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Hepatoprotective and antioxidant potential of *Chloroxylon swietenia (Rutaceae)* On Acetaminophen Induced toxicity in Male Albino Rats

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ABSTRACT: *Chloroxylon swietenia* is a folklore medicinal plant that is commonly used for antimicrobial, antifertility, analgesic, insecticidal, antifeedant activities The present study was aimed to investigate the hepatoprotective and antioxidant activities of ethanol extract of *Chloroxylon swietenia* (250mg/kg & 500 mg/kg B/W) on acetaminophen (APAP) induced rat hepatic injury. The development of hepatotoxicity induced by APAP is promoted by oxidative stress. APAP treated group significantly ($p<0.01$) elevated the serum enzymatic levels like serum glutamate pyruvate transaminase (ALT), serum glutamate oxaloacetate transaminase (AST), serum alkaline phosphatase (SALP) and total bilirubin, which were restored towards normalization significantly ($p<0.01$) by the ethanol extract of *Chloroxylon swietenia* in a dose dependent manner. In addition, this plant extract significantly ($p<0.01$) elevated the decreased level of antioxidant enzymes such as glutathione peroxidase (GPX), catalase (CAT), glutathione-s-transferase (GST) superoxide dismutase (SOD) and reduced glutathione (GSH). Biochemical and Histopathological observations confirmed the beneficial roles of ethanol extract of *Chloroxylon swietenia* against acetaminophen induced liver injury in rats. Histological analysis of the liver of these rats revealed marked necro-inflammatory changes by APAP and ethanol extract of *Chloroxylon swietenia* attenuated the necro-inflammatory changes in the liver. The activity of ethanol extract of *Chloroxylon swietenia* (500 mg/kg B/W) was comparable to the standard drug silymarin (25mg/kg B/W).
Key words: *Hepatoprotective, Chloroxylon swietenia, acetaminophen, silymarin, antioxidant.*

INTRODUCTION

Chloroxylon swietenia DC belonging to the family Rutaceae is commonly known as East Indian Satin Wood. It is a moderate sized tree with straight cylindrical stems having glaucous pinnate leaves and finds immense application as a phyto pharmaceutical formulation for therapeutic use particularly in southern parts of India[1].*Chloroxylon swietenia is a* folklore medicinal plant [2] which is distributed through out the India and other Asian countries [3] . This plant is used for the treatment of various ailments [4]. Ethanolic extract of the leaves exhibited good analgesic activity [5]. Stem and bark part of powdered and the juice applied for ophthalmic infection and cataract by Malayalis. Various parts of the plant are traditionally used in rheumatism and the leaves are applied to cuts wounds [6-9]. This plant extract is also used in antifertility activity [10] and Wood resistant to the attack of termites [11]. The leaves and stem oil exhibit bioactive properties against the larvae of a. Egyptian [12]. The essential oils obtained by hydro distillation of leaves and stems of *Chloroxylon*

swietenia is used for antimicrobial activity [1&13], insecticidal activity [12].

This species has been extensively investigated and a number of chemical constituents from the leaves, bark and roots of the plant have previously reported in a number of instances which includes alkaloids, [12& 14] cumarin [15-16] furoquinolines[7] and lignans[17]. Some active constituents such as coumarins xanthyletin, xanthoxyletin and 7-demethylsuberosin [18] and alkaloid skimmianine. [19] have been extracted from hardwood.

Acetaminophen is an antipyretic analgesic drug that is available over-the-counter, and an overuse excessive use of APAP can cause overproduction of ROS during formation of N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450 [20]. This mechanism has been suggested to participate in the development of oxidative stress and injury in APAP-induced hepatotoxicity[21].. Many studies have demonstrated that overproduction of Reactive oxygen species (ROS) [such as super oxide anion, hydroxyl radical and hydrogen peroxide] can further aggravate the oxidative stress and the result is a unifying mechanism of injury that occurs in many developments of clinical disease processes, such as heart disease, diabetes, liver injury, cancer, aging, etc [21-22]. Hepatotoxicity arises from infectious diseases and oxidative damages, etc. Most of the reported biological activities and active constituents of *Chloroxylon swietenia* species may be related to its antioxidant nature, but limited work has been done from the *Chloroxylon swietenia.* Keeping this in view, the present study has been undertaken to investigate hepatoprotective and antioxidant activities role of the ethanol extract of *Chloroxylon swietenia* against acetaminophen induced liver damage in rats.

