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Comparative Effect of *Terminalia belerica* Fruit Extract and its Active Principle Against Carbon Tetrachloride-Induced Toxicity in Rats.

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The protective effect of *Terminalia belerica* fruit extract and its active principle (gallic acid: 3,4,5-trihydroxybenzoic acid) were investigated against carbon tetrachloride induced toxicity in rats. Carbon tetrachloride caused significant increase in the activity of alkaline phosphatase, transaminases and protein content. Hepatic lipid peroxidation increased significantly while reduced glutathione level in liver decreased after toxicant administration. Considerable inhibition was observed in glycogen content as well as in the activity of alkaline phosphatase, succinic dehydrogenase and adenosine triphosphatase in liver and kidney. Activity of acid phosphatase elevated significantly in both the organs. Recoupment was seen in almost all the parameters by therapy with extract and active principle intoxicant induced subjects. The degree of protection conferred by active principle was more as compared to ethanolic extract of *Terminalia belerica.*

Terminalia belerica Roxb. (TB, Combretaceae, '*bahera*') is distributed throughout the forests of India. Among the various medicinal properties attributed to its significance, one is its therapeutic value in the treatment of liver disorders and indigestion[1]. The fruits of TB are reported to have purgative[2], cardiac depressant and choleretic effects[3]. It is one of the ingredients of ayurvedic purgative medicament *triphala*. Gallic acid (GA: 3,4,5-trihydroxybenzoic acid) is an active principle of *Terminalia belerica* fruit. Therefore, present investigation aims to evaluate the protective potential of *Terminalia belerica* fruit extract and its active principle against carbon tetrachloride (CCl_4) intoxication.

MATERIALS AND METHODS

Preparation of the extract:

Fruits of *Terminalia belerica* were procured from authenticated Ayurvedic dealer and were identified by the taxonomist of Botany department of Jiwaji University, Gwalior. A voucher specimen (No. 336) has been deposited in Herbarium (Acronym JUG) of Jiwaji University, Gwalior. Fruits were dried, chopped and ethanolic extract was prepared (17.6% w/v). An aqueous suspension of crude extract in 2% gum acacia was administered to the animals orally. Gallic acid was procured from Sigma-Aldrich (Bangalore) and CCl_4 was procured from Ranbaxy (New Delhi). The other chemicals used in the study were procured from Sigma-Aldrich (Bangalore) and E-Merck (Germany). Toxicity was induced by 1.5 ml/kg (ip) CCl_4 mixed with liquid paraffin. Equal amount of liquid paraffin[4] was given as vehicle.

Female rats of Sprague Dawley strain (130±10 g) were used for hepatoprotective studies. Animals were housed under standard conditions (25±2°, 60-70 % relative humidity and 14 h light and 10 h dark. The rats were fed on standard pellet diet (Hindustan Liver Ltd., New Delhi) and water *ad libitum.* Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Control and Supervision of Experiments on Animals (CPCSEA), Chennai. Experimental protocol for treating animals was approved by IAEC (Institutional Animals Ethics Committee).

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TABLE 1: EFFECT OF TREATMENTS ON THE ACTIVITY OF SERUM TRANSAMINASES.

Treatments	AST (IU / L)	ALT (IU / L)
Normal Control	65.5±3.36	47.4±2.51
CCl_4	228.4±18.6*	436.0±22.0*
CCl_4+Extract	168.0±9.77**	247.0±17.0**
CCl_4+AP	109.0±9.39**	126.0±6.95**
CCl_4+Sily	79.8±6.28**	117.0±10.8**
One-way ANOVA df	6, 20	6, 20
F	49.03	153.87
P	<0.05	<0.05

AP=active principle, Sily=silymarin, Values are mean±SEM, n=5, * $P \leq 0.05$, when compared with control, ** $P \leq 0.05$, when compared with CCl_4-treated group.

Treatments:

Animals were divided into five groups of five animals each. Group 1 served as normal control. Other four groups were administered CCl_4 (1.5 ml/kg, ip). Group 2 was treated as experimental control. Groups 3-4 were administered *Terminalia belerica* extract (400 mg/kg, po) and active principle (200 mg/kg, po) respectively after 24 h of CCl_4 administration. Group 5 received a reference drug silymarin (50 mg/kg, po) and served as positive control[5]. All the animals were sacrificed after 24 h of last treatment.

