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ANTIOXIDANT, ANTI-INFLAMMATORY AND HEPATOPROTECTIVE ACTIVITIES OF *TERMINALIA BELERICA* AND ITS BIOACTIVE COMPOUND GALLIC ACID AGAINST CCL_4 INDUCED OXIDATIVE STRESS AND HEPATOTOXICITY IN RAT MODEL

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Abstract

The hepatoprotective activity of the alcoholic extract of Terminalia belerica (TB) and its active principle Gallic acid (GA): 3,4,5-tri hydroxy benzoic acid was investigated against carbon tetrachloride induced hepatic damage.

Female albino rats weighing 130±10g were randomly divided into four groups of five animals each. Group 1 served as normal control. Groups 2 – 4 were administered carbon tetrachloride (1.5 ml/kg, i.p.). Group 2 was treated as experimental control. Group 3 and 4 were administered with Terminalia belerica and Gallic acid respectively after 24 hours of carbon tetrachloride administration. Animals were sacrificed 24 hours after the last treatment.

Carbon tetrachloride administration caused significant decrease in Hb %, blood sugar and activity of serum alkaline phosphatase on the contrary, the serum transaminases and protein content elevated significantly. Significant decrease was observed in the glycogen content, activity of alkaline phosphatase and glutathione level of liver and kidney. Activity of acid phosphatase, glucose 6 phosphatase, triglyceride level, lipid peroxidation and bromosulphalein retention time enhanced significantly. A significant decrease were observed in drug metabolizing enzymes i.e. amidopyrine N-demethylase and aniline hydroxylase. Chloretic activity of gallic acid was also performed. Recoupment was seen in almost all the parameters by therapy with TB and GA intoxicant induced subject. The degree of protection conferred by GA was more as compared to ethanolic extract of TB.

Key Words: *CCl_4, Terminalia belerica (TB), Gallic Acid (GA)*

INTRODUCTION:

Liver plays an important role in the removal of substances from the portal circulation and is susceptible to first and persistent attack by offending agents like chemicals, toxins, drugs and environmental pollutants. A global estimate indicates that there are about 18,000 deaths every year due to liver diseases. Botanical medicines have been used traditionally by herbalists and indigenous healers world wide for the prevention and treatment of liver

diseases. WHO is an inventory of medicinal plants of over 20,000 species. As a part of strategy to reduce financial burden on developing countries which spend 40-50% of their total health budget on drugs, it currently encourages, recommends and promotes inclusion of herbal drugs in National Health Care Programmes. Clinical research in this century has confirmed the efficacy of several plants in the treatment of liver disease, while basic scientific research has uncovered the mechanism by which some plants provide their therapeutic effects.

Terminalia belerica roxb. (combretaceae) commonly known as `Bahera', is distributed through out India. The fruit of the plant is used for various ailments in the indigenous system of medicine (Kirtikar and Basu, 1933). The fruit has astringent, tonic, laxative, antipyretic activities, and is used in piles, dropsy dysentery, diarrhoea, leprosy, biliousness, dyspepsis and headache. It is a powerful rejuvenative herb that nourishes the lungs, throat, eyes and hair. It is the single herb for controlling Kapha. It excels at removing stones and accumulations of toxins (mucus, cholesterol, minerals deposits) in the digestive urinary and respiratory tracts (Nandkarni, 1954). It is one of the ingredients of ayurvedic purgative medicament Triphala. Gallic acid (3,4,5-Trihydroxy benzoic acid) is an active principle of *Termialia belerica*.

Therefore present investigation aims in identifying the hepatoprotective potential of *Terminalia belerica* and Gallic acid on experimental liver injury induced by carbon tetrachloride.

MATERIALS & METHODS:

Preparation of the extract

Fruits of *Terminalia belerica* were collected from the plants growing locally and were identified by the Botany Department of Jiwaji University, Gwalior. Fruits were dried, chopped and ethanolic extract was prepared.

Animals:

Female albino rats of *sprague dawley* strain (120±10) and swiss albino mice (30±5) were used for hepatoprotective studies. Animals were housed under standard conditions (25±2 °C temperature, 60%-70% relative humidity and 12 hr. photoperiod) and allowed free access to food and water *ad libitum*.

Hepatotoxin:

Hepatic injury was induced by 1.5 ml/kg carbon tetrachloride (Sharma *et al* 1989, Janbaz and Gilani 1995) mixed with equal amount of liquid paraffin administered intraperitoneally (*i.p.*). Vehicle received equal amount of liquid paraffin.