MATERIALS AND METHODS

Plant material

The whole plant of *Chloroxylon swietenia* was collected from Coimbatore district, Tamil Nadu, India in the month of March. The plant material was taxonomically identified and authenticated by the botanist and the Voucher specimen (AECBT-06/2007-2008) has been retained in the Anna bioresearch foundation, Arunai engineering college, Tiruvannamalai, Tamilnadu, India.

Extraction

The whole plant of *Chloroxylon swietenia* was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60^{o}C [23]. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccator.

Animals

Studies were carried out using Wistar albino male rats (150-200g), obtained from Indian Veterinary Preventive medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2^{o}C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Poultry Research Station, Nandhanam, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Drugs and Chemicals

Silymarin was purchased from Micro labs, Tamilnadu. India. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total protein kits were procured from Span Diagnostics, Surat, India, and the rest of the chemicals utilized were of analytical grade and were obtained from Ranbaxy research laboratory, Hyderabad, India.

Experimental treatments

Animals were divided into five groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II treated with a single dose of acetaminophen (APAP) of 750mg/kg body weight was kept as toxin control. Group III and IV were treated with ethanol extract of *Chloroxylon swietenia* at two different doses of 250 and 500 mg/kg body wt plus APAP. Group V were fed with standard drug silymarin 25mg/kg daily for seven days. The extract was administered by oral gavages 1 h before APAP administration [24].

Preparation of serum from blood

After 24 h, animals were sacrificed by chloroform anaesthesia. Blood was collected by heart puncture. The blood samples of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600×*g* for 15 min and analyzed for various biochemical parameters including serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT)[25],alkaline phosphatase (ALP)[26], bilirubin and total protein . [27]

Preparation of liver homogenate

Hepatic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra

acetic acid (EDTA; pH 7.4) and centrifuged at 12,000×g for 60 min. The supernatant was used for assay of the marker enzymes (glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase), reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

Biochemical estimation of markers of oxidative stress

MDA content was measured according to the earlier method reported [28].SOD activity was determined according to previous report [29].CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method [30]. GPX activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3**[31]**. Glutathione reductase activity was assayed according to previous reports[32] Protein content in the tissue was determined by earlier method reported [33], using bovine serum albumin (BSA) as the standard.

Histopathological study

The liver was removed and stored immediately in 10 percent formalin. The tissue was subsequently put in paraffin. Thin (5µm) sections were drawn using a microtone and then stained with hematozylin and eosin and mounted in neutral di-styrene-dibutyl propylene (DPX) medium and examined using photomicroscopy .[34]

Statistical analysis

The obtained results were analyzed for statistical significance using one way ANOVA followed by Dunnet test using the graph pad statistical software for comparison with control group and acetaminophen treated group. $P < 0.05$ was considered as significant.

RESULTS

Biochemical observations

The effect of ethanol extract of *Chloroxylon swietenia* on serum marker enzymes is presented in fig (1,2 &5) The serum levels of GOT, GPT, ALP and total bilirubin were markedly ($p< 0.01$) elevated fig (1,2 &5) and that of protein levels significantly ($p< 0.01$) decreased (fig.3) in acetaminophen treated animals, indicating liver damage. Administration of ethanol extract of *Chloroxylon swietenia* at the doses of 250 and 500 mg/kg remarkably significantly ($p< 0.05$; $p< 0.01$) prevented hepatotoxicity induced by acetaminophen.

Acetaminophen treatment caused a significant ($P<0.01$) decrease in the level of SOD, catalase, GPX and GST in liver tissue when compared with control group. The treatment of ethanol extract of *Chloroxylon swietenia* at the doses of 250 and 500 mg/kg resulted in a significant ($P<0.05$; $P<0.01$) increase of SOD, catalase, GPX and GST when compared to Group II (Fig 3 & 4). The standard drug, silymarin treated animals also showed a significant ($P<0.01$) increase in antioxidant enzymes levels compared to Group II.

Analysis of MDA levels by thiobarbituric acid reaction showed a significant ($P<0.01$) increase in the acetaminophen treated rats. Treatment with ethanol extract of *Chloroxylon swietenia* (250 mg/kg & 500 mg/kg) significantly ($P<0.01$; $P<0.01$) prevented the increase in MDA level which was brought to near normal (fig 1).

