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Research Article

HEPATOPROTECTIVE ACTIVITY OF SILYMARIN IN CARBONTETRACHLORIDE (CCL4)–INTOXICATED RATS

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ABSTRACT

Hepatoprotective property of silymarin at graded doses (40, 60 and 80 mg/kg body weight) was investigated using the model of CCl_4-induction in wistar strain of albino rats. Significant ($p<0.05$) rise in the serum levels of transaminases (alanine and aspartate amino transaminases), alkaline phosphatase, total bilirubin and lipid peroxide values were observed in the liver of rats group treated with CCl_4. However, there were sharp decrease in the serum activity of the transaminases, alkaline phosphatase, total bilirubin and peroxide values on the administration of silymarin at different doses and a concomitant increase in the serum levels of albumin, vitamin C and catalase activity significantly ($p<0.05$) different from experimental group treated with CCl_4 only. Balanced in the serum equilibrium levels in all the groups of experimental rats treated simultaneously with CCl_4 and silymarin to that effects, similar to what were experienced in liquid paraffin administered (at 1ml/kg body weight) (control) group. This observation attest to the fact that, silymarin though in its lowest doses could still retain its hepatoprotective and therapeutic potential on the liver against exogenous toxic onslaught.

INTRODUCTION

Medicinal plants and its derivatives are increasingly valued in the areas of alleviating ailments, providing sucour for eternal health, longevity, relieving pains and comfort (Suruchi *et al* 2013). Free radicals have been implicated in cellular injury through covalent binding and lipid peroxidation being major routes of propagation. Free radicals scavengers are electrons donating agents and are known to quicking the regeneration of liver cells from various cellular injury (Singh *et al*., 2009 and Ahn *et al*., 2014). Natural products of plants origin and their purified compounds have received much attention as an alternative solution to numerous health challenges and as well, a preferred agents recently that can withstand the growing trend of these health complications (Mates *et al*., 2009). Their antioxidant potentials have been shown to inhibit the initiation and generation of free radicals with maximum protection on the liver against hepatocellular damage (Manubolu *et al*., 2014). Antioxidants and free radical scavengers have been employed to study the mechanism of carbon tetrachloride (CCl_4)- toxicity and protect the liver cells against CCl_4-induced damaged done through termination of lipid peroxidation reaction (Huo *et al*., 2011).

Silymarin is a polyphenol flavonoids, isolated from *Silybum marianum* commonly known as milk thistle in the *Asteraceae* (*Compositae*) and one of the largest families of plants (Bisset, 1994; Tumova *et al*., 2010**).** It is a potent hepatoprotective agent, used as supportive treatment against liver diseases of different etiologies. Silymarin scavenges reactive oxygen species (ROS) and converts them into less reactive and harmless compounds. It boost the *in vivo* antioxidants (glutathione, superoxide dismutase) activities, ensure their constant levels and conform them to their biological roles (El-Shitany *et al*., 2008; Psotova *et al*., 2002; Nencini *et al*., 2007).

Hepatoprotective potential of silymarin have been demonstrated and documented by several researchers at various time, with different models (Hiroshi *et al*., 1984; Dehmlow *et al*., 1996; Flora *et al*., 1998; Pradhan and Girish, 2006; Hassan *et al*., 2015). This study aimed at establishing and affirming the hepatoprotective potential of silymarin at lower doses by employing CCl_4 –induced hepatoprotective model in *wistar* strain of experimental rats.

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MATERIAL AND METHODS

Chemicals

All chemicals used were of analytical grade.

Animals

Twenty five (25) male of *wistar* albino rats (*Rattusnorvegicus*) weighing 150–190g were obtained from animal house, Department of Biochemistry, Kogi State University, Anyigba, Nigeria. The animals were kept in wire meshed cages with beddings, which were adequately changed throughout the period of work. The animals were acclimatized for two weeks in standard laboratory conditions and maintained on standard animal feeds and clean tap water *ad libitum*.

