Journal of Applied Pharmaceutical Science Vol. 3 (02), pp. 099-104, February, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.30217
ISSN 2231-3354 CC BY-NC-SA

Evaluation of the Antioxidant and Hepatoprotective Activity of *Cryptolepis Buchanani*

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ARTICLE INFO

Article history:
Received on: 17/01/2013
Revised on: 31/01/2013
Accepted on: 15/02/2013
Available online: 27/2/2013

Key words:
Hepatoprotective, *Cryptolepis buchanani*, paracetamol, silymarin, Antioxidant.

ABSTRACT

Cryptolepis buchanani (Berberidaceae) is a climbing tree, leaves is widely used in folk medicine in Southeast Asia. The alcoholic extract of stem of this plant is commonly used for the treatment of inflammatory conditions such as arthritis, and muscle and joint pain. The development of hepatotoxicity induced by acetaminophen is promoted by oxidative stress. The aim of the present study was to investigate the hepatoprotective effect and antioxidant activities of the ethanolic extract of *Cryptolepis buchanani* on acetaminophen induced hepatotoxicity in rats. We observed that the ethanolic extract of *cryptolepis buchanani* conferred hepatoprotectivity. Biochemical observations confirmed the beneficial roles of *Cryptolepis buchanani* and silymarin against acetaminophen induced liver injury in rats.

INTRODUCTION

A naturally derived herbal medicine has a biological activity for use in pharmaceutical drug discovery and drug design. These drugs useful for treating or preventing life style related disorders. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. *Cryptolepis buchanani* (Asclepiadaceae), a climbing tree, is widely used in folk medicine in Southeast Asia. In Thailand, the alcoholic extract of stem of this plant is commonly used for the treatment of inflammatory conditions such as arthritis, and muscle and joint pain (Panthong *et al*., 1986).

A weak inhibition of eicosanoid generation from rat leukocytes was previously reported (Laupattarakasem *et al*., 2003). The aqueous plant extract posses broad spectrum of medicinal properties ranging from antibacterial to anti-inflammatory, the root extract is analyzed for its immuno potentiating properties. The plant as a hole is used as cardiotonic. It also plays a great medicinal value in treating arthritis. (Laupattarakasem *et al*., 2003). Preliminary phytochemical chemical study confirmed the presence of alkaloids form this plant. Alkaloids related compounds has good potential for treating liver diseases. Therefore, we designed experiments to evaluate the hepatoprotective potential of this plant in experimental models.

MATERIALS AND METHODS

Plant Material

The leaves of *Cryptolepis buchanani* was collected from Nilgiri hills, Ooty, TamilNadu region and authenticated through Government Arts College, Ooty. Voucher specimen (AWC-01/2011-2012) has been retained in the PG Dept of Biochemistry Akilandeswari Womens College, Wandavasi, India.

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Extraction

The leaves of *Cryptolepis buchanani* 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°C (Chattopadhyay *et al*, 2003). The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccators.

GC-MS Analysis

The GC-MS analysis of the ethanolic extract of *Cryptolepis buchanani* was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary coloum (5% phenyl 95% dimethyl polysiloxane) (30nm X 0.25mm ID X 0.25μmdf) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carrier gas at a flow rate of 1 ml/min. The injector was operated at 290ºC and the oven temperature was programmed as follows; 50ºC at 8ºC/min to 200ºC (5min) at 7ºC/min to 290ºC (10min).

Identification of Components

Interpretation on mass spectrum of GC-MS was done using the database of Dr.Dukes ethanobotanical databases. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained

Experimental Animals

Studies were carried out using male Wistar albino 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 ± 20ºC) 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.

Drugs and Chemicals

Silymarin was purchased from Micro labs, Tamilnadu, India. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkalinephosphatase (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.

Determination of Hepatoprotective Effect of *Cryptolepis Buchanani*

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 (AAP) of 750mg/kg body weight was kept as control (Palani *et al*., 2010). Group III and IV were treated with extract of *Cryptolepis buchanani* 250 and 500 mg/kg body wt plus AAP and Group V were fed with standard drug silymarin 25mg/kg daily for seven days (Palani *et al*., 2009). The extract was administered by oral gavages 1 h before AAP administration.