Morphological observations showed an increased size and enlargement of the liver in acetaminophen treated groups. These changes were reversed by treatment with silymarin and also *Chloroxylon swietenia* at the two different doses in tested groups.

Histopathological observations

The normal histological liver structure showed in Fig. 6(a) (control group; group I) Marked inflammatory changes associated with fatty changes are seen in liver sections of the APAP treated group (group II , Fig 6(b)) . The liver sections of *Chloroxylon swietenia* extract treated group (group III & Group IV) showed periportal lymphocytic and neutrophilic infiltration with out any lesions in the hepatocytes (fig. 6(c &d). Lesser degree of inflammation was seen in the silymarin treated group when compared with control (Fig. 6 (e) Group V). All these results indicate a hepatoprotective potential by the ethanol extract of *Chloroxylon swietenia*.

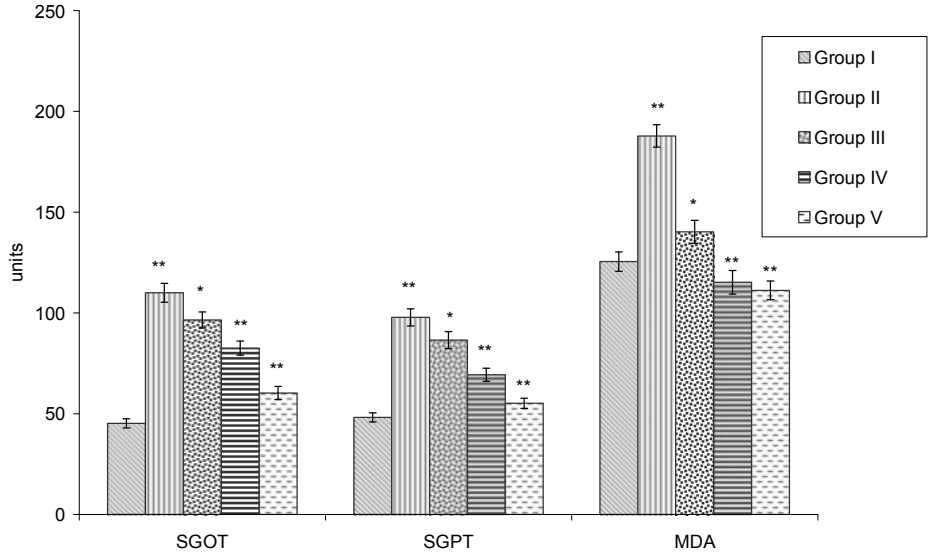

Fig.1. Effect of ethanolic extract of *Chloroxylon swietenia* and silymarin (standard drug, (25 mg/kg)) on serum levels of SGOT (IU/L), SGPT (IU/L) and MDA (nM/mg of protein) [Lipid peroxidation (LPO)] level of hepatic tissue during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

