www.sphinxsai.com

PharmTech
International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 573-584, Jan-Mar 2010

Hepatoprotective, Antioxidant and Phytochemical Properties of Leaf Extracts of *Newbouldia Laevies*

Hassan S.W.*[1], Salawu, K.[1], Ladan M.J.[1] ,Hassan, L.G.[2], Umar, R.A.[1] and Fatihu, M.Y.[3]

[1]Department of Biochemistry, [2]Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

[3]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

Corresponding author e-mail: hassanwara@yahoo.com

Abstract: Hepatoprotective effect and antioxidant properties of leaf extracts of *Newbouldia laevies* (NL) were investigated against carbon tetrachloride (CCl_4)-induced acute hepatic injury in rats. Phytochemical constituents were also evaluated. Significantly ($p<0.05$) increased levels of transaminases, alkaline phosphatase, albumin, bilirubin and cholesterol in CCl_4 intoxicated rats were brought to normal levels in rats treated with the extract and CCl_4 in a dose dependant manner. Reduced enzymic and non enzymic antioxidants levels and elevated lipid peroxides levels were restored to normal by administration of the extract at 300 mg/kg. Histopathological examination further confirmed the hepatoprotective activity of NL when compared with CCl_4 treated group. Phenolics, glycosides, balsams, resins and volatile oils were detected in the extract. The lethal dose (LD_{50}) of the plant was greater than 3000 mg/kg. The study suggests that CCl_4-induced damage in rats can be ameliorated by treatment with the leaf extracts of *Newbouldia laevies*.
Keywords: Hepatoprotective effect, *Newbouldia laevies* , antioxidant properties, lipid peroxidation.

Introduction

Scientific validations are being made globally to get evidences for traditionally used herbal plants. There still exist a large number of tropical trees with tremendous medicinal potentials but with no empirical proof to support claims of efficacy. Liver diseases are largely attributable to the increasing number of chemical compounds and environmental pollution[1]. Free radicals or oxidative injury are critically involved in various pathological conditions such as cancer, neurological disorder, arthritis, inflammation and liver diseases[2,3]. However, there are still no specific treatments in modern medicine that give protection to the liver against damage or help to regenerate hepatic cells[4,5]. Because of this fact efforts are being made to find suitable curative agents for the treatment of liver diseases in natural plants products[6]. Chemicals and drugs such as CCl_4 and paracetamol catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causing the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes in to the blood[7]. Thus, CCl_4 was chosen in this study as model for investigating radical-induced damage and its prevention in wistar albino rats.

A large number of medicinal plants have been found to offer some hepatoprotection[1,8,9,10,11,12]. Flavonoids and other phenolics of plant origin have been reported to have roles as scavengers and inhibitors of lipid peroxidation[13,14] .

Newbouldia laevies (family Bignoniaceae) is distributed mainly in the tropics and forms an important part of the vegetation. Bignoniaceae is of little economic value. Some members such as *Catalpa* and *Tecoma* sp. yield timber and are also cultivated for ornament[15] . In Nigeria and Ghana, the bark and leaves of *Newbouldia laevies* are used for the treatment of breast cancers[16]. Previously, it has been reported that the extracts of the leaf[17] , stem bark[18] and root[19] have strong antibacterial activities. Azuine reported that *Newbouldia laevies* possess strong antineoplastic activity and provides credence to the herbal use of the

plant for breast cancer treatment[20]. Ethanolic leaf extract of the plant has been shown to have antimalarial activity in *Plasmodium falciparum in vitro* model[21] .

To the best of our knowledge, there is no scientific report available in support of the hepatoprotective activity of *Newbouldia laevies* against CCl_4-induced hepatotoxicity in rats. The hepatoprotective activity of the plant reported in this study would provide scientific evidence of its claimed medicinal properties.

Materials and methods

Chemicals

All chemicals used were of analytical grade.

Plant material

The leaves of *Newbouldia laevies* were collected from Ngaski local government, Kebbi state, Nigeria. The plant was botanically authenticated at the Herbarium, Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen of the plant was deposited for reference. This research was conducted between June and July 2008 at Usmanu Danfodiyo University, Sokoto, Nigeria.

Preparation of plant extract

The leaves were open-air-dried under the shade, cut in to small pieces and pulverized in to coarse powder (using wooden pestle and mortar) and stored until required for use. Two hundred grams (200 g) of the powdered leaves were extracted with two liters of 50 % ethanol-water (2000 ml of 1:1) at room temperature for 24 hours. The extract was filtered with Whatman filter paper (No.1) and concentrated under reduced pressure. The yield of the extract was 14.16 % (w/w).

Animals

Wister strains of albino rats of either sex weighing 200-300g were purchased from animal house, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. They were kept in wire mesh cages in a well ventilated room, allowed access to free food and water and kept for one week to acclimatize. The animals were maintained on standard animal feeds (Bendel feeds and flour mills, Edo state, Nigeria) and clean tap water *ad libitum*, before and after daily administration of the plant extract between 10.30 to 11.30 h. Experiment was performed according to ethical guidelines[22] .