TABLE 2: EFFECT OF TREATMENTS ON SERUM ALKALINE PHOSPHATASE AND SERUM PROTEIN CONTENT.

Treatments	SALP (mg Pi/100 ml/h)	Serum Protein (mg/100 ml)
Normal Control	204.0±12.9	37.5±2.61
CCl_4	1085.0±83.5*	88.6±6.41*
CCl_4+Extract	481.4±24.4**	57.4±3.91**
CCl_4+AP	386.0±36.4**	47.5±4.24**
CCl_4+Sily	334.0±19.15**	38.2±3.74**
One-way ANOVA df	6, 20	6, 20
F	77.88	29.03
P	<0.05	<0.05

AP=active principle, Sily=silymarin, Values are mean±SEM, n=5, *$P \leq 0.05$, when compared with control, **$P \leq 0.05$, when compared with CCl_4-treated group.

Just before the necropsy, blood was collected by puncturing the retero-orbital sinus, serum was separated at 2000 rpm. and various parameters *viz* - alkaline phosphatase[6], protein[7] and transaminases[8] were processed. Immediately after necropsy, liver and kidney were removed. Fresh tissues were processed for the estimation of glycogen[9]. The quantitative measurement of lipid peroxidation was done by measuring the concentration of thiobarbituric acid reactive species (TBARS) in liver[10]. Reduced glutathione was estimated in the liver homogenate using dithionitrobenzoic acid (DTNB)[11]. Tissue homogenates were prepared in ice-cold hypotonic solution for the estimation of alkaline and acid phosphatase[12], adenosine triphosphatase[13] and succinic dehydrogenase[14]. Data were expressed as mean±SEM. and were statistically assessed by one-way analysis of variance (ANOVA). Difference between, animals of treated and control groups were calculated by Student's 't' test. $P \leq 0.05$ was taken as significant[15].

RESULTS

Tables 1 and 2 demonstrate that administration of CCl_4 caused significant increase in the activity of transaminases, alkaline phosphatase and protein content in experimental control group. Because CCl_4 is associated with a variety of biochemical abnormalities and these are attributed to the release intracellular constituents into the circulation. Thus elevated level of these enzymatic variables clearly indicated the cellular leakage and loss of the functional integrity of the cell membranes in liver. With the treatment of active principle significant recoupment was observed when compared with the crude extract of *Terminalia belerica*. CCl_4 caused decrease in the activity of alkaline phosphatase whereas acid phosphatase activity increased significantly in liver and kidney. Active principle was found to be significantly effective in both the organs (Table 3). Appreciable fall was observed in glycogen content, activity of adenosine triphosphatase and succinic dehydrogenase after CCl_4 administration. Extract caused a marked reversal in the inhibitory effect of the enzymatic variables while active principle was found to be more effective and the values were very near to control in both the organs (Table 4). Toxicant exposure caused significant inhibition in the glycogen content in liver and kidney ($P \leq 0.05$). These parameters were significantly recovered by the therapy of active principle but extract therapy was not effective at this

TABLE 3: EFFECT OF TREATMENTS ON ACID AND ALKALINE PHOSPHATASE.

Treatments	Acid phosphatase (mg Pi/100 g/h)		Alkaline phosphatase (mg Pi/100 g/h)	
	Liver	Kidney	Liver	Kidney
Normal Control	234.6±16.3	267.6±18.3	76.2±5.42	2680±261
CCl_4	389.0±28.6*	369.6±19.0*	43.6±3.83*	1289±115*
CCl_4+Extract	277.0±14.7**	299.0±22.5**	55.6±4.19	1666±102**
CCl_4+AP	258.0±23.9**	292.0±21.3	60.0±4.00**	2209±131**
CCl_4+Sily	260.0±24.3**	271.0±23.0**	73.0±6.02**	2198±184**
One-way ANOVA df	6, 20	6, 20	6, 20	6, 20
F	9.33	4.84	9.79	12.61
P	<0.05	<0.05	<0.05	<0.05

AP=active principle, Sily=silymarin, Values are mean±SEM, n=5, *$P \leq 0.05$, when compared with control, **$P \leq 0.05$, when compared with CCl_4-treated group.

level (Table 5). There was a significant increase in lipid peroxidation on the contrary decrease was observed in the glutathione content after CCl_4administration. Active principle and extract were effective in recouping these variables significantly (Table 6). The protective effects of these therapeutic agents were also compared with silymarin treated animals, which is used as a reference drug.