Experimental Design

Carbon tetrachloride induction of hepatotoxicity was done according to reported procedures of Guntupalli *et al*., (2006) and Hassan *et al*., (2010). After acclimatization, the animals were randomly divided into five (5) groups containing five animals in each group with adequate matching of weight. The experimental design is as follows;

- ***Group I:*** (normal control untreated rats) - received a daily dose of liquid paraffin for five days (1 ml/kg body weight).
- ***Group II:*** (Induced control) were administered 30% carbon tetrachloride (CCl_4) in liquid paraffin daily for five days (1 ml/kg body weight, intraperitoneally).
- ***Group III:*** received silymarin orally at a dose of 40mg/kg body weight from the first day to the fifth day, and CCl_4 as group II from the second day to the fifth day, simultaneously.
- ***Group IV:*** received silymarin orally at a dose of 60mg/kg body weight from the first day to the fifth day, and CCl_4 as group II from the second day to the fifth day, simultaneously.
- ***Group V:*** received silymarin orally at a dose of 80mg/kg body weight from the first day to the fifth day, and CCl_4 as group II from the second day to the fifth day, simultaneously .The experimental animals were sacrificed on the sixth day.

The experimental animals were made unconscious by anaesthetizing them with diethyl ether. This was done by placing them in a glass desiccators containing cotton wool soaked in diethyl ether solution. The organ of interest (liver) was removed and perfused with 0.9% normal saline to completely remove all the red blood cells.

Evaluation of hepatoprotective activity

A section of the liver was weighed, suspended in the ratio 1g: 10ml of 0.1M phosphate buffer (pH 7.4), homogenized using an automated homogenizer and centrifuged to obtain post mitochondrial supernatant for the determination of biochemical parameters, enzymic and non enzymic antioxidants.

Alanine aminotransferase (ALT) and Aspartate aminotranseferase (ASAT) Estimations

Reitman and Frankel (1957) technique was employed.

Alkaline Phosphatase (ALP) Estimation

Colorimetric method by Sood (1999) was employed in the estimation.

Albumin and Total bilirubin

Doumas *et al.* (1971) and Kaplan *et al*.,(1984) were employed respectively for the determination

Vitamin C Estimation

Method of Baker and Frank (1968) was adopted.

Catalase Activity

Was determined using Beers and Sizer (1952) method.

Lipid peroxide level

Hartman (1983); Abubakar *et al*., (2004) methods were adopted.

Statistical analysis

Data are expressed as mean ± standard error of mean. Results were analyzed statistically by one-way Analysis of Variance (ANOVA), using Graph pad Instat, Bonferroni compare all columns (San Diego, USA). A value of $p< 0.05$ was considered statistically significant.

RESULTS

Rats intoxicated with carbontrachloride (CCl_4) showed elevated levels of activity of Alanine amino transferases (ALT), Aspartate amino transferases (AST), Alkaline phosphatase (ALP), Lipid peroxide value, serum total bilirubin (Table 1). However, Serum albumin, Vitamin C and Catalase were significantly ($p<0.05$) decreased in CCl_4- treated rats (Table 2).

Table 1 Effects of Silymarin on Liver Marker Indices in CCl_4–Induced Rats

Groups	ALT (µ/l)	AST (µ/l)	ALP(µ/l)	Albumin (g/dl)	TB (mg/dl)
Group I	3.40±0.22	2.91±044	24.84±3.38	3.84±0.41	6.75±1.65
Group II	$6.35±0.78^{a}$	$4.54±0.53^{a}$	$84.82±50.15^{a}$	$2.28±0.59^{a}$	$11.57±2.65^{a}$
Group III	$2.69±0.74^{bx}$	$3.01±0.39^{bx}$	$23.46±9.33^{bx}$	$3.52±0.21^{bx}$	$5.88±0.76^{bx}$
Group IV	$3.41±1.08^{bx}$	$3.18±0.60^{bx}$	$33.12±7.22^{bx}$	$3.72±0.11^{bx}$	$6.71±1.78^{bx}$
Group V	$3.32±0.65^{bx}$	$3.45±0.46^{by}$	$49.68±20.03^{bx}$	$4.14±0.32^{bx}$	$6.88±2.11^{bx}$

Results are expressed in mean ± SEM (n=5). a= significantly: ($p<0.05$) different vs I: b=non-significantly ($p<0.05$) different vs I; x= significantly ($p<0.05$) different vs II; y= not significantly ($p<0.05$) different vs II: using analysis of Variance (ANOVA). Bonferroni multiple comparison compare all pairs of columns using Instat Graph Software (San Diego, USA).