Experimental procedure

Carbon tetrachloride induction of hepatotoxicity was done according to reported procedures of Guntupalli[23] with some modifications. Animals were randomly divided into six groups of six animals. Group 1 (normal control untreated rats) received a daily dose of liquid paraffin for five days (1 ml/kg body weight, *per os*). Group II (Induction control) were administered 30 % carbon tetrachloride in liquid paraffin from day two to five (1 ml/kg body weight, intra peritoneal). Group III received CCl_4 and Silymarin, a known antihepatotoxic drug at a dose of 100mg/kg *per os*, from the second to the fifth day. Groups IV, V and VI (test groups) were treated with CCl_4 and a daily dose of 100, 200 and 300 mg/kg body weight (orally) of leaves of *Newbouldia laevies* respectively, from the second to the fifth day. All the animals were sacrificed on the 6^{th} day. The blood collected was allowed to clot for 30 minutes. Serum was separated by centrifuging at 37^0C for estimation of biochemical parameters. A section of the liver tissue was quickly removed and fixed in 10% formalin for histopathological study.

Assessment of hepatoprotective activity

The activities of serum aspartate and alanine transaminases were assayed by the method (Randox assay kit) of Reitman and Frankel[24] . Alkaline phosphatase (ALP), albumin, total protein, cholesterol and serum total bilirubin were estimated by the methods of Sood[25] , Bigg[26] , Gornall[27] , Allain[28] and Varley[29] respectively. All these parameters were used to assess the acute hepatic damage caused by CCl_4.

Assessment of antioxidant activity

Another section of the liver was perfused with 0.86 % cold saline to completely remove all the red blood cells. It was then suspended in 10 % (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) and cut into small pieces. The required amount was weighed, homogenized using a Teflon homogenizer and centrifuged to obtain post mitochondrial supernatant for estimation of enzymic and non enzymic antioxidants such as vitamin C[30, 31] , reduced glutathione[32], catalase[33] and lipid peroxide level[34] .

Phytochemical analysis.

The methods of Harborne[35] , Sofowora[36] and EL-Olemyl[37] were employed.

Acute toxicity studies (Determination of LD_{50})

Aqueous ethanolic extract of *Newbouldia laevies* (3000mg/kg body weight) was administered orally to five groups of rats (one after the other at a grace of 48hours) in a single dose using a feeding needle. The control group received distilled water. Observation of toxic symptoms was made and recorded systematically one, two, four and six hours after administration.

Finally, the number of survivors was noted after 48 hours for each group of animals. The toxicological effect was assessed on the basis of mortality and expressed as LD_{50} and calculated using the limit test dose, up and down procedure of Organization for Economic and Cultural Development[38]

Statistical analysis

The data are expressed as mean ± standard deviation. Results were analysed statistically by one-way Analysis of variance (Anova), using Graph pad Instat, Benferoni compare all columns (San Diego, USA). A value of $P < 0.05$ was considered statistically significant.

Results

Groups of rats treated with carbon tetrachloride exhibited significant ($P < 0.05$) increase in the activity of alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), serum total bilirubin (TB) and peroxide value (PV) when compared to normal control rats (Table 1). However, serum total protein (TP), albumin (AB), vitamins C and E levels were significantly ($P<0.05$) reduced in carbon tetrachloride treated rats (Table 2).

The liver damage was evaluated by the measurement of serum levels of ALP, AST, ALT, TB, AB and TP. Rats that were orally administered leaf extracts of *Newbouldia laevies* (100, 200 and 300 mg kg^{-1}) and CCl_4 and the standard silymarin and CCl_4 showed significant ($P< 0.05$) decrease in serum enzymes activities, TB, and peroxide value. Increase in the levels of total protein, albumin, vitamins C and E were also observed compared to CCl_4 treated group (Table 1 and 2). Alkaloids, saponins, glycosides, volatile oils, flavonoid aglycones, flavonoid glycosides, saponin glycosides, anthraquinones, balsams, resins, triterpenoids and terpenoids were detected in the leaf extracts (Table 3). The lethal dose (LD_{50}) of *Newbouldia laevies* leaves extract determined was greater than 3000mg /kg in rats.

The CCl_4 induced acute hepatic injury in rats was manifested by the significant increase in the serum enzyme activities, total bilirubin and peroxide values.

Discussion

Acute toxicity test at 3000mg/kg of leaf extracts of *Newbouldia laevies* produced no mortality after 48 hours of observation. The median lethal dosage (LD_{50}) of the aqueous leaves extract was greater than 3000 mg/kg body weight. The extract did not produce any grossly negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma. However, a reduction in body weights of the rats was observed. The reduction in weight may be due to reduced fluid and water intake, which may be secondary to feeling of fullness and loss of appetite after administration of the extract[39,40]. Despite the above side effects, the very high value of the LD_{50} indicated that the extract of *Newbouldia laevies* is practically non-toxic.

The effect of *Newbouldia laevies* extracts at three dose levels (100, 200 and 300 mg/kg, *per os* and CCl_4 on serum marker enzymes, total protein, albumin, and bilirubin in CCl_4-induced hepatic injury are shown in table 2. Hepatic injury induced by CCl_4 caused significant ($p < 0.05$) rise in ALT, AST, ALP, cholesterol and bilirubin, but decreases in levels of total proteins and albumin were observed. Administration of the plant extract and CCl_4 at three dose levels resulted in recovery as indicated by the decrease in the hitherto increase of the serum parameters, produced by CCl_4. The effect was almost similar to that produced by silymarin treatment.