Preparation of Serum from Blood

After 24 h, animals were sacrificed by chloroform anesthesia. 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) (Reitman and Frankel (1957), alkaline phosphatase (ALP) (King *et al*., (1934), bilirubin (Malloy *et al*.,1937) and total protein (Gornall *et al*.1949).

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

Measurement of MDA content was according to the method of Zhang (1992). SOD activity was analyzed by the method described by Rai *et al*., (2006). CAT activity was determined from the rate of decomposition of H_2O_2 by Bergmeyer *et al*., method (1974). GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and $NaNO_3$ (Hafemann *et al*., 1974). Glutathione reductase activity was assayed by the method of (Carlberg and Mannervik 1975) and modified according to Mohandas *et al.,* (1984). Protein content in the tissue was determined (Lowry *et al*., 1951) using bovine serum albumin (BSA) as the standard.

Statistical Analysis

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

Results

GC-MS chromatogram of the ethanolic leaf extract of *Cryptolepis buchanani* (Fig. 1) showed 20 peaks indicating the presence of twenty compounds. The chemical compounds identified in the ethanolic extract of the *Cryptolepis buchanani*

are presented in Table 1. GC-MS analysis revealed that the presence of 2-Octenoic acid, 4,5,7-trhydroxy, 1-Amino-2,6-dimethylpiperidine, à-Methyl-D-mannopyranoside, Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl- [Synonyms: à-Ionene] 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- D-Galactose, 6-deoxy- [Synonyms: D-Fucose], 1,6-Anhydro-á-D-glucopyranose (Synonyms: levoglucosan, Dodecanoic acid, Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester, (1R,3R,4R,5R)-(-)-Quinic acid, Tetradecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid (Z,Z)- Oleic Acid, Octadecanoic acid, 4,8,12,16- Tetramethylheptadecan -4- olide, 1,2-Benzenedicarboxylic acid, di isooctyl ester. The GC-MS analyses revealed that the alcoholic extract is mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons. These phtochemicals are responsible for various pharmacological actions like antimicrobial, anti-oxidant, hepatoprotective and antiinflammation activities. This study is only a preliminary study of the occurrence of certain properties of *Cryptolepis buchanani* extract an in-depth study will provide a good concrete base for all the biochemical and phytochemical functions mentioned above.

The acetaminophen induction resulted in a marked increase in the specific activities, serum SGOT, SGPT, SALP and total bilirubin levels (Fig. 2&3). Maximum hepatoprotective activity was observed at 500 mg/kg dose level of ***Cryptolepis buchanani*** which was comparable to that of silymarin. Activities of SOD, CAT, LP, GSH and GPX activities were significantly ($p<0.05$) enhanced only in the high dose of *Cryptolepis buchanani* plus acetaminophen treated group. However the levels of hepatic GSH and GST were found to be not significant compared to control group (Fig. 4 & 5). Hepatic LP levels was significantly ($p<0.01$) elevated in the control group (Fig.6). Moreover, the activity of CAT in the 500mg/kg *Cryptolepis Buchanani* and acetaminophen treated group were significantly increased from the control group ($p<0.05$) (Fig .6).