The extent of liver damage was evaluated by measuring the serum levels of ALT, AST, ALP, Albumin and Total bilirubin as shown below. Rats that were both administered silymarin (40, 60 and 80mg/kg) orally and CCl_4 intraperitoneally

concurrently, showed, significant ($p<0.05$) decreased levels in enzymes activity.

Table 2 Effects of Silymarin on Liver Enzymatic and Non-enzymatic Antioxidant levels in CCl_4 –Intoxicated *Wistar* Albino Rats.

Groups	Vitamin C (mg/dl)	Catalase (U/mg tissue)	Lipid peroxides (nmoles of MDA/g tissue)x10^{-5}
Group I	92.32±3.23	11.20±0.90	1.19±0.34
Group II	55.57±2.37^{a}	7.50±0.10^{a}	2.25±0.14^{a}
Group III	60.05±4.74^{xb}	7.93±0.09^{by}	1.83±0.11^{bx}
Group IV	70.81±4.99^{bx}	8.40±0.23^{by}	1.44±0.03^{bx}
Group V	81.56±4.74^{ax}	10.05±0.37^{bx}	1.19±0.23^{ax}

Values are presented in mean ± SEM at $p<0.05$; number of replicate(n)=5; a=significant difference vs I: $p<0.05$; b=non-significant difference vs I. x=significant difference vs II and y= non-significant difference vs II, using analysis of variance (ANOVA), Bonferroni multiple comparison compare all pairs of columns Instat graph pad software (San Diego, USA).

There were elevated levels in liver marker indices upon administration of CCl_4 only on CCl_4- treated group. Mild activity of these endogenous proteins in silymarin and CCl_4 co-administered groups were recorded, prominent at a higher doses of silymarin, a standard hepatoprotective agent.

CCl_4-mediated acute hepatic damages in *wistar* strain of albino rats is taught to be through increase in the activities of the liver marker enzymes, total bilirubin and lipid period values (Hassan *et al*., 2010).

DISCUSSION

The effects of administration of silymarin at doses 40, 60 and 80 mg/kg *per os* and CCl_4 were felt on the activities of transaminases, serum albumin and total bilirubin levels *in vivo* (Table 1). Induction of liver damages initiated by the hepatotoxic model, CCl_4 results in an increased activities of both the enzymatic (catalase) and non-enzymatic (vitamin C) antioxidants species and extent of the damages incured to the membrane, an index of lipid peroxidation (Table 2). It is obvious as seen in the results presented above that, administration of silymarin to CCl_4 treated animals reduced these sudden and continous elevation of these parameters produced by CCl_4.

The most important markers of liver damages are the increased activities of ALT, AST, ALP and DBL and are regarded as conventional indicators of liver injury (Achiliya *et al*., 2004). It is probable that, decreased in the amount of serum albumin is as a result of the free radicals generated under condition of severe oxidative stress leading to the modification and ultimately affects the biological activity of these proteins, particularly albumin. The amino acids sequence of this protein may be damaged completely via specific interaction of oxidants (Narasimhanaidu and Ponnaian, 2006).

Catalase, an enzymatic antioxidant widely distributed in all animal tissues, with the highest activity in the liver, decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Gaetani *et al*., 1996). However, our findings of a significant ($p<0.05$) decrease in both catalase and vitamin C levels (a strong non-enzymic antioxidant) in the liver of rats treated with CCl_4 suggest a compromised function and a depletion of physiological protective moieties (Narasimhanaidu and Ponnaian, 2006).

The hepatotoxic effects of CCl_4 are mostly due to its active metabolite, trichloromethyl radical produced to initiate and propagate free radicals in the system (Johnson and Kroening, 1998). These radicals are activated, bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (PUFAs). This leads to the formation of lipid peroxides. This lipid peroxidative degeneration of biomembranes is one of the major causes of hepatotoxicity of CCl_4 (Kaplowitz *et al*., 2006). In this study, there were significant ($p<0.05$) increase in peroxide values in the liver of rats administered with CCl_4. Elevated values of lipid peroxides signifies lipid peroxidation leading to hepatocellular damage and an adverse failure of antioxidant defense system to inhibit and curtail the formation of excessive free radicals (Achiliya *et al*., 2004). The results generated in the present study conform to the postulation of Bakirel *et al*., (2008), that reactive oxygen species (ROS) react with all biological substances; with polyunsaturated fatty acids being the chief target, a major components of cell membrane, leading to lipid peroxidation.