Reduced activities of enzymatic and non-enzymatic antioxidants and elevated activity of lipid peroxidation were seen in the CCl_4 treated group, whereas the standard silymarin and CCl_4 and the extract and CCl_4 treated groups showed significant ($p < 0.05$) rise in antioxidants levels with reduction in lipid peroxidation levels when compared with the CCl_4 treated control group. From the results, it is clear that the leaf extracts showed dose-dependent hepatoprotective activity. It was shown that at 200mg/kg, the extract exhibited a relatively high protective action. However, the extract at 300mg/kg, exhibited a higher protective action than the standard drug, silymarin (100mg/kg).

Since the changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis[41], CCl_4 mediated hepatotoxicity was chosen as the experimental model. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which that have been disturbed by a hepatotoxin, is the index of its protective effects[42] . The hepatotoxicity induced by CCl_4 is due to its metabolite CCl_3, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage[24,43,44,45]. Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood[46] . Increased levels of ALT, AST, ALP, TBL and DBL are conventional indicators of liver injury[47] .

Under condition of severe oxidative stress, free radical generation leads to protein modification. Protein may be damaged directly by specific interaction of oxidants or free radicals with particularly

susceptible amino acids[48] . This may be the cause of decrease in total protein and indirectly affected the levels of albumin. Our results show significant ($p<0.05$) increase in cholesterol in the CCl_4 treated group. It has been shown that micro-viscosity of a membrane increase markedly with increases in cholesterol to phospholipids ratio thus leading to cellular rigidity[49] . Intoxication of rats with CCl_4 may have altered membrane structure and function as suggested by the increase in cholesterol level[50].

Antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 -induced hepatopathy[52] .
The body has an effective defence mechanism to prevent and neutralize free radicals-induced damage. This is accomplished by a set of endogeneous antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase. These enzymes constitute a mutually supportive team of defense against reactive oxygen species (ROS)[53] . In CCl_4 - induced hepatotoxicity, the balance between ROS production and antioxidant defenses may be lost hence oxidative stress results which, through a series of events deregulate cellular functions leading to hepatic necrosis. The reduced activities of catalase observed point out the hepatic damage in the rats administered with CCl_4. But the extract treated groups showed significant ($P<0.05$) increase in the level of these enzymes, which indicates the antioxidant activity of the plant extract.

Regarding non-enzymic antioxidants, reduced GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4[54] . In the present study, a decrease in hepatic tissue GSH level was observed in the CCl_4 treated groups. The increase in hepatic GSH level in the rats treated with the plant extract and CCl_4 may be due to *de novo* GSH synthesis or GSH regeneration. Our findings of a significant ($p<0.05$) decrease in the levels of vitamins C and E (strong non-enzymic antioxidant) in treated CCl_4 group when compared to the normal control and extract with CCl_4 treated group, was corroborated with the findings of Narasimhanaidu and Ponnaian[48], who show that under condition of severe oxidative stress, massive production of reactive species may also lead to the depletion of protective physiological moieties such as glutathione, vitamins C and E and ceruloplasmin in rats. Glutathione through its significant reducing power contributes to the recycling of other antioxidants such as vitamins C and E, that have become oxidized[54].

The level of lipids peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, significant ($p<0.05$) elevation of lipid peroxidation in the liver of rats treated with CCl_4 was observed. The increase in MDA levels in the liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals[47]. This is also in agreement with the findings of Moscrella[55] and Patrick-Iwuan[56] who established that high serum levels of MDA in patients with liver cirrhosis was correlated with lower serum levels of vitamin E. Treatment with *Newbouldia laevis* plant extract significantly reversed these changes.

Histopathological studies showed that CCl_4 caused fatty degeneration, necrosis and balloning degeneration of hepatocytes. Pre-treatment with *Newbouldia laevies* plant extracts exhibited protection, which confirmed the results of biochemical studies. The results of our study indicated that simultaneous treatment with the plant extracts protects the liver against CCl_4 - induced hepatotoxicity.

Presence of flavonoids and other phenolic compounds in this plant might have scarvenged the free radicals thus offering hepatoprotection. Flavonoids, phenolic acids and some terpenoids have been reported to possess antioxidant activities by different mechanisms[57]. Phytochemicals have also been reported to have medicinal uses[58]. This present study has provided an empirical basis for the use of *Newbouldia laevies* in traditional medicinal practices. Studies on structural elucidation of the active component(s) and mechanism of action of *Newbouldia laevies* are there fore recommended.