DISCUSSION

The results of the present study clearly demonstrate that the various biochemical alterations produced by CCl_4 in the serum and tissue were reversed significantly by the administration of the extract and active principle of *Terminalia belerica*. Results reveal that the administration of CCl_4 caused significant increase in the level of serum proteins, activity of serum alkaline phosphatase and

TABLE 4: EFFECT OF TREATMENTS ON ADENOSINE TRIPHOSPHATASE AND SUCCINIC DEHYDROGENASE

Treatments	Adenosine triphosphatase (mg Pi/100 g/h)		Succinic dehydrogenase (n mol $K_3Fe(CN)_6$/min/mg protein)	
	Liver	Kidney	Liver	Kidney
Normal Control	2008±138	2480±130	45.2±2.96	39.0±2.82
CCl_4	997±57.0*	1033±61.4*	28.0±2.00*	26.8±2.67*
CCl_4+Extract	1685±97.4**	1649±96.3**	35.8±3.36	35.0±2.44
CCl_4+AP	1947±100**	1809±120**	40.6±2.25**	37.8±2.65**
CCl_4+Sily	2099±127**	2200±121**	44.0±2.57**	38.8±3.02**
One-way ANOVA df	6, 20	6, 20	6, 20	6, 20
F	21.41	32.32	8.59	4.36
P	<0.05	<0.05	<0.05	<0.05

AP=active principle, Sily=silymarin, values are mean±SEM, n=5, *$P \leq 0.05$, when compared with control, **≤P0.05, when compared with CCl_4-treated group.

TABLE 5: EFFECT OF TREATMENTS ON GLYCOGEN CONTENT

Treatments	Liver glycogen (mg/100 g)	Kidney glycogen (mg/100 g)
Normal Control	2801±135	86.4±4.95
CCl_4	1548±120*	58.0±3.12*
CCl_4+Extract	1919±121	72.0±3.65**
CCl_4+AP	2203±155**	79.0±5.91**
CCl_4+Sily	2435±165**	76.2±4.70**
One-way ANOVA df	6, 20	6, 20
F	14.49	6.60
P	<0.05	<0.05

AP=active principle, Sily=silymarin, values are mean±SEM, n=5, *P≤0.05, when compared with control, **P≤0.05, when compared with CCl_4-treated group.

transaminases patterns. These findings are also supported by various authors[16-18].

Due to liver injury, the transport function of the hepatocytes gets disturbed, resulting in the leakage of serum alkaline phosphatase in plasma membrane thereby causing an elevation[19]. 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 release into circulation after cellular damage. It is generally accepted that the toxicity of CCl_4 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[20,21]. In the present study author has also observed a rise in the level of AST and ALT in CCl_4-treated rats. The recoupment seen with gallic acid may be due to the substantial antioxidant properties. It may combine with reactive metabolites and lead to inactivate them, which may suppress the intracellular concentration of free radicals produced by CCl_4. Thus it may prevent the acute organ dysfunction and cellular injury thereby inhibiting the rapid leakage of these enzymes. Recoupment with the administration of different plant preparations are reported, e.g. *Schisandra chiensis*[22], *Cappris spinosa*[23], *Withania somnifera*[24], *Andrographis paniculata*[25], *Emblica officinalis*[26], *Mallotus japonicus*[27].

It is also observed that CCl_4 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. CCl_4 brings about a rise in cytosolic free calcium, which may lead to glycogen mobilization, thus causing depletion in hepatic glycogen content[28]. It may be assumed that active principle being an antioxidant reduces the stress to a considerable extent thereby reducing the demand for the excess sugar and recouping the glycogen content.