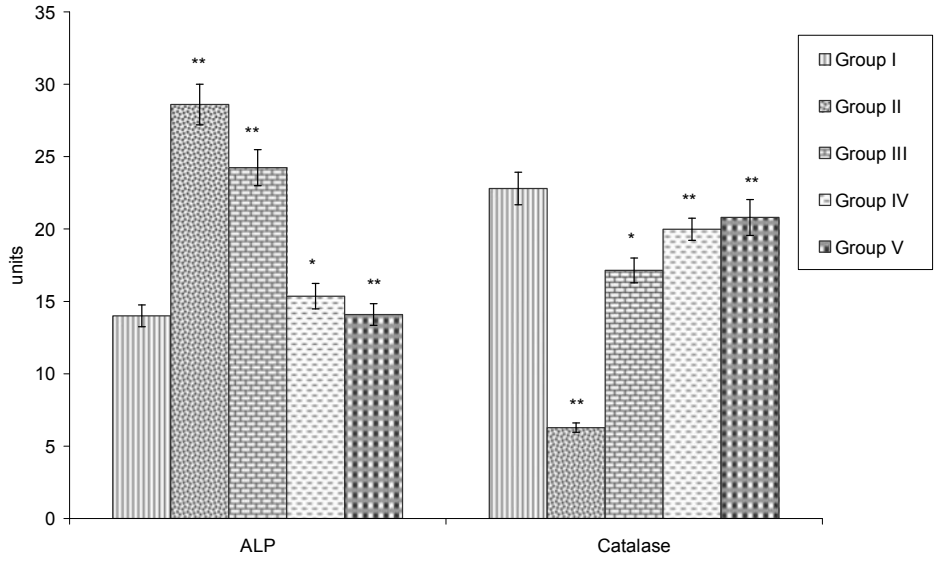

Fig.2. Effect of ethanolic extract of *Chloroxylon swietenia* and silymarin (standard drug, (25 mg/kg)) on serum levels of alkaline phosphotase (ALP) (IU/L) and hepatic levels of CAT (U/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

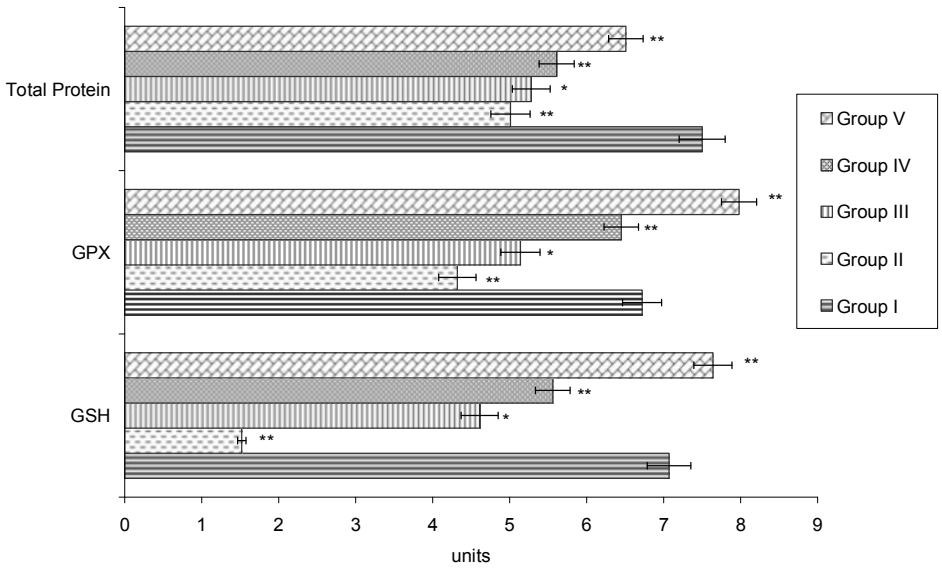

Fig.3. Effect of ethanolic extract of *Chloroxylon swietenia* and silymarin (standard drug, (25 mg/kg)) on serum levels of total protein and hepatic levels of GSH (U/mg protein) and GPX (micrograms of glutathione utilized/min/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

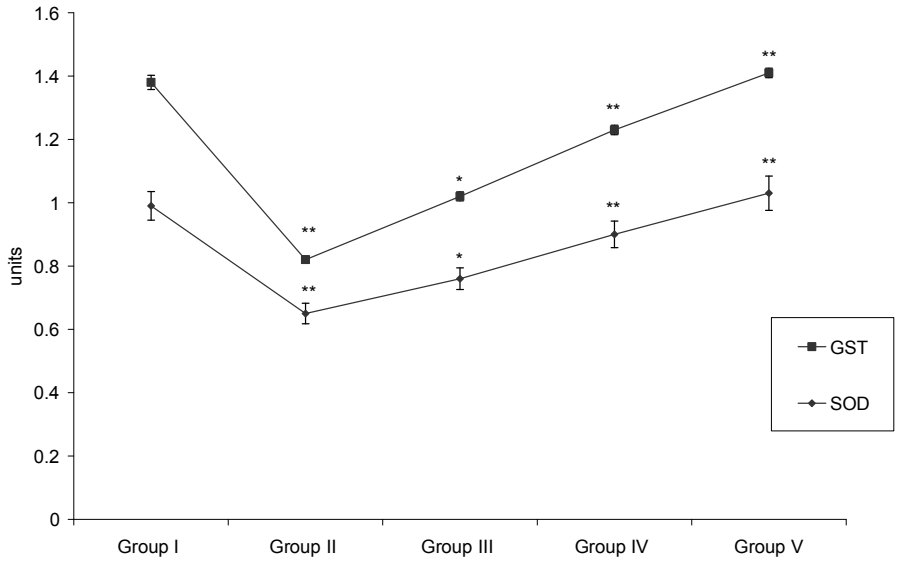

Fig.4. Effect of ethanolic extract of *Chloroxylon swietenia* and silymarin (standard drug, (25 mg/kg) on hepatic levels of SOD(units of activity/mg protein) & GST (Units/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