Table 1. Effect of leaf extracts of *Newbouldia laevies* on serum liver function indices in CCl_4 - induced acute hepatic injury in rats

GROUPS	ALAT(u/l)	ASAT(u/l)	T.P(g/dl)	ALB(g/dl)	TBL(µmol/l)	DBL(µmol/l)	ALP(u/l)	CHOL(mmol/l)
Group I	18.51±0.53	13.84±0.94	9.89±0.63	4.50±0.31	17.88±0.78	12.51±1.36	104.88±2.67	2.48±0.10
Group II	34.38±1.45	30.53±1.99$^{\alpha}$	6.94±0.36$^{\alpha}$	2.76±0.06$^{\alpha}$	23.42±1.00$^{\alpha}$	14.88±1.25$^{\alpha}$	321.54±4.78$^{\alpha}$	4.04±0.18$^{\alpha}$
Group III	17.05±0.87*$^{\beta}$	13.84±0.68*$^{\beta}$	10.12±0.23*$^{\beta}$	4.48±0.35*$^{\beta}$	14.08±0.96$^{\alpha\beta}$	10.17±0.27$^{\alpha\beta}$	139.38±2.11$^{\alpha\beta}$	2.68±0.13$^{\alpha\beta}$
Group IV	18.12±0.59*$^{\beta@}$	21.10±0.49$^{\alpha\beta\gamma}$	9.71±0.48*$^{\beta@}$	3.81±0.37$^{\alpha\beta\gamma}$	16.28±0.40$^{\alpha\beta\gamma}$	12.77±0.22*$^{\beta\gamma}$	193.20±1.59$^{\alpha\beta\gamma}$	2.85±0.11$^{\alpha\beta@}$
Group V	16.43±1.05$^{\alpha\beta@}$	12.75±0.99*$^{\beta@}$	10.14±0.62*$^{\beta@}$	4.55±0.28*$^{\beta@}$	14..59±0.76$^{\alpha\beta@}$	11.46±0.65*$^{\beta\gamma}$	128.62±6.32$^{\alpha\beta\gamma}$	2.40±0.12*$^{\beta\gamma}$
Group VI	16.33±0.95$^{\alpha\beta@}$	11.69±0.61$^{\alpha\beta\gamma}$	10.56±0.41*$^{\beta@}$	4.88±0.36*$\beta\gamma$	10.62±0.44$^{\alpha\beta\gamma}$	9.68±0.12$^{\alpha\beta@}$	107.64±2.67*$^{\beta\gamma}$	2.32±0.07*$^{\beta\gamma}$

Values are means ±standard error of the mean, n=6, Comparison were made between:
"a" - Group I vs II, III, IV, V and VI; "b" - Group II vs III, IV, V and VI; "c" - Group III vs IV, V, and VI, using Duncan's multiple comparison test.
ALAT= Alanine transaminase, ASAT= Aspartate transaminase, TP= Total Protein, ALB = Albumin, TBL = Total bilirubin, DBL= Direct bilirubin
ALP= Alkaline phosphatase, CHOL= Cholesterol.

α= represents statistical significance vs I: $p<0.05$
β = represents statistical significance vs II: $p<0.05$
γ = represents statistical significance vs III: $p<0.05$
*= represents non statistical significance vs I: $p>0.05$
@= represents non statistical significance vs III $p>0.05$

Table 2. Effect of leaf extracts of *Newbouldia laevies* on liver enzymatic and non-enzymatic antioxidant levels in CCl_4 -induced hepatic injury in rats.

Groups	Vitamin C (mg/dl)	Vitamin E(mg/dl)	Catalase(U/mg tissue)	Reduced glutathione (mg/100ml)	Lipid peroxides (nmoles of MDA/g tissue)x10^{-5}
Group I	69.58±4.67	4.90±0.30	1.18±0.03	218.59±26.32	0.176±0.04
Group II	26.34±0.83$^{\alpha}$	2.41±0.05$^{\alpha}$	0.63±0.03*	129.69±11.06$^{\alpha}$	1.232±0.02$^{\alpha}$
Group III	50.83±0.53$^{\alpha\beta}$	5.58±0.66*$^{\beta}$	1.43±0.21*$^{\beta}$	217.70±16.64*$^{\beta}$	0.138±0.05*$^{\beta}$
Group IV	44.83±4.03$^{\alpha\beta\gamma}$	7.46±0.64$^{\alpha\beta\gamma}$	1.72±0.01* $^{\beta@}$	260.65±12.30$^{\alpha\beta\gamma}$	0.203±0.04*$^{\beta\gamma}$
Group V	75.43±0.43$^{\alpha\beta\gamma}$	9.53±1.19 $^{\alpha\beta\gamma}$	2.11±0.19$^{\alpha\beta\gamma}$	269.62±11.93 $^{\alpha\beta\gamma}$	0.101±0.02$^{\alpha\beta@}$
Group VI	77.50±0.91$^{\alpha\beta\gamma}$	9.53±1.19 $^{\alpha\beta\gamma}$	2.63±0.41 $^{\alpha\beta\gamma}$	324.89±7.92 $^{\alpha\beta\gamma}$	0.087±0.04 $^{\alpha\beta\gamma}$

Values are means ± standard error of the mean; n=6, Comparisons were made between: "a"- group I vs II, III, IV, V, IV; "b"- Group II vs III, IV, V and VI; "c"- Group III vs IV, V, VI, using Duncan's multiple comparison test.

