu BP. 1994. Cellular defences against damage from reactive oxygen species. Biol Rev. 74: 139-162.