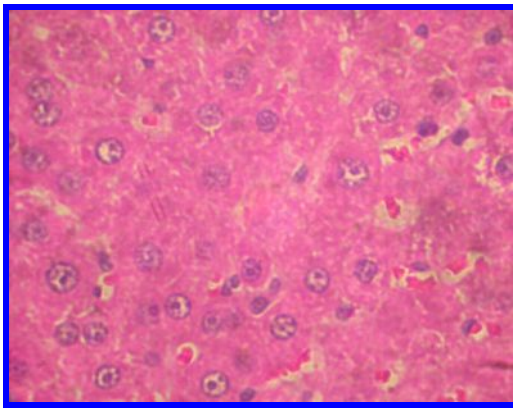

Fig. 6(a)

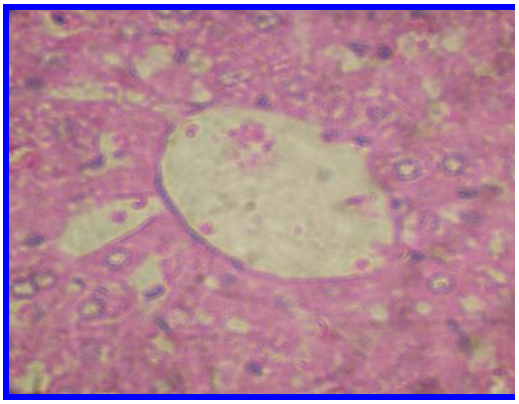

Fig. 6(b)

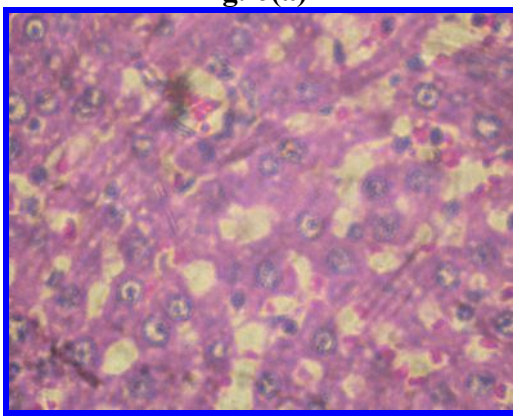

Fig. 6(c)

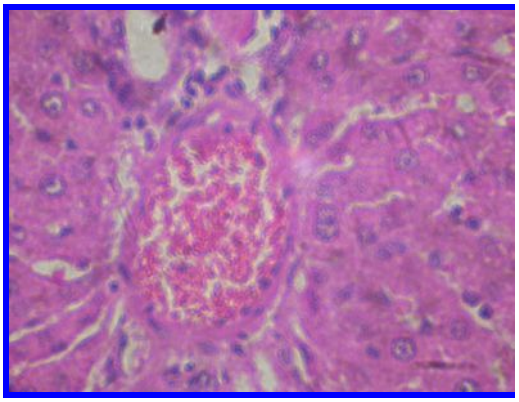

Fig. 6(d)

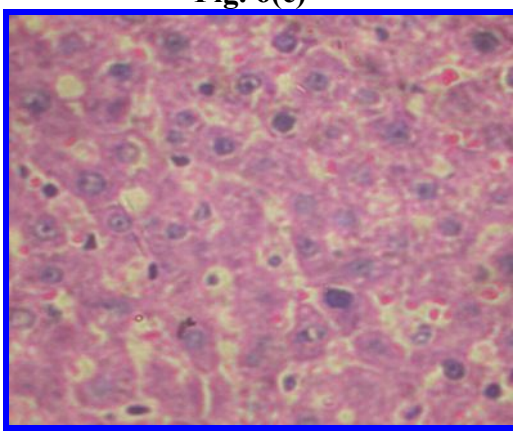

Fig. 6(e)