α =Represents statistical significance vs I: $p < 0.05$

β =Represents statistical significance vs II: $p < 0.05$

γ=Represents statistical significance vs III: $p < 0.05$

* =Represents non statistical significance vs I: $p > 0.05$

@ =Represents non statistical significance vs III: $p > 0.05$

Table 3. Phytochemical constituents of leaf extracts of *Newbouldia laevies*.

ALK	SAP	TAN	GLY	VLO	FLA	FLG	CGS	SAG	ATQ	BAL	RES	TRP	TER	STR	CYG
+++	+++	-	+++	+++	+++	+	-	+++	+++	+++	+++	+++	+++	-	-

-= Absence, + = Presence in trace amount, +++ = Presence, ALK = Alkaloids, SAP=Saponins, TAN=Tanins, GLY=Glycosides, VLO=Volatile oil, FLA=Flavonoid aglycones, FLG=Flavonoid glycosides, CGS=Cardiac glycosides, SAG=Saponin glycosides, ATQ=Anthraquinone, BAL=Balsams, RES=Resins, TRP=Triterpenoids, TER=Terpenoids,STR=Steroids, CYG=Cyanogenic glycosides.

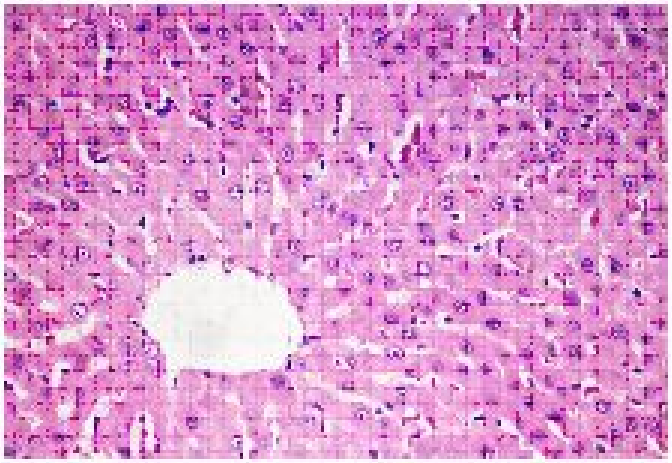

Fig.1. Liver sections of normal rat (control) administered with liquid paraffin (1ml/kg)showing no histopathological lesion.Hematotoxylin and Eosin (H & E)x400.

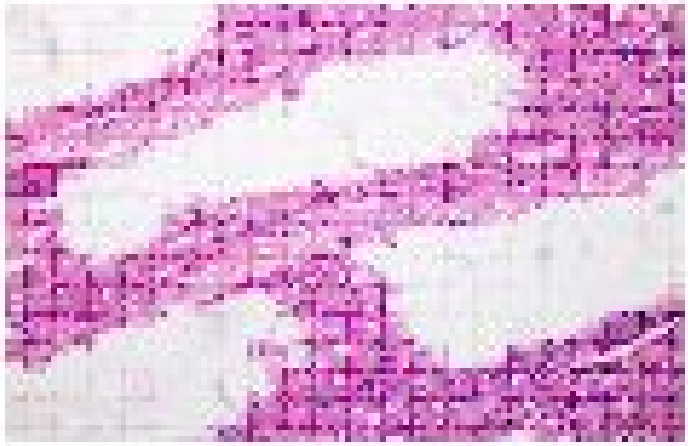

Fig. 2. Liver section of rat administered with CCl_4 (1ml/kg,i.p) showing diffuse severe peripheral necrosis, massive fatty change and ballooning degeneration of hepatocytes.Hematotoxylin and Eosin (H & E)x400.

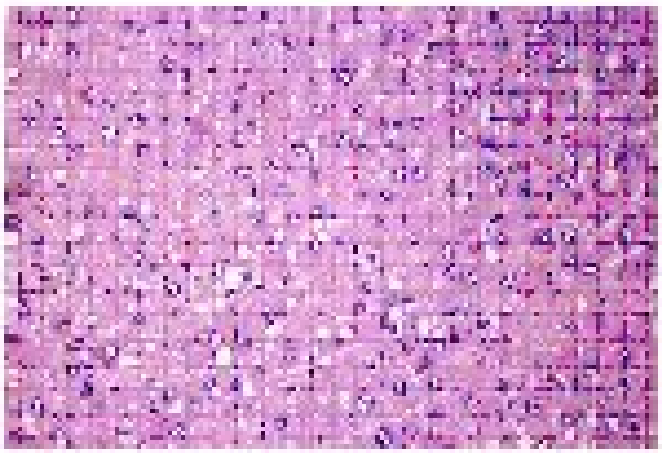

Fig. 3. Liver section of rats treated with Silymarin (100mg/kg,p.o.) + CCl_4 (1mn/kg, i.p.) X 6 days, showing very mild peripartal fatty change, ballooning degeneration and hepatocellular necrosis. Hematotoxylin and Eosin (H & E)x400.