References

Abubakar, M.G., Taylor A. and Ferns G.A., (2004). The effects of aluminum and Selenium Supplementation on Brain and Liver Antioxidant Status in Rats. School of Biomedical and Life Sciences, University of surrey, Guilford 7x h U.K.

Achiliya, G. S., Wadodkar, S. O. and Dorle, A. K. (2004). Evaluation of hepatoprotective effects of *Amakadi Ghrita* against Carbontetrachloride induced hepatic damage in rats. *Journal of Ethnopharmacology*. **90**:229-232.

Ahn, M., Park, J.S., Chae, S., Kim, S., Moon, C., Hyun, J.W., Shin,T. (2014). Hepatoprotective Effects of Lycium Chinese Miller fruit and its constituent betaine in CCl_4-induced hepatic Damage in Rats. *Acta Histochem*.**116:**1104–1112.

Baker, H. and Frank, O. (1968). Clinical Vitaminology, Method and interpretation. InterScience Publishers. New York. Pp:172-173.

Bakirel, T., Bakirel, U., Keles, O.U., Ulgen, S.G., Yardibi, H. (2008). *In vivo* Assessment of Anti-diabetic and Antioxidant Activities of Rosemary (*Rosariusofficialis*) in Alloxan-Induced Diabetic rabbits. *Journal of Ethnopharmacology*.**116** (1)**:** 64-73.

Beers R. F. and Sizer I.W. (1952). A spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase *J. Biol. Clem.* **195**: 130-140.

Bisset, N.G. (1994). *Herbal Drugs and Phytopharmaceuticals*; CRC Press: Boca Raton. FL.USA. Pp. 121-123.

Dehmlow, C., Erhard, J., De Groot, H.(1996). Inhibition of Kupffer Cell Functions as an Explanation for the Hepatoprotective Properties of Silybinin. *Hepatology* **23**, 749-754.

Doumas, B.T., Watson W. and Briggs H.G. (1971). Albumin Standards, Formulation against CCl_4-induced Liver Injury and Survival in Rats. *J. of ethnopharmacol.* **56:**87-96.

El-Shitany, N.A., El-Haggar, S., El-Desoky, K. (2008). Silymarin prevents adriamycin-induced cardiotoxicity and nephrotoxicity in rats.*Food Chemical Toxicology*.***46*:** 2422-2428.

Flora, K., Hahn., M, Rosen, H., Benner, K. (1998). Milk Thistle (*Silybum marianu)* for the Therapy of Liver Disease. *American Journal of Gastroenterology* **93**, 139-143.

Gaetani, G., Ferraris, A.,Rolfo, M.,Mangerini, R., Arena, S., Kirkman, H. (1996)."Predominant Role of Catalase in the Disposal of Hydrogen Peroxide within Human Erythrocytes"*Blood*. **87**(4): 1595–9

Guntupalli, M.M.R., Chandana, V.R., Palpu, P. and Anine, S. (2006). Hepatoprotective effects of Rubiadin, a Major Constituent of *Rubia* cordifolia *Linn. J Ethnopharmacol.* **103**:484- 490.

Hartman, P. E., (1983). Putative Mutagens and Carcinogens in Foods, Iv. Malondialdehyde. *Environ.Mutagen*., **5** (4) 603-607.

Hassan S.H., Ayeni G., Wasagu R.S.U., Umar R.A., Agaie B.M., Farouk U.Z. (2015). Hepatoprotective Effect of Leaf Extracts of *Newbouldia laevis* on Carbontetrachloride- intoxicated Rats. *Journal of Global Biosciences.* **4**(5):2258-2281

Hassan S.W, Salawu,K, Hassan. L.G, Umar R.U, Fatihu M.Y. (2010). Hepatoprotective, Phytochemical and Antioxidant Properties of Leaf Extracts of *Newbouldia Laevis*. *International Journal of Pharm Tech Research*. CODEN (USA). Vol.2, No. 1, 573-584.