Biochemical parameters:

Animals were divided into four groups. Group 1 served as normal control. Other three groups were administered CCl_4 (1.5 ml/kg, *i.p.).* Group 2 was treated as experimental control. Groups 3-4 were administered *Terminalia belerica* and Gallic acid respectively after 24 hours of CCl_4 administration. Animals were necropsied 24 hours after the last treatment.

Just before the necropsy, blood was collected by puncturing the retero-orbital sinus and various haematological parameters *viz* - blood sugar (Asatoor and King, 1954), serum alkaline phosphatase (Fiske and Subbarow, 1925), serum protein (Lowry *et al*, 1951) and transaminases (Reitmen and Frankel, 1957) were processed. Immediately after necropsy, liver and kidney were removed. Fresh tissues were processed for the estimation of glycogen (Seifter *et al*, 1950).

Tissue homogenates were prepared in ice-cold hypotonic solution and protein (Lowry *et al*, 1951), alkaline and acid phosphatase (Hawk *et al*, 1954), adenosine triphosphatase (Seth and Tangari, 1966), succinic dehydrogenase (Slatter and Bonner, 1952) Glucose-6-Phosphatase (Duve *et al.,* 1955) Triglyceride (Neri *et al.,* 1973) were determined. Hepatic lipid peroxidation (Sharma and Krishnamurthy, 1968) and reduced glutathione (Brehe and Burch, 1976) were measured in liver.

Statistical Analysis

Results were analyzed by Student's t test. P values ≤ 0.05 were taken as significant. This was followed by one way Analysis of Variance (ANOVA, Snedecor and Cochran, 1994)

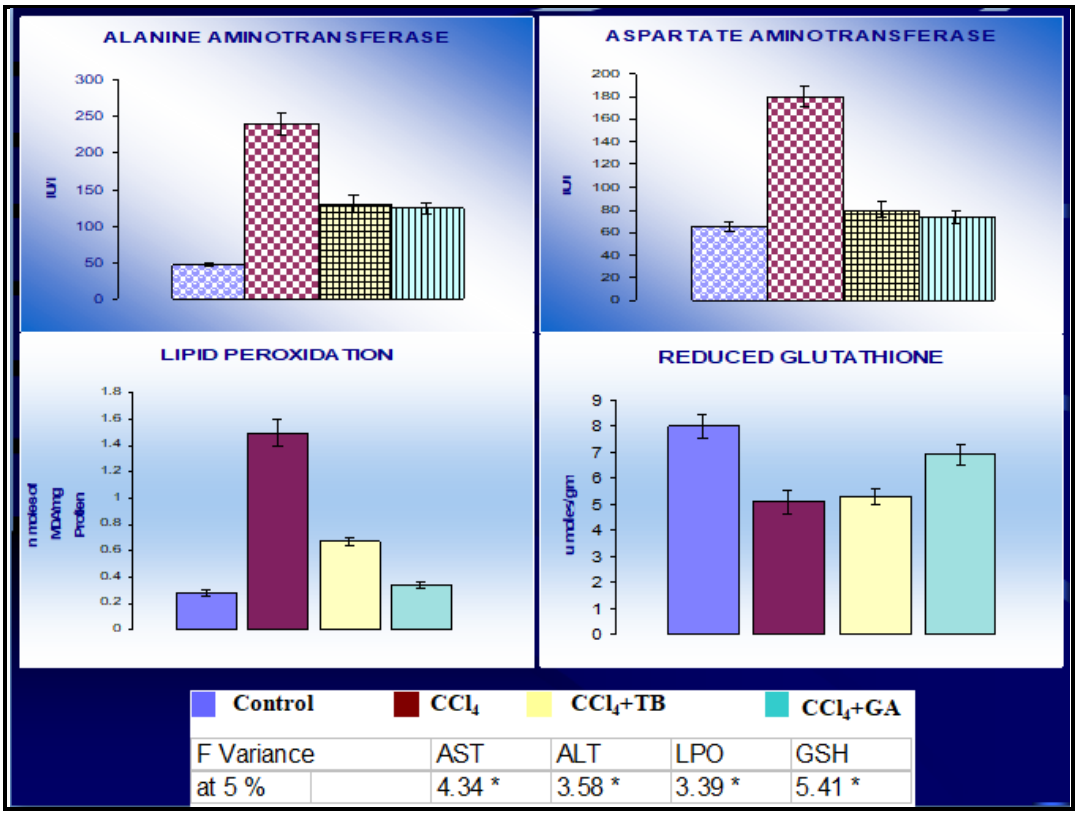
RESULTS:

Results showed that CCl_4 administration caused changes in the blood biochemical variables ie. AST and ALT. After the administration of toxicant, significant elevation in the activity of serum transaminases were seen. Administration of extract of TB at 400mg/kg and GA at 200 mg/kg showed considerable therapeutic effect. With the treatment of GA marked recoupment was observed when compared with the crude TB extract. There was a significant increase in lipid peroxidation on the contrary decrease was observed in the glutathione content after carbon tetrachloride administration. GA and TB were effective in recouping these variables near control (Graph 1-4).