Fig.6. Hepatoprotective effect of ethanol extract of *Chloroxylon swietenia* against acetaminophen (AAP) induced acute hepatotoxicity in rats. Liver sections were stained with H&E 100X. (a) Normal; (b) APAP; (c) *Chloroxylon swietenia* (500 mg/kg body wt) AP ; (d) *Chloroxylon swietenia* (750 mg/kg body wt) + APAP and (e) Silymarin (25 mg/kg body wt) + APAP

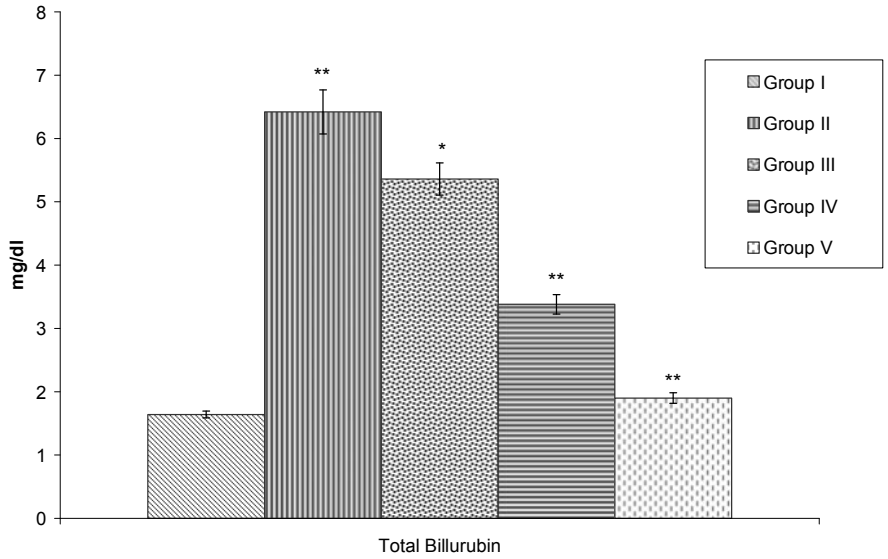

Fig.5. Effect of ethanolic extract of *Chloroxylon swietenia* and silymarin (standard drug, (25 mg/kg)) on serum levels of total bilurubin (mg/dl) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

DISCUSSION

Acetaminophen is a widely used antipyretic analgesic drug produces acute hepatic damage on accidental over dosage. The hepatic damage is established such that a fraction of acetaminophen is converted via the cytochrome P450 pathway to a highly toxic metabolite; N–acetyl–p–benzoquinamine (NAPQI) [20] which is normally conjugated with glutathione and excreted in urine. In overdose situations, however, glutathione levels are exhausted and NAPQI can directly modify susceptible protein residues in what is widely believed to be the first step in a cascade of biochemical events leading to hepatocyte death [35-36]

In the present study, rat treated with single dose of APAP treated animals developed a **s**ignificant hepatic damage and oxidative stress, resulted in a marked increase in serum SGOT, SGPT, SALP and total bilirubin levels. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver [37]. However the total protein level was decreased. There was a significant (P<0.01) restoration of these enzyme levels on administration of the ethanol extract of *Chloroxylon swietenia* in a dose dependent manner and also by silymarin at a dose of 25 mg/kg. The reversal of increased serum enzymes in acetaminophen induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [38]. Effective control of ALP, bilirubin and total protein levels points towards an early improvement in the secretary mechanism of the hepatic cells, as well as repair of hepatic tissue damage caused by APAP. This indicates the anti-lipid per oxidation and/or adaptive nature of the systems as brought about by ethanol extract of *Chloroxylon swietenia* against the damaging effects of free radical produced by APAP.

The increase in MDA levels in liver induced by acetaminophen suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with ethanol extract of *Chloroxylon swietenia* significantly reverses these changes. Hence it is likely that the mechanism of hepatoprotection of *chloroxylon swietenia* is due to its antioxidant effect.

Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in live injury [39]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. In ethanol extract of *Chloroxylon swietenia* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [40] .Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. A higher dose (500 mg/kg) of ethanol extract of *Chloroxylon swietenia* and silymarin significantly increases the level of CAT.

Both reductions of GPX &GSH activity AAP-treated rats as observed in this study indicate the damage to the hepatic cells. Administration of ethanol extract of *Chloroxylon swietenia* promoted the reactivation of hepatic glutathione reductase enzyme in AAP-treated rats. The restoration of GSH level after the administration of plant extract to such AAP treated rats due to the protective effect.