Hiroshi, H., Yoshinobu, K., Wagner, H., Manfred, F. (1984). Antihepatotoxic Actions of Flavonolignans from *Silybum marianum* fruits. *Planta Medica* **52**, 248-250.

Huo, H.Z., Wang, B., Liang, Y.K., Bao, Y.Y., Gu, Y. (2011). Hepatoprotective and Antioxidant Effects of Licorice Extract against CCl_4-induced Oxidative Damage in Rats. *International Journal of Molecular Science.***12**:6529–6543.

Johnson, D.E., Kroening, C. (1998). Mechanism of Early CCl_4 Toxicity in Cultured Rat Hepatocytes. *Pharmacology toxicology.* **83:**231-239.

Kaplan, A., Rubalteli, F.F., Hammerman, C., Vilie, M.T., Leiter, C., and Abramov, A. (1984). Bilirubin. *Clin. chem.* The C.V Mosby Co. St.Louis. Toronto Priceton. Pp: 436-650 and 1238-1241.

Kaplowitz, N., Aw, T.Y., Simon, F.R., Stolz, A. (2006). Drug Induced Hepatotoxicity. *Annual International Medicine*.**104:**826-839.

Manubolu, M., Goodla, L., Ravilla, S., Thanasekaran, J., Dutta, P., Malmlöf, K., Obulum, V.R. (2014). Protective Effect of Actiniopterisradiata (Sw.) against CCl_4 Induced Oxidative Stress in Albino Rats. *Journal of Ethnopharmacology*.**153**. 744–752.

Mates, J. M., Segura, J.A., Alonso, F.J.,Márquez, J. (2009). Natural Antioxidants:Therapeutic Prospects for Cancer and Neurological Diseases. *Mini Review of Medical Chemistry*.**9:** 1202–1214.

Narasimhanaidu, K. and Ponnaian, S.M.P. (2006). Antihyperglycaemic and Antioxidant Effect of Rutin, a Polyphenolic flavonoids in *streptozotocin*-induced Diabetic *Wistar* rats. *Journal of Basic and Clinical Pharmacology and Toxicology.* **98**:97-103.

Nencini, C.,Giorgi, G.,Micheli, L. (2007). Protective effect of Silymarin on Oxidative Stress in Rat Brain. *Phytomedicine.***14**. 129-135.

Psotova, J., Chlopcikova, S.,Grambal, F.,Simanek, V.,Ulrichova, J. (2002). Influence of Silymarin and its Flavonolignans on Doxorubicin-iron Induced Lipid Peroxidation in Rat Heart Microsomes and Mitochondria in Comparison with Quercetin. *Phytother.Res.***16:**63-67.

Pradhan, S.C., and Girish, C. (2006). Hepatoprotective Herbal Drug, Silymarin from Experimental Pharmacology to Clinical Medicine. *Indian J Med Res* **124**, 491-504.

Reitman, S. and Frankel, S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxaloacetic and Glutamic pyruvic Transaminases. *Amer. J. chlin. Pathol.* **28**: 56-63.

Singh, D., Singh, R., Singh, P., Gupta, R.S. (2009). Effects of Embelin on Llipid Peroxidation and Free Radical Scavenging Activity against Liver Damage in Rats. *Basic Clinical Pharmacology Toxicology.***105**. 243–248.

Sood, R., (1999): Medical Laboratory Technology, Method and Interpretation, 5ed. Jaypee Brothers Medical Publishers Ltd., Pp: 488-490.

Suruchi Singh, Maryam Bincy Thomas, Sharada Pal Singh, Bhowmik. D. (2013). Plants Used in Hepatoprotective Remedies in Traditional Indian Medicine. *Indian Journal of Research in Pharmacy and Biotechnology*. Volume 1 Issue 1. P 58.

Tůmová, L.,Tůma, J., Megušar, K., Doležal, M. (2010). Substituted Pyrazinecarboxamides as Abiotic Elicitors of Flavolignan Production in *Silybum marianum* (L.) Gaertn cultures *in vitro.Molecules* **15**.331-340.

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