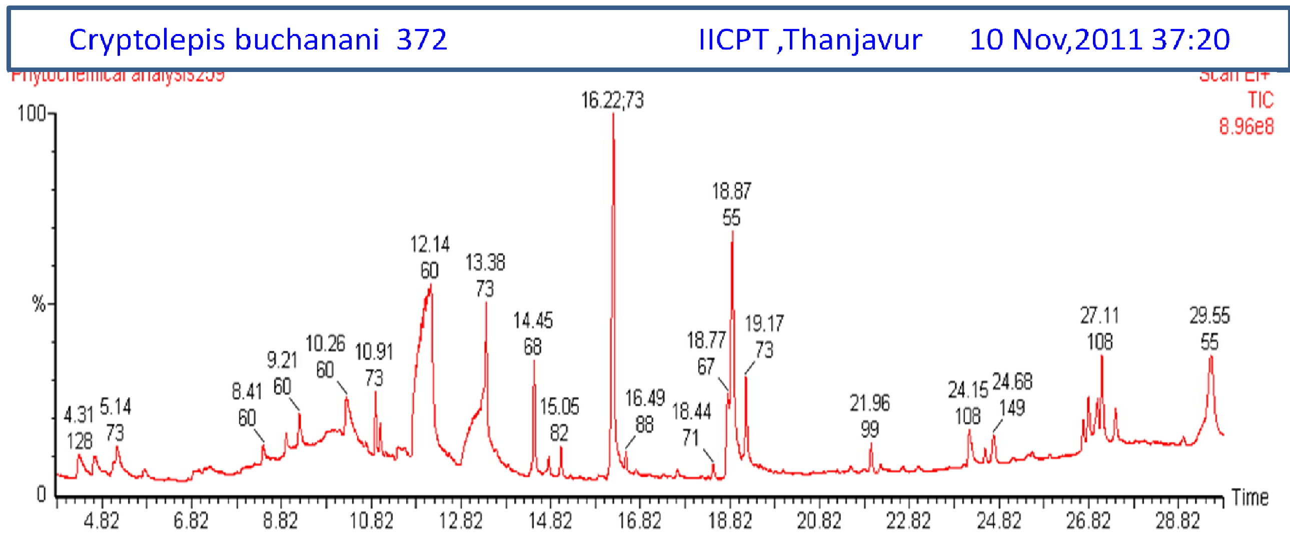

Fig 1: The chromatogram showing Quinic acid (12.14), n-Hexadecanoic acid (16.22), Oleic Acid (18.87) and Octadecanoic acid (19.17).

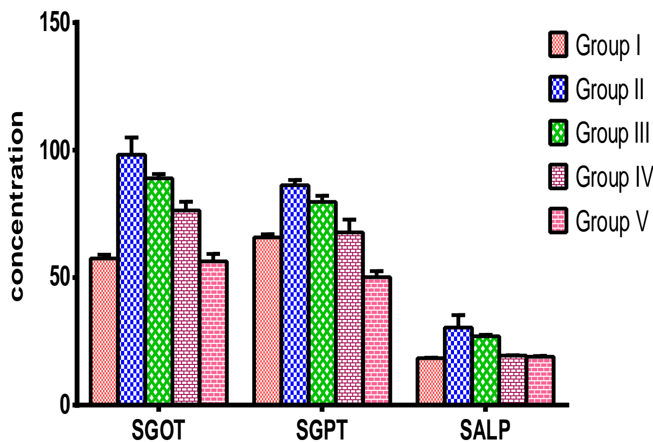

Fig 2. Effect of ethanol extract of Cryptolepis buchanani and silymarin on serum enzymes (SGPT, SGOT and ALP), on acetaminophen induced hepatotoxicity in rats.

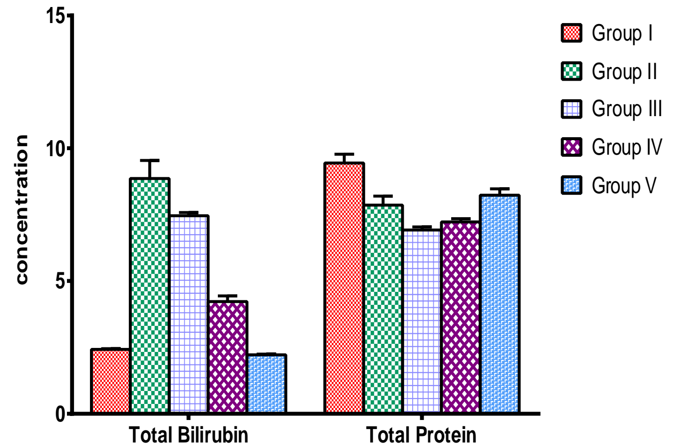

Fig. 3: Effect of ethanol extract of Cryptolepis buchanani and silymarin on serum enzymes total bilirubin and total protein on acetaminophen induced hepatotoxicity in rats.

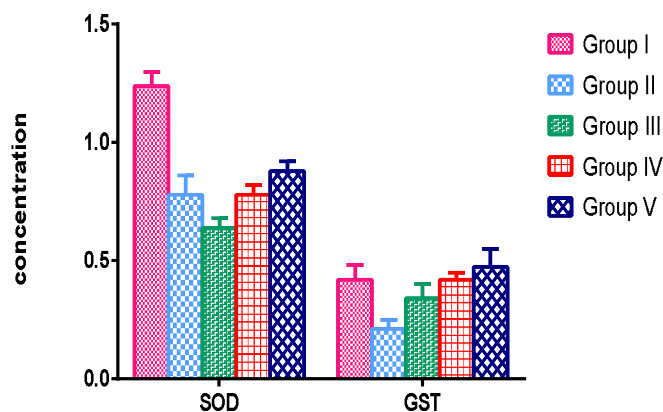

Fig. 4: Effect of ethanol extract of Cryptolepis buchanani & silymarin oantioxidants levels (SOD, & GST) in acetaminophen induced heapatotoxicity in rats.