The blood sugar level declined significantly after the administration of toxicant. TB extract and GA active principle were effective in recouping the blood sugar level. Carbon tetrachloride caused decrease in the glycogen content in liver and kidney. GA was found to be significantly effective in both the organs (Graph 5-7). (Graph 8-11) Acid and alkaline phosphatase activity revealed a marked rise after CCl_4 treatment, except in the activity of alkaline phosphatase of kidney. Effectiveness of extract and its active principle was observed in both the organs. Appreciable fall was observed in the activity of adenosine triphosphatase and succinic dehydrogenase after carbon tetrachloride administration. Extract caused a marked reversal in the inhibitory effect of the enzymatic variables and thus the values were very near to controls in both the organs. GA was found to be more effective (Graph 12-15).

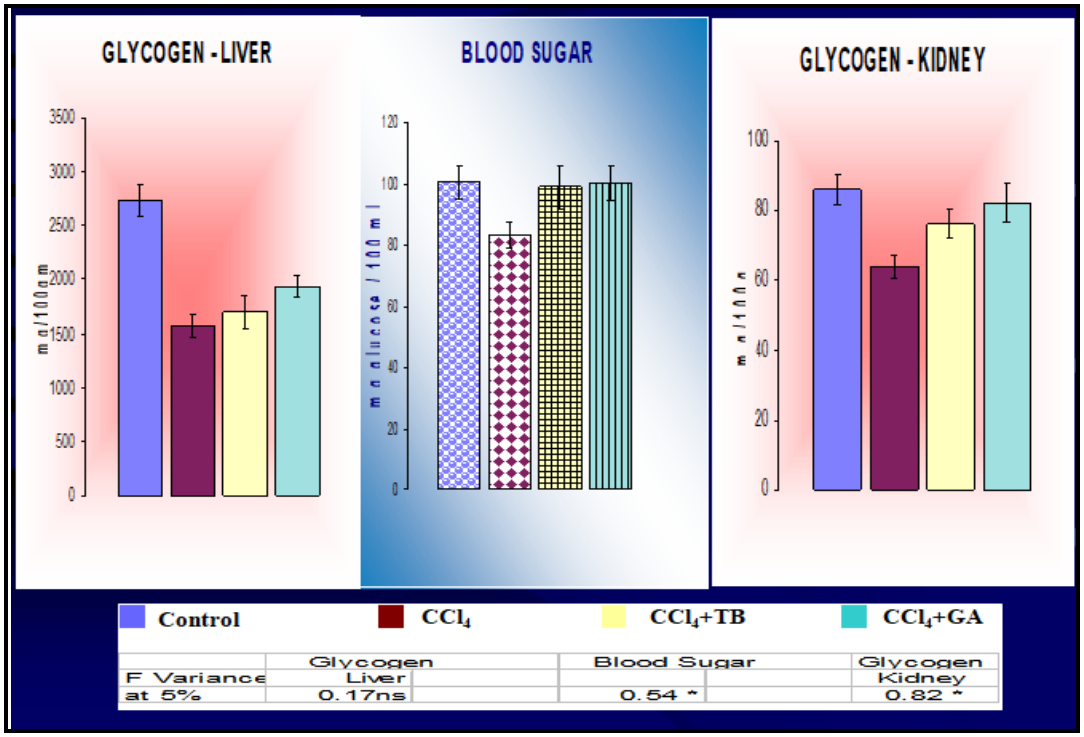
Activity of glucose 6-phosphatase and triglyceride contents of liver increased significantly. TB and its active principle Gallic acid showed marked reversal in both the parameters. Carbon tetrachloride exposure decrease the activity of drug metabolizing enzymes i.e. amidopyrine N-demethylase and aniline hydroxylase in liver microsomal fraction which were recouped by the treatment of GA (Graph 15-19).