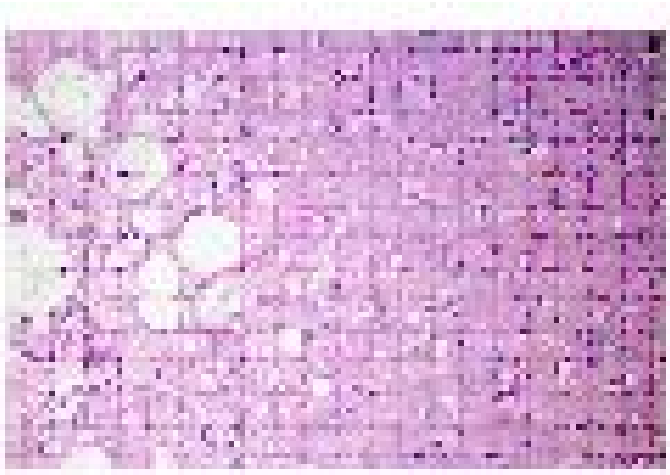

Fig.4. Liver section of rats treated with plant extract (100mg/kg. P.O)+CCl_4 (1ml/kg, i.p.) X 6 days, showing: fatty change, ballooning degeneration and peripheral hepatocellular necrosis. Hematotoxylin and Eosin (H & E)x400.

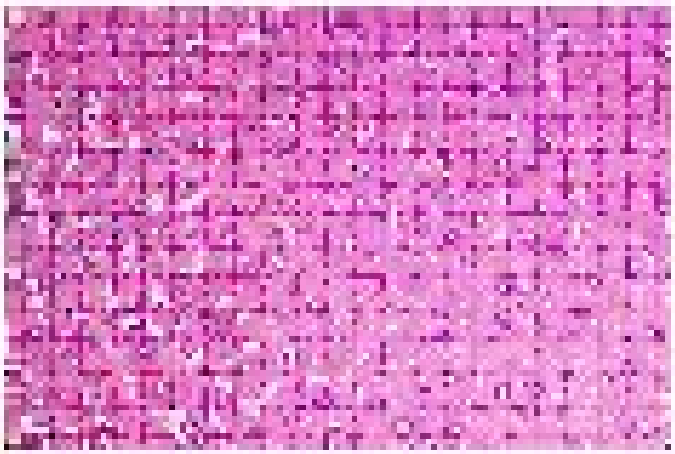

Fig. 5. Liver section of rats treated with plant extract (200mg/kg, p.o.) + CCl_4 (1ml/kg,i.p) x 6 days showing focal areas of fatty change and no significant lesion. Hematotoxylin and Eosin (H & E)x400.

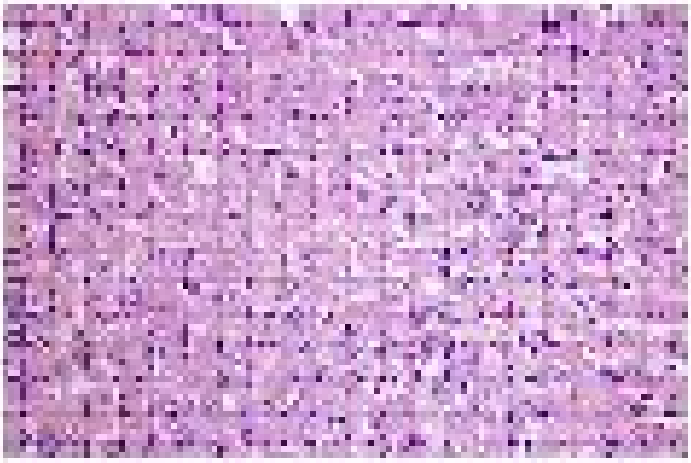

Fig. 6. Liver section of rats treated with plat extract (300mg/kg, p.o.)+CCl_4 (1ml/kg, i.p.) X 6 days, showing: very mild fatty change at portal areas but no significant lesion.Hematotoxylin and Eosin (H & E)x400