TABLE 6: EFFECT OF TREATMENTS ON HEPATIC LIPID PEROXIDATION AND REDUCED GLUTATHIONE

Treatments	Hepatic lipid peroxidation (n mol of MDA/ mg protein)	Hepatic reduced glutathione (μ mol/g)
Normal Control	0.27±0.02	8.63±0.48
CCl_4	1.60±0.15*	3.70±0.20*
CCl_4+Extract	1.03±0.08**	5.20±0.31**
CCl_4+AP	0.58±0.04**	6.30±0.52**
CCl_4+Sily	0.35±0.03**	7.00±0.62**
One-way ANOVA df	6, 20	6, 20
F	57.10	20.86
P	<0.05	<0.05

AP=active principle, Sily=silymarin, values are mean±SEM, n=5, *P≤0.05, when compared with control, **P≤0.05, when compared with CCl_4-treated group.

CCl_4 also caused significant increase in the activity of acid phosphatases. It 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 thus increases the activity of acid phosphatase[29]. Extract may possess anti-inflammatory and lysosomal stability properties and obstructs the rise in the enzymatic activity. The increase in the activity of the lysosomal enzymes, in the organs after treatment with the CCl_4 suggested increased tissue catabolism and autophagy, which are possible sequences leading to renal damage[30]. Alkaline phosphatase has been reported to be involved in the transport of metabolites across the cell membranes, synthesis of protein and 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.

Adenosine triphosphatase is lipid dependent membrane bound enzyme any alteration in membrane lipid leads to change in membrane fluidity, which in turn alters the adenosine triphosphatase, mediated cellular functions. In the present investigation, significant fall was observed in the enzyme activity on CCl_4 exposure. This inhibition of adenosine triphosphatase by CCl_4 exposure has also been confirmed in other studies[28,31]. Therapy of active principle showed significant improvement in the activity of enzyme. Succinic dehydrogenase is a mitochondrial enzyme tightly bound to the inner mitochondrial membrane. It plays an important role in energy conversion. A significant fall in the succinic dehydrogenase activity could result in serious impairment of mitochondrial function and metabolic turnover. This may be due to the structural and functional disorganization of the mitochondrial assembly. Active principle may possibly play a role in retaining the impairment of mitochondrial function.

Considerable lowering of reduced glutathione 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[32-35] 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 active principle has antioxidant property, it may prevent free radical generation thereby reducing oxidative stress. Administration of active principle 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. Here the role of TB extract and its active principle in reversing the elimination of hydrogen peroxide free radicals may be visualized as a form of adaptation on the part of GSH dependent defense system against lipid peroxidation. It also appears that certain constituents of *Andrographis paniculata* prevented liver membrane from undergoing lipid peroxidation and showed improvement in the reduced glutathione level[25].

Thus in the case of beneficial effect of the therapeutic agents three types of possible interventions are expected. (I) The chemical ingredients in the extracts have membrane stabilizing effect. (II) Gallic acid may possess the ability to block the bioactivation of CCl_4 by inhibiting P_{450}2E1 activity and its expression. (III) Due to the presence of -OH and -COOH groups GA may directly combine with free radicals ($^{\bullet}CCl_3$) and form free radical adduct. Thus it may be concluded that extract and active principle possess protective potential but effect of active principle was more pronounced in ameliorating CCl_4-induced toxicity.