Graph (1-4)

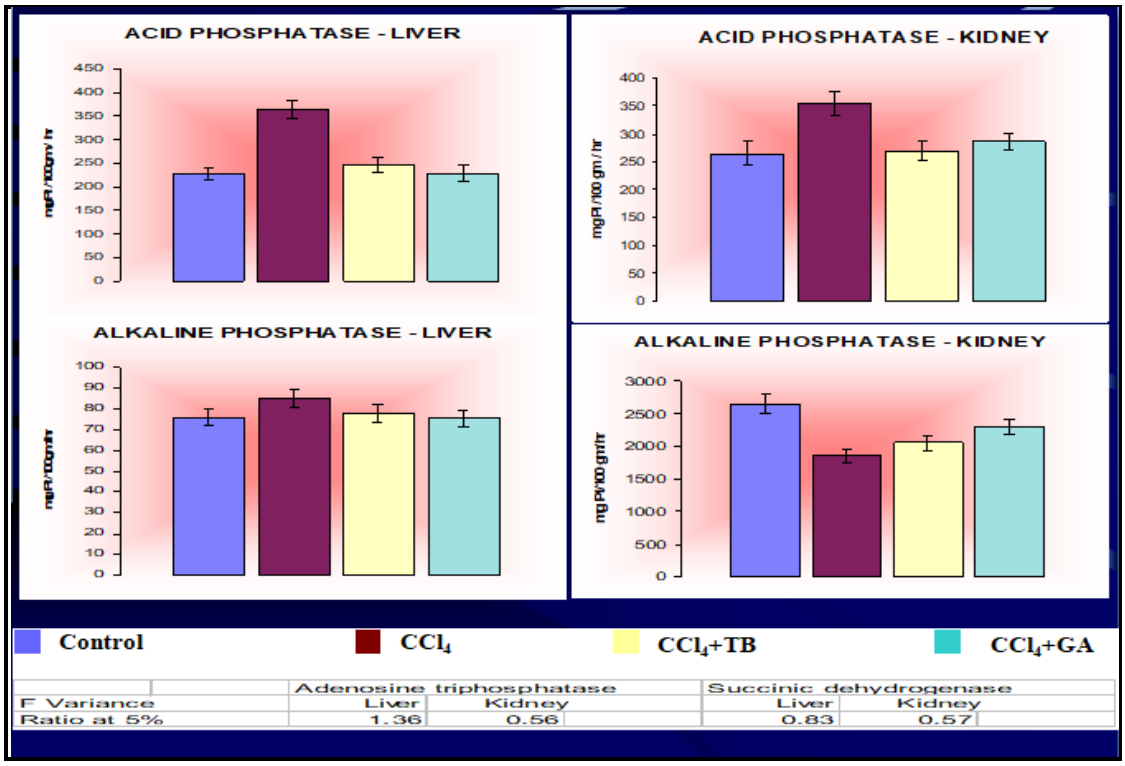


F Variance	AST	ALT	LPO	GSH
at 5 %	4.34 *	3.58 *	3.39 *	5.41 *

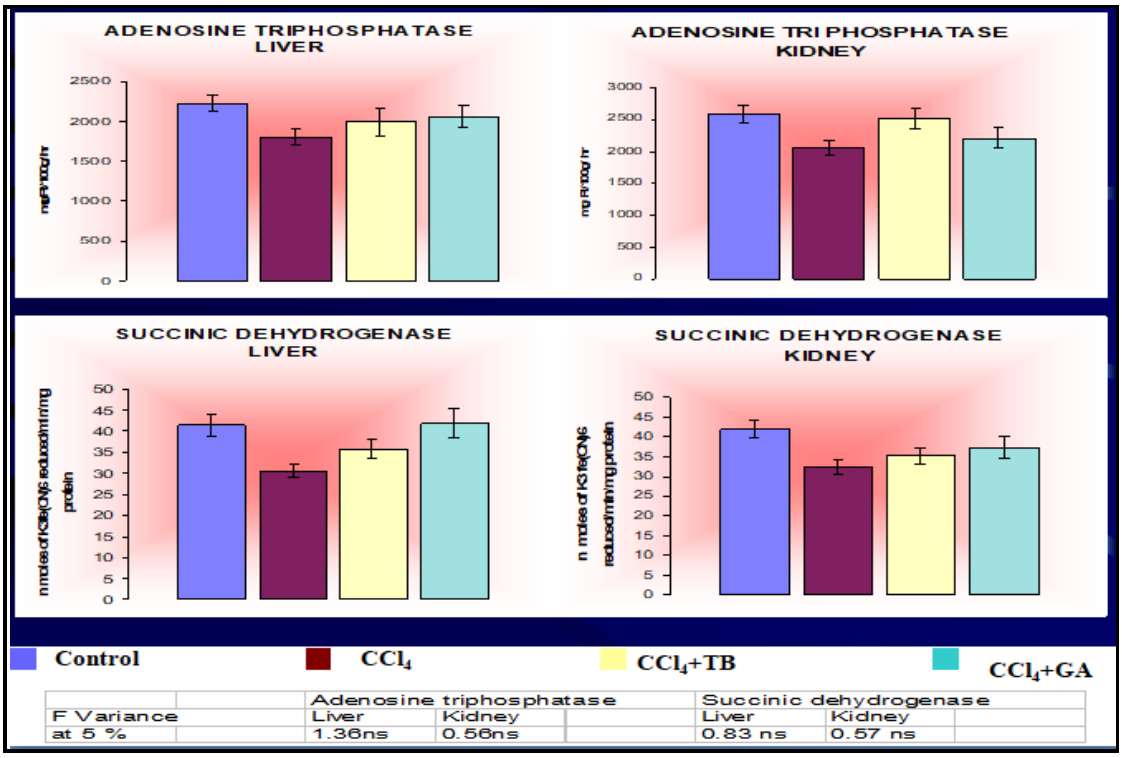
Graph (5-7)