The histopathological findings in liver shows severe centrilobular necrosis and fatty infiltration in in hepatocytes was produced by acetaminophen. Treatment with different doses of ethanolic extract of *Chloroxylon swietenia* produced only mild degenerative changes and absence of centrilobular necrosis, indicating *Chloroxylon swietenia* treatment significantly rescured these signs of inflammation and necrosis. This results indicated that *Chloroxylon swietenia* treatment conferred hepatoprotectivity.

In conclusion, ethanol extract of *Chloroxylon swietenia* significantly protects against liver injuries as well as oxidative stress, resulting in improved serum biochemical parameters such as SGOT, SGPT and SALP. The reduced levels of parameters such as SOD, CAT, GSH, GPX, and GST in acetaminophen-treated rats were significantly increased by treatment with ethanol extract of *chloroxylon swietenia*. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

REFERENCES

[1]. Ravi Kiran, S. Sita Devi, E P. Janardhan Reddy,E K .Evaluation of in vitro antimicrobial activity of leaf and stem essential oils of Chloroxylon swietenia DCWorld J Microbiol Biotechnol (2008) 24:1909–1914.

[2]. Sivakumar T. Kanagasabai R. Sampathkumar R. Perumal P. Sivakumar, Gupta P.M. Mazumder U. K. (2008) 11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar p.201-213

[3]. Nantel F., denis d., gordon R., northey A., cirino, M., metters K.M., chan, C.C., *Br. J. Pharmacol*., 128, 853– 859 (1999).the wealth of india: raw materials, vol., I CSIR, New Delhi India, 1950, P. 131

[4]. Anand, R.M.; Nandakumar, N.; Karunakaran, L.; Ragunathan, M.; Murugan, V. ASurvey of medicinal plants in Kollimalai hill tracts, Tamil Nadu. Natural Product Radiance v. 5(2): p. 139-143, 2006.

[5]. Senthil raja A. ; Ramkumar R. Analgesic activity of chloroxylon swietenia .(2003) Indian drugs 2003, vol. 40,: 134-36 .

[6]. Saily, A.Sahu, R.Gupta, B.Sondhi, S.M. Analysis for mineral elements of medicinal plants used for the treatment of asthma, syphilis, diarrhoea, skin diseases and rheumatism. Hamdard Medicus. v. 37(4): p. 18-22, 1994.

[7]. Vorkoc J ., sedmera, p., evaluation of anti-inflammatory activity and toxicity related studies of *chloroxylon sweitenia* phytochem, 11,2647,(1977).

[8]. Girach, R.D., Ahmed, A., 1998. Medical ethnobotany of Sundargarh, Orissa, *India. Pharmaceutical Biology* 36, 20–24.

[9]. Panda, D.C., Das, P., 1999. Medicinal plant-lore of the tribals of Baliguda subdivision, Phulbain, District, Orissa. Journal of Economic and TaxonomicBotany 23, 515–521.

[10]. Sharma AK, Chandan BK, Anand KK and Pushpangadan P (1989). Antifertility activity of *Chloroxylon swietenia* DC. In Rats. *Zoologica Orietalis*. 4(2): 50-55.

[11]. Harris W. V, *Termites. Theb Reco,.ynition and Control,* p. 187, Longmans, London (1961).

[12]. Ravi kiran S. ; shiva reddy A. ; sita devi P. ; janardhan reddy K. ; insecticidal, antifeedant and oviposition deterrent effects of the essential oil and individual compounds from leaves of Chloroxylon swietenia DC . Pest management science. 2006:62- 1116-1121.

[13]. Bakkali a,b,F., Averbeck a,S., Averbeck a,D.,* Idaomar,M . Biological effects of essential oils – A review. Food and Chemical Toxicology 46 (2008) 446–475.

[14]. Talapatra S.K., Bhattacharya M., Talapatra B.,Das, B.C.,*J.Indian Chem. Soc*.,45,861 (1968).

[15]. Mujumdar R.B., Rama Rao A.V., Rathi, S.S.Venkataraman,K. *Tetrahedron Lett*., 11 867(1975).

[16]. Govindachari T.R.,Sathe, S.S. & Viswanathas N., *Tetrahedron Lett*., 42 ,4183 (1967).