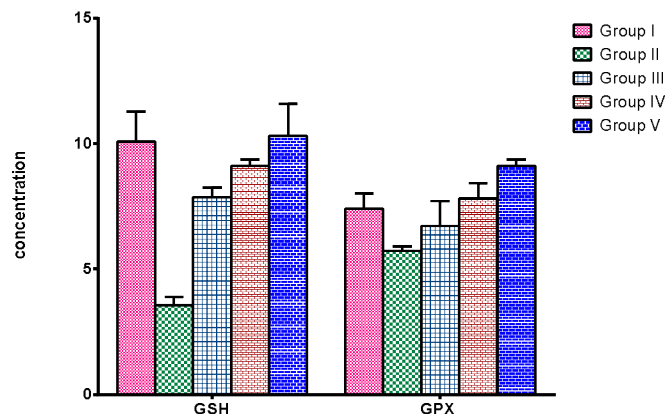

Fig 5. Effect of ethanol extract of Cryptolepis buchanani & silymarin on antioxidants levels (GSH, GPX) in acetaminophen induced heapatotoxicity in rats.

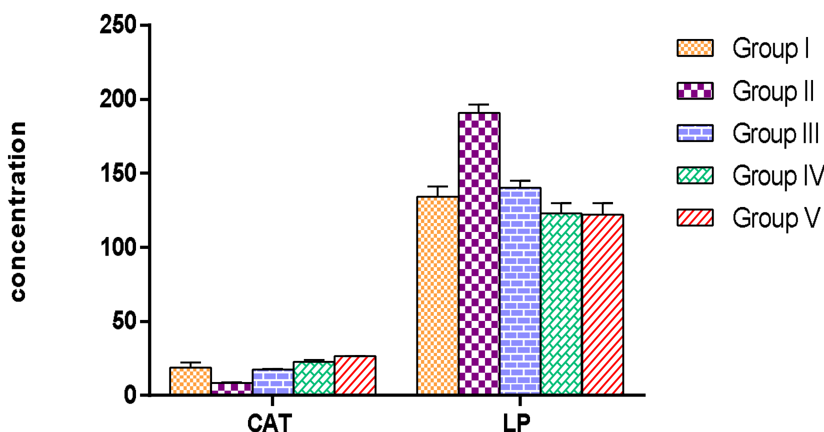

Fig 6. Effect of ethanol extract of Cryptolepis buchanani and silymarin on antioxidants levels (CAT, MDA) in acetaminophen induced heapatotoxicity in rats.

Table. 1: Phyto-Components identified in the *Cryptolepis buchanani* [GC MS study].

No.	RT	Name of the compound	Molecular	MW	Peak Area %
1	4.31	2-Octenoic acid, 4,5,7-trhydroxy	$C_7H_{16}N_2$	128	1.52
2	4.65	1-Amino-2,6-dimethylpiperidine	$C_8H_{14}O_5$	190	1.9
3	5.14	à-Methyl-D-mannopyranoside	$C_7H_{14}O_6$	194	2.12
4	5.75	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl- [Synonyms: à-Ionene]	$C_6H_8O_4$	144	0.38
5	8.92	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_{13}H_{18}$	174	2.94
6	9.21	D-Galactose, 6-deoxy-[Synonyms: D-Fucose]	$C_6H_{12}O_5$	164	6.06
7	10.26	1,6-Anhydro-á-D-glucopyranose (Synonyms: levoglucosan)	$C_6H_{10}O_5$	162	5.85
8	10.91	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.83
9	11.02	Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester	$C_9H_{18}FO_2P$	208	0.50
10	12.14	(1R,3R,4R,5R)-(-)-Quinic acid	$C_7H_{12}O_6$	192	26.04
11	13.38	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	15.69
12	14.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.55
13	16.22	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.44
14	16.49	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.88
15	18.44	Phytol	$C_{20}H_{40}O$	296	0.32
16	18.77	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.85
17	18.87	Oleic Acid	$C_{18}H_{34}O_2$	282	7.70
18	19.17	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.89
19	21.96	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.65
20	24.68	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.93

**Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

DISCUSSION

In this study , we show that ethanolic extracts of *Cryptolepis Buchanani* protected against acetaminophen induced hepatotoxicity. This suggests that a fat-soluble metabolite or metabolites constitutes the active ingredient. Candidate molecules include alkaloids, flavonoids, etc,. In the Preliminary phytochemical studies reveal the presence of alkaloids and flavonoids in ethanolic extract of *Cryptolepis Buchanani* , these molecules are reported to have hepatoprotective activity (Seevola *et al*., 1984; Wegner and Fintelmann, 1999). The hepatic damage is established that, a fraction of acetaminophen is converted via the cytochrome P450 pathway to a highly toxic metabolite; N–acetyl–p– benzoquinamine (NAPQI) (Dahlin *et al*., 1984) 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. (Bessems *et al*.,2001; Nelson and Bruschi,2002; Hinson *et al*., 1981; Nelson and Pearson 1990; Potter *et al*.,1973; Jollow *et al*.,1973; Pierce *et al*.,2002; and Adams *et al*., 2001).

In this study, rat treated with single dose of AAP 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 (Drotman and Lawhorn, 1978). However the total protein level was decreased.

The significant (P<0.01) restoration of these enzyme levels on administration of the leaf extract 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 the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987; Maiti *et al*., 2005).

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 acetaminophen..

During hepatic injury, superoxide radicals generate at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages liver. Decreased CAT activity is linked up to exhaustion of the enzyme as a result of oxidative stress caused by Acetaminophen. The SOD and CAT activities were brought to near normal after pretreatment with extract in APA-treated rats evidently shows the antioxidant property of the extract against oxygen free radicals.

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

CONCLUSION

Ethanolic leaf extract of Cryptolep is Buchanani significantly protects against liver injuries as well as oxidative stress, resulting in increased serum biochemical parameters such as SGOT, SGPT and SALP. The reduced levels of SOD, CAT, GSH, GPX, and GST in acetaminophen treated rats were significantly increased by treatment with the extract. Further studies to characterize the active chemical compounds and to elucidate the mechanism are in progress.

REFERENCES

Adams, ML., Pierce, RH., Vail, ME. Enhanced acetaminophen hepatotoxicity in transgenic mice overexpressing bcl-2. Mol Pharmacol. 2001 ;60:907–915.

Bergmeyer, HU, Goweh, K, Grassel, H. In: Bergmeyer HU, editor. Methods of enzymatic analysis. Weinheim: Verlag Chemine.1974; 438-439.

Bessems, JG., Vermeulen, NP. Acetaminophen (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit Rev Toxicol. 2001;3:55–138.

Carlberg, I., Mannervik, B. Glutathione reductase levels in rat brain. Journal of Biological Chemistry. 1975; 250: 5475–5479.

Chattopadhyay, RR. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II. J. Ethnopharmacol. 2003; 89: 217–219.

Dahlin, D., Miwa,G., Lee, A. N-acetyl-pbenzoquinonamine: a cytochrome P450 dependent oxidation product of acetaminophen. *Proc. Natl. Acad. Sci.* 1984 ; 81: 327-331.

Dahlin, DC., Miwa, GT., Lu, AYH., Nelson, SD. N-acetyl-p benzoquinone imine: a cytochrome P-450 mediated oxidation product of acetaminophen. Biochemistry. 1984;8:1327–1331.

Drotman, RB., Lawhorn, GT. Serum enzymes as indicators of chemical induced liver damage. Drug and Chemical Toxicology. 1978;1 :163–171.

Gornall, AG., Bardwill, CJ., David, MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem. 1949; 177: 751-756.

Hafemann, DG., Sunde, RA., Houestra, WG. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr. 1974; 104:580–584.

Hinson, JA., Pohl, LR., Monks, TJ., Gillette, JR. Acetaminophen- induced hepatotoxicity.Life Sci. 1981;29 :107–116.

Jollow, DJ., Mitchell, JR., Potter, WZ., Davis, DC., Gillette, JR., Brodie, BB. etaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo.J Pharmacol Exp Ther. 1973;187:195–202.