Graph (8-11)

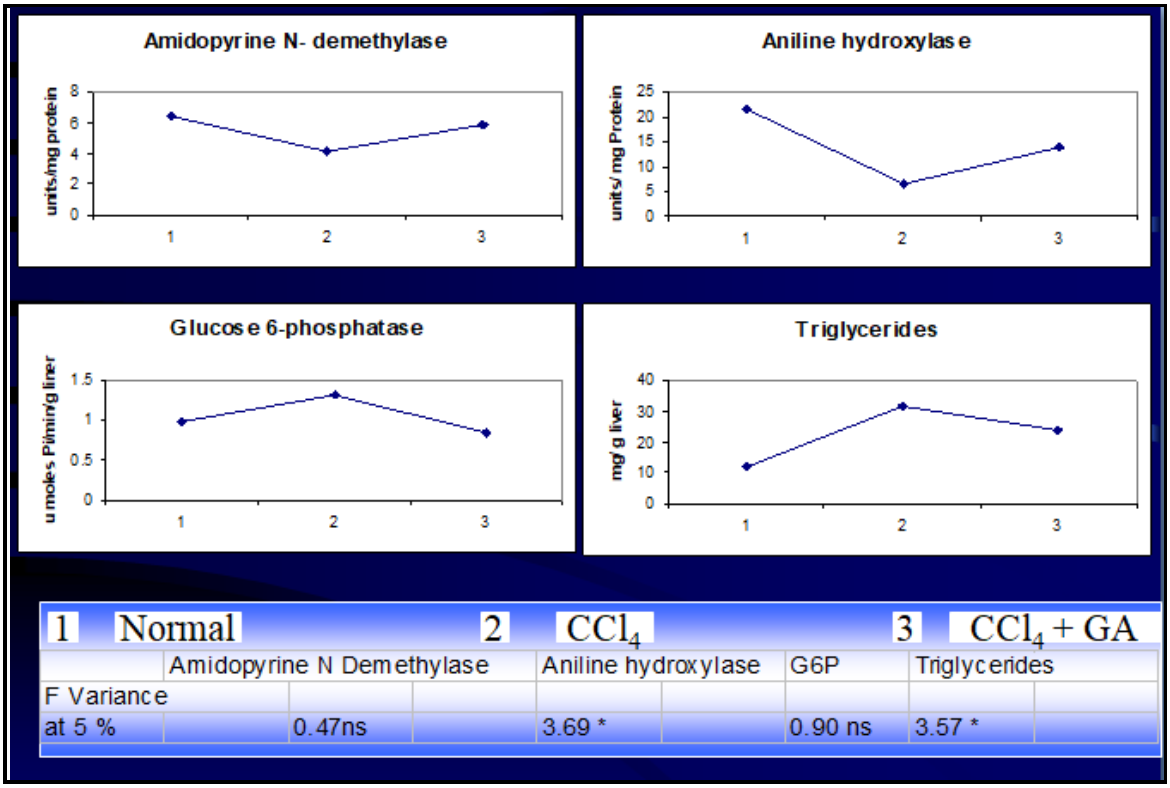


Graph (12-15)