[17]. Lee K., Soine T.O., *J. Pharm. Sci*., 58 , 681, (1969).

[18]. King, F. E., Housley, J. R. and King, J, T,. J. *Chem. Sot.* 1392 (1954).

[19]. Mookerjee, A. And Bose, P. K,. *J . Indian Chem. Sot. 23, 1 (1946).*

[20]. Dahlin D, Miwa G, Lee A. N-acetyl-pbenzoquinonamine: a cytochrome P450 dependent oxidation product of acetaminophen. *Proc Natl Acad Sci U S A* 1984; 81: 327-331.
[21]. Rolo AP, Palmeira CM. Diabetes and mitochondrial function, role of hyperglycemia and oxidative stress. *Toxicol Appl Pharmacol* 2006; 212: 167–178.

[22]. James LP, Mayeux PR, Hinson JA. Acetaminophen-induced hepatotoxicity. *Drug Metab Dispos* 2003; 31: 1499–1506.
[23]. Chattopadhyay RR. (2003) Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract: Part II. *J. Eth.phar.col*. 89, 217–219.
[24]. Deepak K Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam D, Sengupta P, Maiti BC and Maity TK. (2007) Evaluation of hepatoprotective and antioxidant activity of Ichnocarpus frutescens (Linn.) R.Br on paracetamol-induced hepatotoxicity in rats**.** *Trop. J .Pharm Res*. **6**, 755-765.
[25]. Reitman S, Frankel SA.(1957) Colourimetric method for the determination of serum oxaloacetatic and glutamic pyruvic transaminases. Am .*J .Clin Pathol*. 28, 56–63.
[26]. King EJ, Armstrong AR. (1934) A convenient method for determining of Serum and bile phosphatase activity. *J. Canad Med Assoc*. 31, 376-381.
[27]. Malloy HT, Evelyn KA.(1937) The determination of bilirubin with the photometric colorimeter. *J. Biol Chem*. 119, 481-490.
[28]. Zhang ,X Z. (1992) Crop Physiology Research Methods. China Agricultural Press Beijing. 131–207.
[29]. Rai S, Wahile A, Mukherjee K, Saha BP, Mukherjee PK. (2006) Antioxidant activity of Nelumbo nucifera (sacred lotus) seeds. J. *Ethnopharmacol*. 104, 322–327.
[30] Bergmeyer, H.U., K. Gowehn, and H. Grassel. 1974. 22. Methods of Enzymatic Analysis, ed. H.U. Bergmeyer, 438–9. Weinheim: Verlag Chemine.
[31]. Hafemann DG, Sunde RA, Houestra WG. (1974) Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *J. Nutr*. 104, 580–584.
[32]. Carlberg I, Mannervik B. (1975) Glutathione reductase levels in rat brain. *J. Biol Chem*. 250,5475–5479.
[33] Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193: 265–75.

[34] Strate T , Mann O, Kleighans H, Rusani S, Schneider C, Yekebas E, Freitag M, Standi T, Bloechle C, Izbicki J.R.,2005. Micro circulatory function and tissue damage is improved after the repeutic injection of bovine hemoglobin in server acute rodent pancreatitis. Pancreas.30 (3):254-259.
[35] Pierce RH, Franklin CC, Campbell JS. Cell culture model for acetaminophen induced hepatocyte death in vivo. *Biochem Pharmacol* 2002; 64: 413–424.
[36] Adams ML, Pierce RH, Vail ME. Enhanced acetaminophen hepatotoxicity in transgenic mice overexpressing bcl-2. *Mol Pharmacol* 2001; 60: 907–915
[37] Drotman RB, Lawhorn GT. Serum enzymes as indicators of chemical induced liver damage. Drug Chem Toxicol 1978; 1: 163–171.
[38] Maiti K, Mukherjee, K, Gantait A, Ahamed HN, Saha BP, Mukherjee PK Enhanced therapeutic benefit of quercetin-phospholipid complex in carbon tetrachloride induced acute liver injury in rats: a comparative study. *Iran J Pharmacol Ther* 2005; 4: 84–90.
[39] Curtis JJ, Mortiz M. Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. *Gastroenterol* 1972; 62: 84-92.
[40] Chance B, Greenstein DS. The mechanism of catalase actions-steady state analysis. *Arch Biochem Biophys* 1992; 37: 301-339.

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