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REFERENCES

1. Nadkarni, A.K., In; Indian Materia Medica, 3rd Edn., Vol. I, Dhoota Papeshwar Prakashan, Mumbai, 1954, 1202.
2. Chakravarti, M.D. and Tayal, J.N., **Sci. Cult.,** 1947, 13, 122.
3. Siddiqui, H.N., **Indian J. Pharmacol.,** 1963, 25, 297.
4. Sharma, A.K., Anand, K.K., Pushpangadan, P., Chandan, B.K., Chopra, C.L., Prabhakar, Y.S. and Damodaran, N.P., **J. Ethnopharmacol.,** 1989, 25, 93.
5. Anand, K.K., Singh, B., Saxena, A.K., Chandan, B.K., Gupta, V.N. and Bhardwaj V., **Pharmacol. Res.,** 1997, 36, 315.
6. Fiske, C.H. and Subbarow, Y., **J. Biol. Chem.,** 1925, 66, 375.
7. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J., **J. Biol. Chem.,** 1951, 193, 265.
8. Reitman, S. and Frankel, S.A., **Amer. J. Clin. Pathol.,** 1957, 28, 56.
9. Seifter, S., Dayton, S., Novic, B. and Muintwyler, E., **Arch. Biochem.,** 1950, 25, 151.
10. Sharma, S.K. and Krishnamurthy, C.R., **J. Neurochem.,** 1968, 15, 147.
11. Brehe, J.E. and Burch, H.B., **Anal. Biochem.,** 1976, 74, 189.
12. Hawk P.B., Oster, B.L. and Summerson, W.H., In: The Practical Physiological Chemistry, 14th Edn., Mc Graw Hill Book Company, New York, 1984, 1123.
13. Seth, P.K. and Tangari, K.K., **J. Pharm. Pharmacol.,** 1966, 18, 831.
14. Slatter, E.C. and Bonner, W.D., **Biochem. J.,** 1952, 82, 185.
15. Snedecor, G.W. and Cochran, W.G., In: Statistical Method, 8th Edn. Affiliated East-West Press, 1994.
16. Balasubramaniam, P., Pari, L. and Menon, V.P., **Phytother. Res.,** 1998, 12, 434.
17. Chung, M.H., Ob, H.S. and Lin, J.H., **Korean J. Pharmacol.,** 1998, 29, 402.
18. Sharma, A., Mathur, R. and Shukla, S., **Indian Drugs.,** 1994, 2, 120.
19. Jafri, M.A., Jalis, M., Javed, K.S. and Singh, S., **J. Ethnopharmacol.,** 1999, 66, 355.
20. Zafar, R. and Ali, S.M., **J. Ethnopharmacol.,** 1998, 63, 227.
21. Ahmed, B., Alam, T. and Khan, S.A., **J. Ethnopharmacol.,** 2001, 76, 187.

22. Zhu, M., Lin, K.F., Yeung, R.Y. and Li, R.C., **J. Ethnopharmacol.,** 1999, 67, 61.
23. Gadgoli, C. and Mishra, S.H., **J. Ethnopharmacol.,** 1999, 66, 187.
24. Rasool, M.K., Latha, L.M. and Varalakshmi, P., **Pharm. Pharmacol. Commun.,** 2000, 6, 187.
25. Trivedi, N. and Rawal, U.M., **Indian J. Pharmacol.,** 2000, 32, 288.
26. Jose, J.K. and Kuttan, R., **J. Ethnopharmacol.,** 2000, 72, 135.
27. Lim, H.K., Kim, H.S., Choi, H.S., Oh, S. and Cho, J., **J. Ethnopharmacol.,** 2000, 72, 469.
28. Rastogi, S. and Rana, S.V.S., **Indian J. Exp. Biol.,** 1990, 28, 794.
29. Bhadauria, M., Jadon, A., Sharma, A. and Shukla, S., **Indian J. Exp. Biol.,** 2002. 40, 1254.
30. Abraham, P. and Wilfred, G., **Indian J. Pharmacol.,** 2000, 32, 250.
31. Kim, H.J., Bruckner J.V., Dallas, C.E. and Gallo J.M., **Toxicol. Applied Pharmacol.,** 1990, 102, 50.
32. Tripathi, Y.B. and Pandey, E., **Indian J. Exp. Biol.,** 1999, 37, 567.
33. Shenoy, A. and Bairy, K.L., **Indian J. Pharmacol.,** 1999, 31, 79.
34. Lin, C.C., Yen, M.H., Lin, J.M. and Lo, T.S., **J. Ethnopharmacol.,** 1998, 60, 9.
35. Vijayapadma, V., Saju, V., Devi, S. and Prema, C.S., **Fitoterapia.,** 1998, 69, 520.