	Adenosine triphosphatase		Succinic dehydrogenase	
F Variance	Liver	Kidney	Liver	Kidney
at 5 %	1.36ns	0.56ns	0.83 ns	0.57 ns

Graph (16-19)



	Amidopyrine N Demethylase	Aniline hydroxylase	G6P	Triglycerides
F Variance at 5 %	0.47ns	3.69 *	0.90 ns	3.57 *

DISCUSSION:

The results of the present study clearly demonstrate that the various biochemical alterations produced by carbon tetrachloride in the serum / blood and tissue were reversed significantly by the administration of the extract (400-mg/kg) and Gallic acid (200 mg/kg). Results reveal that the administration of carbon tetrachloride caused significant decrease in blood sugar level and serum alkaline phosphatase. On the contrary it caused remarkable increase in the level of serum proteins and transaminases patterns. These finding are also supported by various authors (Balasubrmanian *et al.*, 1998, Chung *et al.*, 1998). (Sharma *et al.*, 1994).

Damage to the structural integrity of liver is reflected by an increase in the level of serum transaminases because these are cytoplasmic in location and released into circulation after cellular damage. It is generally accepted that the toxicity of carbon tetrachloride depends on the cleavage of the carbon-chlorine bond to generate trichloromethyl and trichloromethyl peroxy radicals, which may contribute to the hepatotoxicity and subsequent increase in hepatic enzymes (Zafar *et al.*, 1998, Ahmed *et al.*, 2001). Author also observed a rise in the level of AST and ALT in carbon tetrachloride treated rats. GA may prevent the acute organ dysfunction and cellular injury thereby inhibiting the rapid leakage of enzymes. Recoupment with the administration of different plant preparations are reported, e.*g. Schisandra chiensis* (Zhu, *et al*, 1999*), Cappris Spinosa* (Gadgoli, *et al*, 1999), *Cassia Tora* (Maity, *et al*, 1998), *Ginkgo biloba* (Shenoy, *et al*, 1999), *Withania somnifera* (Rasool, *et al*, 2000), *Andrographis paniculata* (Trivedi, *et al*, 2000), *Emblica officinalis* (Jose, *et al* 2000), *Mallotus japonicus* (Lim, *et al*, 2000),

It is also observed that carbon tetrachloride caused significant decrease in the glycogen content of liver and kidney. The reason may be the disruption of glycogen storage, which is associated with dysfunctional and dystrophic changes in the liver and kidney due to inhibition of key enzymes in carbohydrate metabolism. Rastogi and Rana (1990) reported that carbon tetrachloride bring about a rise in cytosolic free calcium, which may lead to glycogen mobilization, thus causing depletion in hepatic glycogen content. It may be assumed that GA being an antioxidant reduces the stress to a considerable extent thereby reducing the demand for the excess sugar and recouping the glycogen content.

Carbon tetrachloride also caused significant increase in the activity of acid and alkaline phosphatases. As acid phosphatase is a lysosomal enzyme, the increase in the activity of acid phosphatase may be due to the lysosomal imbalance resulting in destruction

of the intact membranes. Administration of CCl_4 led to the assimilation of fat in the liver and kidney and demonstrates continuous process of autophagy and increased the activity of acid phosphatase (Bhadauria *et al.,* 2002), and extract may possess anti-inflammatory and lysosomal stability properties and obstructs the rise in the enzymatic activity. Abraham and Wilfred, (2000) suggested that the increase in the activity of the lysosomal enzymes, i.e. acid phosphatase in the organs after treatment with the carbon tetrachloride suggested increased tissue catabolism and autophagy, which are possible sequences leading to renal damage. As alkaline phosphatase have been reported to be involved in the transport of metabolites across the cell membranes, protein synthesis, synthesis of certain enzymes, secretary activities and glycogen metabolism. Thus the alterations in the enzymatic activity may be due to the disturbance in the secretary activity or in the transport of metabolites or may be due to altered synthesis of certain enzymes.

Treatment of carbon tetrachloride also caused centrilobular necrosis, which results in the accumulation of fat in liver. Fat from the peripheral adipose tissue is translocated to the liver and leading to its accumulation during toxicity. This is an evident from the elevated level of triglyceride in the liver of the carbon tetrachloride treated groups as seen in this study. These changes were almost completely restored with GA treatment. Glucose 6-phosphatase is located on the luminal side of the smooth membrane of endoplasmic reticulum. It catalises the ultimate biochemical reaction of both glucogenolysis and gluconeogenesis. It allows the gluconeogenic lesion which it is specifically expressed to release glucose in blood. In the present investigation carbon tetrachloride administration significantly increases the activity of this enzyme, which was recouped by the therapy of GA.