References

1. Raju R.W., Radhika S.S., Kunal Mahesh T., Kalpana S.P., Sunil S.J.,. Screening of roots of *Baliospermum montanum* for hepatoprotective activity against paracetamol- induced liver damage in albino rats. Int.. J. Green Pharm. October-December, 2008, 220-223.
2. Quambo X., Koji H., Yasuhiro T., Tadata T., Tsuneo N. and Shigetoshi K., Hepatoprotective activity of phenyl ethanoids from *Cistanches deserticola*. Planta med., 64, 1998, 120-125.
3. Atawodi S.E., Antioxidant potentials of African plants, Afr. J. Biotechnol. 4(2), 2005, 128-133.
4. Chatterjee T.K., Medicinal plants with hepatoprotective properties in herbal Opinions. Vol. III. Books and Allied (P) Ltd., Calcutta, 2000, 135.
5. Chattopadhyay R.R., Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract. Part II., J. Ethnopharmacol., 89 (2-3), 2003, 217- 219.
6. Bhandarkar M. and Khan A., Antihepatotoxic effect of *Numphaen stellata* wild against carbon tetrachloride hepatic damage in albino rats, J. Ethnopharmacol., 91, 2004, 61-64.
7. Singh B.,. Saxena A.K, Chandan B.K., Anand K.K, Suri O.P., Suri K.A. and Satti N.K., Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia., 59(2), 1998, 135-140.
8. Sibel K. and Canan K., The protective effects of *Achillea L*. species native in Turkey against H_2O_2 - induced oxidative damage in human erythrocytes and leucocytes. J. Ethnopharmacol., 102, 2005, 221- 227.
9. Kharpate S., Vadnerkar G., Deepti J. and Jain S., Evaluation of Hepatoprotective activity of ethanol extract of *Ptrospermum acerifolium* Ster Leaves, Indian J. Pharm Sci. November-December, 2007, 850-852.
10. Ramnik S. and Harwinder S.R., Hepatoprotective effect of the pulp/seed of *Aegle marmelos correa* ex Roxb against carbon tetrachloride induced liver damage in rats. October-December, 2008, 232-234.
11. Singal A. and Kumar V.I., Effect of aqueous suspension of dried latex of *Calotropis procera* on hepatorenal functions in rat, J. Ethnopharmacol., 122, 2009, 172- 174.
12. Sisodia S.S. and Bhatnagar M., Hepatoprotective activity of *Eugenia jambolana* Lam. in carbon tetrachloride treated rats. Indian J. Pharmacol. 41(1), 2009, 23-27.
13. Formica J.V. and Regelson W., Review of the biology of quercetin and related bioflavonoids. Food Chem. Toxicol., 33 (12), 1995, 1061-1080.
14. Rice-Evans, C., Miller N. and Paganga G., Antioxidant properties of phenolic compounds. Trends in Plant Sci., 2 (4), 1997, 154-159.
15. Ghazanfar S., Savannah plants. An illustrated Guide.Macmillan publishers Ltd. London, 1989, 157- 158.
16. Burkill H.N., The useful plants of west Tropical Africa. The white friars press limited, London.
17. Ogunlana E.O. and Ramstard E., Investigations into the antibacterial activities of local plants Planta Med., 72, 1975, 354-347.
18. Malcolm S.A. and Sofowora E.A., Antimicrobial activity of selected Nigerian folk remedies and their constituent plants. Lloydia 32, 1968, 512-517.
19. Le Grand A., Wondergem P.A., Verpoormte, R. and Pousset, J.L., Anti-infectious phytotherapies of the tree savannah of Senegal [West Africa] Antimicrobiol activity of 33 species. J. Ethnopharmacol., 22, 1988, 25 -31.
20. Azuine M.A., Ibrahim K., Enweren M., Gamaniel K. and Wambebe C.,Cytotoxic and anticancer activity of *Newbouldia laevies* in mice, West Afr. J. Pharmacol. Drug Res., 11, 1995, 72-79.
21. Gbeassor M., Kedijagni A.Y., Kovmaglo K., Despvza C., Agbo K., Aklikolou K. and Amegbo K. A., *In vitro* antimalarial activity of six medicinal plants. Phytother. Res., 43, 1990, 116-117.

22. Zimmerman, M. Z., Ethical guidelines for investigation of experimental pain in conscious animal. Pain, 16 (2), 1983, 109-110.

23. Guntupalli M.M.R., Chandana V.R., Palpu P. and Annie S., Hepatoprotective effects of *rubiadin*, a major constituent of *Rubia cordifolia Linn*. J. Ethnopharmacol., 103, 2006, 484-490.

24. Reitman S. and Frankel A.S., A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase, Am. J. Clin. Pathol., 28(1), 1957, 56-63.

25. Sood R., Medical Laboratory Technology, Methods and Interpretation. 5th ed.. Jaypee Brothers. Medical Publishers Ltd., New Delhi, 1999, 488-490.

26. Bigg H. G., Doumas, B.T. and Watson W.A., Clinical chemistry; A J. Clin. Chem., 31, 1971, 81.

27. Gornall A.G., Bardawill C.S. and David M.M., Determination of serum proteins by means of the biuret reaction, 1949, J. Biol. Chem., 177 (2), 1949, 751-766.

28. Allain C.C., Poon L.S., Chan C.S.G., Richmond W. and Fu P.C., Enzymatic Determination of total serum cholesterol, Clin. Chem., 20, 1974, 470-473.

29. Varley H., Gewenlock A.H. and Bell M., Practical Clinical Biochemistry. 5th Ed., CBS Publishers and Disytributors. Delhi, 1, 1991, 741-742.

30. Urbach C. Hickman K., and Harris P.L., Effects of individual vitamins A,C, E and carotene administered at high levels and their concentration in the blood. Exp. Med. and Surg., 10(1), 1951, 7-20.

31. Baker H.O., Frank B.A. and Feingold S., Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr. Rep. Int., 21(2), 1980, 531-536.

32. Patterson J.W. and Lazarow A., Methods of Biochemical analysis. Glick D. (ed.) New York, Inter Science. .2, 1955, 259.

33. Aebi H., Catalase in viitro methods in enzymology, 1984, 105, 121-126.

34. Hartman P.E., Putative mutagens and carcinogens in foods, iv.Malonaldehyde (Malondialdehyde).Environ Mutagen. 5(4), 1983, 603 - 607.

35. Harborne J. B., Phytochemical methods. A Guide to Modern Technique of plant Analysis, 3rd ed. Chapman & Hill, London, 1998, 285.

36. Sofowora A., Medicinal plants and traditional medicine. John Wiley and Sons, 1991, 66- 79.

37. El-Olemyl M.M., Al-Muhtadi F.J and Afifi A.A., Experimental Phytochemistry. A Laboratory Manual, College of Pharmacy, King Saud University. King Saud University Press, 1994, 1-134.