King , EJ., Armstrong, AR. A convenient method for determining of Serum and bile phosphatase activity. J Canad. Med. Assoc.1934 ; 31: 376-381.

Laupattarakasem, P., Houghton, PJ., Hoult, JR., Itharat A. An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. Journal of Ethnopharmacology.2003; 85:207–215.

Lowry, OH., Rosebrough, NJ., Farr, AL., Randal, RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry.1951 ; 193: 265–275.

Maiti, K., Mukherjee, K., Gantait, A., Ahamed, HN., Saha, BP., Mukherjee, P. Enhanced therapeutic benefit of quercetin-phospholipid complex in carbon tetrachloride induced acute liver injury in rats: a

comparative study. Iranian Journal of Pharmacology and Therapeutics. 2005; 4:84–90.

Malloy, HT., Evelyn, KA. The determination of bilirubin with thephotometric colorimeter. J Biol Chem. 1937 ;119: 481-490.

Mohandas, J., Marshall, JJ., Duggin, GG., Horvath, JS., Tiller,D. Differential distribution of glutathione and glutathione related enzymes in rabbit kidney: possible interactions in analgesic neuropathy. Cancer Research. 1984; 44:5086–5091.

Nelson, SD., Bruschi, SA. Mechanisms of acetaminophen-induced liver disease. In:Kaplowitz N, DeLeve LD, eds. Drug-Induced Liver Disease. New York and Basel:Marcel Dekker. 2002; 287–325.

Nelson, SD., Pearson, PG. Covalent and noncovalent interactions in acute lethal cell injury caused by chemicals. Annu Rev Pharmacol Toxicol. 1990; 30:169–195.

Palani S, Raja S, Praveen Kumar R, Venkatesan D, Devi K, Sivaraj A, Senthil Kumar B. Therapeutic efficacy of antihepatotoxic and antioxidant activities of Acorus calamus (AC) on acetaminophen- induced toxicity in rat. International Journal of Integrative Biology. 2009; (l).7, 1-39.

Palani s, Raja S, K Sakthivel, K Devi and Senthil Kumar B. Hepatoprotective and antioxidant effects of Monochoria vaginalis against acetaminophen-induced hepatotoxicity in rats. *Orient. Pharm. Exp. Med*. 2010; 10(1), 29-36.

Panthong, A., Kanjanapothi, D., Taylor, WC. Ethnobotanical review of medicinal plants from Thai traditional books. Part I. Plants with anti inflammatory, anti-asthmatic and antihypertensive properties. Journal of Ethnopharmacology. 1986; 18: 213–228.

Pierce, RH., Franklin, CC., Campbell, JS. Cell culture model for acetaminophen induced hepatocyte death in vivo. Biochem Pharmacol. 2002; 64:413–424.

Potter, WZ., Davis, DC., Mitchell, JR., Jollow, DJ., Gillette, JR., Brodie, BB. Acetaminophen-induced hepatic necrosis. 3. Cytochrome P-450-mediated covalent binding in vitro. J Pharmacol Exp Ther.1973;187:203–210.

Rai, S.,Wahile, A., Mukherjee, K., Saha, BP., Mukherjee, PK. Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. Journal of Ethnopharmacology. 2006; 104: 322–327.

Reitman, S., Frankel, SA. Colourimetric method for the determination of serum oxaloacetatic and glutamic pyruvic transaminases. American Journal Clinical Pathology.1957; 28:56–63.

Seevola, D., Baebacini, GM., Bona, **S.** Flavonoids and hepatic cyclic monophosphates in liver injury. Boll. Ins. Sieroter. Milan.1984; 63:777-782.

Thabrew, M ., Joice, P. A comparative study of the efficacy of *Pavetta indica* and Osbeckia octanda in the treatment of liver dysfunction. *Planta Med*. 1987; 53: 239-241.

Wegner, T., Fintelmann,V. Flavonoids and bioactivity. *Wein. Med. Wochem. Sihr*.1999; 149: 241-247.

Zhang, XZ. Crop Physiology Research Methods. China Agricultural Press, Beijing, pp.1992;131–207.

How to cite this article:

K. Padmalochana, M.S. Dhana Rajan, R. Lalitha, H.Sivasankari., Evaluation of the Antioxidant and Hepatoprotective activity of *Cryptolepis Buchanani.* J App Pharm Sci. 2013; 3 (02): 099-104.