Considerable lowering of GSH level was observed in the liver, on the other hand, there was remarkable increase in the activity of lipid peroxidation. These studies are supported by various authors with the administration of carbon tetrachloride (Tripathi, *et al*, 1999, Shoney, *et al*, 1999, Lin, *et al*, 1998, Vijayapadma, *et al*, 1998). The increased TBARS of liver indicated enhanced lipid peroxidation due to tissue injury and failure of the antioxidant defense mechanism, which prevents the formation of excess free radicals. As GA has antioxidant property, it may prevent free radical generation thereby reducing oxidative stress. Administration of GA may promote the conversion of GSSG into GSH by reactivation of hepatic GSSG reductase enzyme in CCl_4 treated animals. The availability of a sufficient amount of GSH thus increased the detoxification of active metabolites of CCl_4. Similar results were observed by Trivedi, *et al*, (2000) where significant recoupment was observed on administration of *Andrographis paniculata.*

Liver microsomal enzymes such as Amidopyrine N-demethylase and aniline hydroxylase are responsible for the metabolism of drugs. The damage conferred by CCl_4 on hepatocytes as well as hepatic microsomal drug metabolism enzymes (MDME) causes a loss

of drug metabolizing capacity of the liver. Treatment of animals with the GA prevented the CCl_4 induced damage to hepatocytes including MDME such as cytochrome P-450.

As it is well established that the hepatotoxicity by carbon tetrachloride is due to its enzymatic activation of CCl_3 free radical, which in turn disturb the structure and function of lipid and protein macromolecules in the membranes of the cell organelles and induced microsomal lipid peroxidation. Hepatoprotective activity of the active principle may be may be due to its strong free radical scavenging activity thereby conferring its protective effect.

REFERENCES:

Abraham, P., Wilfred, G. Lysosomal enzymes in the pathogenesis of carbon tetrachloride induced injury to the kidney and testes in rats. Indian Journal of Pharmacology (2000) 32, 250-251.

Ahmed, B., Alam, T. and Khan, S. A. Hepatoprotective activity of Luffa echinata fruits. Journal of Ethnacopharmacology (2001), 76, 187-189.

Asatoor, A.M. and King, E. Simplified colorimetric blood sugar method. Biochem. J., (1954) 44,56.

Balasubramaniam, P.; Pari, L.; Menon, V. P.; Protective effect of carrot (Daucus carota L.) Against Lindane induced hepatotoxicity in rats. Phytotherapy Research 1998,12 (6): 434-436.

Bhadauria, M., Jadon, A., Sharma, A. and Shukla, S. Effect of propriety herbal formulation against chronic carbon tetrachloride induced hepatotoxicity. Indian Journal of Experimental Biology (2002), 40, 1254-1259.

Brehe, J.E. and Burch, H.B. (1976). Enzymatic assay for glutathione. Anal. Biochem., 74, 189. Asatoor, A.M. and King, E. (1954). Simplified colorimetric blood sugar method .Biochem.J.,44,56.

Chung, M. H.; Ob, H. S.; Lin, J. H. Hepatoprotective effect of Angeticae Gigantis Radix extract on hepatic injury induced by toxic drugs in rats. Korean journal of pharmacology. 1998; 29(4) 402-412.

Duvey, C., Pressman, D. and Appelman, R. (1955). Tissue fractionation study intracellular distribution pattern of enzymes in rat liver tissue. Biochem. J., 60, 604.

Fiske, C.H. and Subbarow, Y. The colorimetric determination of phosphates. J. Biol., Chem. 1925, 66 : 375-400.

Gadgoli, C.; Mishra, S.H. Antihepatotoxic activity of p-methyl benzoic acid from Capparis spinosa. Journal of ethnopharmacology. 1999;66(2):187-192.

Hawk, P. B., Oster, B.L., Summerson, W. H., 1984. The Practical Physiological Chemistry. 14th ed., McGraw Hill Book Co., New York, 1123.

Janbaz, K. H. and Gilana, A. H. Evaluation of protective potential of Artemisia maritima extract on acetaminophen and CCl_4 induced liver damage. Journal of Ethnopharmacology, (1995) 47, 43-47.

Jose,J. K.; Kuttan R. Hepatoprotective activity of Emblica Officinalis and chyvanaprash. Journal of ethnopharmacology. 2000; 72:135-40.