38. OECD, Guidelines for testing of chemicals. Acute oral toxicity. Up and Down Procedure, 425, 2001, 1-26.

39. Joseph P.K., Rao K.R. and Sundaresh C.S., Toxic effect of garlic extract and garlic oil in rats., Ind. J .Exptal .Biol., 27, 1989, 977-979.

40. Hassan S.W., Umar R.A., Ebbo A.A. and Matazu I.K., Phytochemical, Antibacterial and Toxicity Studies of *Parkinsonia aculaeta L.* (Fabacea). Nigerian journal of Biochemistry and Molecular Biology .20(2), 2005, 89-97.

41. Suja S. R., Latha P. G., Push pangadan P. and Rajasekharan S., Evaluation of hepatoprotective effect of *Helminthostachys zeylanica* (L.) Hook against carbontetrachloride induced liver damage in wistar rats, J.Ethnopharmacol. 92, 2004, 61-66.

42. Yadav N.P. and Dixit V.K., Hepatoprotective activity of leaves of *Khalanchoe pinnata pers* . J. Ethanopharmacol., 86, 2003, 197-202.

43. Bishayee A., Sarkar A. and Chartterjae M., The hepatoprotective activity of carrot (*Daucus carota L.*) against carbon tetrachloride in rats, J. Pharm.Pharmacol.54, 1995, 555-563.

44. Nan J.X., Park E. J., Kim V. C., Ko G. and Sohn D.H., *Scutellaria baicalensis* inhibits Liver Fibrosis induced by bile duct ligation or carbon tetrachloride in rats., J. Pharm. Pharmacol., 54, 2002, 555-563.

45. Sanmugapriya E. and Venkataraman S., Studies on hepatoprotective and antioxidant actions of *Strychnos potatorum* Linn. Seeds on CCl4- induced acute hepatic injury in experimental rats, J. Ethnopharmacol., 105(1-2), 2006, 154-160.

46. Ashok Shenoy K., Somayaji S.N. and Bairy K.L., Evaluation of hepatoprotective activity of *Gingo biloba* in rats, Indian J. Pharmacol., 46(2), 2002, 167-174.

47. Achiliya, G. S., Wadodkar , S. O. and Dorle, A. K., Evaluation of hepatoprotective effect of *Amakadi Ghrita* against carbon tetrachloride induced hepatic damage in rats, J. Ethnopharmacol., 90, 2004, 229-232.

48. Narasimhanaidu K. and Ponnaian S. M. P., Antihytperglycaemic and Antioxidant Effect of Rutin, a Polyphenolic flavonoid in *streptozotocin* - induced Diabetic Wistar rats. J. Basic and Clin. Pharmacol. and Toxicol. 98, 2006, 97-103.

49. Mc Connel H.M. and Hubbell W.L., Molecular Motion in spin-labelled phospholipids and biomembranes. J. Am . Chem. Soc. 93, 1971, 314-326.

50. Ojo O.O., Nadro M.S. and Tella I.O., Protection of rats by extract of some common Nigeria trees against acetaminophen –induced hepatotoxicity. Afric. J. Biotechnol., 5 (9) 2006, 755-760.

51. Castro J.A., Frrya G.C., Casio C.R., Sasame H., Fenos O. M. and Gillete J. R., Prevention of carbon tetrachloride induced necrosis by inhibitors of drug metabolism. Further studies on the mechanism of their action. Biochemical Pharmacology 23, 1974, 295-307.

52. Venukumar M.R. and Latha M.S., Antioxidant activity of C*urculigo orchioides* in carbon tetrachloride- induced hepatopathy in rats. Indian. J. Clin. Biochem., 17, 2002, 80-87.

53. Hewawasam R.P, Jayatilaka K. A. P. W., pathirana C. and Nudduwa L.K.B., Protective effect of *Asteracantha longifolia* extracts on mouse liver injury induced by carbon tetrachlride and paracetamol. J.Pharm. Pharmocol. 55, 2003, 1413-1418.

54. Kidd P.M., Glutathione systemic protestant against oxidative and free radical damage, Altern Med. Rev., 2, 1997, 155-176.

55. Moscrella S., Duchini, A. and Buzzaelli, G., Lipoperoxidation, Trace elements and vitamin E in patients with liver Cirrhosis. Eur. J. Gastroenter., 6, 1994, 633-636.

56. Patrick - Iwuan Yanwu K.C., Wegwu M.O. and Ayalogu E.O., The protective nature of Garlic, Ginger and Vitamin E on CCL_4 - induced hepatotoxicity in rats. Asian J. Biochem. 2(6), 2007, 409-414.

57. Mahmoud A.H., Motawa H.M., Wahba H.E. and Ebrahim A.Y., Study of some Antioxidant parameters in Mice Livers Affected with *Urtica Pilulifera* extracts. Asian J. Biochem. 1(1), 2006, 67-74.

58. Godwin T. W. and Mercer E. I., Introduction to plant Biochemistry 2nd ed. Pergamon press oxford, 1993, 400-480.

*****