Kirtikar, K. R. and Basu, B. D. (1933) Terminalia Linn. In, Indian Medicinal Plants, 2nd ed., Vol. II, pp. 1014-1033. Lalit Mohan Basu, Allahabad, India.

Lim, H. K.; Kim, H. S.; Choi, H. S.; Oh, S.; Cho, J. Hepatoprotective effect of Bergenin a major constituents of Mallotus Japonicus, on carbon tetra chloride intoxicated rats. Journal of ethnopharmacology. 2000; 72: 469-474.

Lin, C. C.; Yen, M. H.; Lin, J. M.; Lo, T. S. Evaluation of the hepatoprotective and antioxidant activity of Boehmeria nivea var nivea and B. nivea var tenacissima. Journal of ethnopharmacology. 1998;60:9-17.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with Folin's phenol reagent. J. Biol. Chem., 1951, 193 : 265-269.

Maity, T. K.; Mandal, S.C.; Pal, M.; Saha, B. P.; Antihepatotoxic activity of cassia tora leaf extract. Natural product Sciences. 1998;4(4): 226-229.

Nandkarni, A. K. (1954) 'Indian Materia Medica' 3rd ed., 1. Dhoota Papeshwar Prakashan, Bombay.

Neri, B. P. and Frings, C. S. (1973). Improved method for determination of triglycerides in serum. Clinical Chemistry 19, 1201.

Rasool, M. K.; Latha, L. M.; and Varalakshmi, P. Effect of Withania somnifera on lysosomal acid hydrolases in Adjuvant- induced Arthritis in rats. Pharm. Pharmacol. Commun. 2000;6:187-190.

Rastogi, S. and Rana, S. V. S. Influence of paratheroidectomy on liver glycogen in rats treated carbon tetrachloride. Indian Journal of Experimental Biology (1990) 28, 794.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28, 56.

Seifter, S., Dayton, S., Novic, B. and Muintwyler, E. (1950). The estimation of glycogen with anthrone reagent. Arch. Biochem., 25, 151.

Seth, P.K. and Tangari, K.K. (1966). Biochemical effects of newer salicylic acid congenesis. J. Pharm. Pharmacol., 18, 831.

Sharma, A. K., Anand, K. K., Pushpangadan, P., chanden, B. K., Chpora C.L., Prabhakar, Y. S. and Damodaran, N. P. Hepatoprotective effect of Wedelia Calendulacea. Journal of Ethinopharmacology 25 (1989) 93-102.

Sharma, A.; Mathur, R. and Shukla, S. Hepatoprotective action of propriety Herbal preparation against carbon tetra chloride intoxication. Indian Drugs 1994;32(3):120-125.

Sharma, S.K. and Krishnamurthy, C.R. (1968). Production of lipidperoxides of brain. J. Neurochemistry, 15, 147.

Shenoy, A.; Bairy, K.L. Effect of Ginkgo- biloba (G B) on carbon tetra chloride induced liver damage in male albimno rats. Indian journal of pharmacology. 1999; 31(1): 79.

Slatter, E.C. and Bonner, W.D. (1952). The effect of fluoride on the succinic oxidase system. Biochem. J., 82, 185.

Snedecor, G. W., Cochran, W. G., 1994. Statistical Method, 8th Edition. Affiliated East-West Press.

Tripathi, Y. B.; Pandey, E. Role of alcoholic extract of shoot of Hypericum perfonatum Linn. On LPO and various species of free radicals in rats. Indian journal of experimental biology. 1999; 37(6): 567-571.

Trivedi, N.; Rawal, U. M. Hepatoprotective and Toxicological evaluation of Andrographis paniculata on severe liver damage. Indian Journal of Pharmacology. 2000; 32:288-293.

Vijayapadma, V.; Saju,V.; Devi, S.; Prema, C. S. Hepatoprotective effcet of Liv-52 on antitubercular drug- induced hepatotoxocity inrats. Fitoteraphia. 1998; 69(6): 520-522.

Zafar, R. and Ali, S. M. Antihepatotoxic effects of root callus extract of Cichorium intybus L. Journal of Ethnopharmacology (1998) 63, 227-231.

Zhu, M.; Lin, K. F.; Yeung, R. Y.; Li, R. C. Evaluation of the protective effect of Schisandra chinensis on phase I drug metabolism using a carbon tetra chloride intoxication model. Journal of ethnopharmacology. 1999;67:61-68.