ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 3, pp. 012-016, April, 2010

RESEARCH ARTICLE

ANTIHEPATOTOXIC EFFICACY OF *NYMPHAEA PUBESCENS* (WILLD.) ON ACETAMINOPHEN INDUCED LIVER DAMAGE IN MALE WISTAR RATS

Minnady Muthulingam

Department of Zoology, Faculty of Science, Annamalai University, Annamalainagar 608 002.

ARTICLE INFO

Article History:
Received 24th February, 2010
Received in revised from
15th March, 2010
Accepted 27th March, 2010
Published online 1st April, 2010

Key words:
Acetaminophen
Bilirubin, Liver
Nymphaea pubescens
Transferases

ABSTRACT

The antihepatotoxic efficacy of aqueous flower extracts of *Nymphaea pubescens* (NP) and Silymarin were investigated against acetaminophen induced liver damage in rats. Acetaminophen at the dose of 3gm/kg body weight orally produced liver damage in rats as manifested by the significant rise in serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, cholesterol and decreases the protein level compared with control. Oral administration of flower extracts of *Nymphaea pubescens* (100, 200, 400mg/kg) and silymarin (25mg/kg) once daily for 28 days to acetaminophen treated rats shows lowered significantly the afore mentioned clinical parameters where as protein level increased. The extract alone treated rats did not adversely affect the serum biochemical estimation. A significant antihepatotoxic efficacy of *Nymphaea pubescens* extracts was reported.

INTRODUCTION

Nymphaea pubescens (Nymphaeaceae) large perennial aquatic herb with short, erect, roundish, tuberous rhizomes; leasves floating, peltate, sharply sinuate-toothed, flowers large, floating, solitary, variable in colour from pure white to deep red; fruits spongy many seeded berries, seeds minute, grayish black when dry with longitudinal striations. *Nymphaea pubescens* found throughout the warmer parts of India, in tanks, ponds and ditches. *Nymphaea pubescens* of rhizome is cooling, sweet, bitter and tonic, and is useful in diarrhoea, dysentery, dipsia and general debility. The flowers are astringent and cardio tonic. The seeds are sweet, cooling, constipating, aphrodisiac, stomachic and restorative. They are useful in vitiated conditions of pita, dipsia, diarrhea and dermatopathy (Prajapathi et al., 2004).

Liver, the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence, this organ is subjected to variety of diseases and disorders. Several hundred plants have been examined for use in a wide variety of liver disorders (Rajkapoor *et al*., 2008). The liver is sometimes referred to as the "great chemical factory" of the body, because the body depends on the liver to regulate, synthesize, store and secrete many important proteins, nutrients, chemical and to purify and clear toxin or unneeded substance from the body. Most importantly, the liver is considered to be the center of metabolic transformation of drugs and other toxins entering from the gastrointestinal tract as such the normal or healthy functioning of the liver determines the health status of an individual (Garba *et al*., 2009).

Acetaminophen (Paracetamol) is an analgesic drug and metabolized by cytochrome P450 system, which leads to the formation of n-acetyl-p-benzoquinoneimine (NAPQI) (Song *et al*., 2004; Masubuchi *et al*., 2005). Paracetomol is a powerful inducer of cytochrome P-450. The action of the P-450 system on paracetomol produces a highly reactive quinoneimine that combines to the sulfhydryl groups of proteins. The toxicity occurs because of its reactive metabolite, N-acetyl-*p*-benzoquinoneimine (NAPQI). NAPQI exerts its toxicity primarily via its oxidative effect on cellular proteins.The inactivation of proteins leads to death of liver cells. The mechanism of hepatotoxicity of paracetomol has been studied extensively (Hazai *et al*., 2002). Pracetamol overdose in both animals and man has been shown to produce hepatic necrosis, ascribed to a toxic metabolite of the parent drug (Thomas, 1993) and it has frequently has been used as an animal model for hapatotoxicity (Visen *et al*., 1993).

Presently, the use of herbal medicines for prevention and control of chronic liver diseases is in the focus of attention for the physicians, pharmaceutical manufacturers and patients; the reasons for such shift toward the use of herbals include the expensive cost of conventional drugs, adverse drug reactions, and their inefficacy (Aghel *et al*., 2007). Many medicinal plants have been recommended in alternative systems of medicine for the treatment of liver diseases. No systematic study has been done on anti hepatotoxic efficacy of *Nymphaea pubescens* flower to treat liver disorder. Therefore, the protective action of

Corresponding Author: muthuau@rediffmail.com

Nymphaea pubescens flower extract was evaluated in acetaminophen induced hepatotoxicity in male albino wistar rats.

MATERIALS AND METHODS

Plant material

Nymphaea pubescens flowers were collected from Chidambaram in Cuddalore district of Tamilnadu, India. The plant was identified and authenticated at the herbarium of Botany Directorate, Faculty of Science, Annamalai University. The flowers were shade dried and powdered was kept in airtight container in a deep freeze until the time of use.

Preparation of aqueous extract

The collected *Nymphaea pubescens* flowers were air dried and powdered. The powdered leaves were kept in airtight containers in a deep freeze until the time of use. A sample containing 250 g of flowers were mixed with 1000 mL of distilled water and stirred magnetically overnight (12 h) at 37°C. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at lower temperature (<40°C) under reduced pressure in rotary evaporator. The residual extract was dissolved in normal physiological saline and used in the study. The yield of the extracts was approximately 17.4 g.

Optimum dosage

The suitable optimum dosage schedule was identified by administration of the aqueous extracts of *Nymphaea pubescens* flowers at different dosages (100, 200, 400 and 800 mg/kg body weight) once daily for twenty eight days. The optimum doses were selected as 100, 200 and 400 mg/kg body weight of the animals for twenty eight days respectively.

Experimental animals

Adult male albino rats (Wistar strain) were collected from Central Animal House, Rajah Muthiah Medical College, Annamalai University and were used for the present study. The rats were housed in polypropylene cages at room temperature (27 ± 2°C). The animals were randomized and separated into normal and experimental groups of body weight ranging from 160-200 g. The animals received a diet of standard pellets (Hindustan Lever Ltd., Bombay). Rats were provided free access to water *ad libitum* and food through the tenure of acclimatization to the environment for a minimum period of two weeks prior to commencement of experiment. All studies involving animals were done according to NIH guidelines, after getting the approval of the Annamalai University Institute's Animal Ethics Committee.

Experimental design

The animals were divided into seven groups of six rats each.

Group 1 : Control rats given physiological saline solution 10 mL/kg body wt.

Group 2 : Rats given paracetamol (3 g/kg body wt one day only) orally was using an intragastric tube.

Group 3: Rats given paracetamol + *N. pubescens* flowers (100 mg/kg body wt. once daily for 28 days) administered orally using an intragastric tube.

Group 4 : Rats given paracetamol + *Nymphaea pubescens* flowers (200 mg/kg body wt. once daily for 28 days) administered orally using an intragastric tube.

Group 5 : Rats given paracetamol + *Nymphaea pubescens* flowers (400 mg/kg body wt. once daily for 28 days) administered orally using an intragastric tube.

Group 6 : Rats given paracetamol + silymarin (25 mg/kg body wt. once daily for 28 days) administered orally using an intragastric tube.

Group 7 : *Nymphaea pubescens* flowers (400 mg/kg body wt. once daily for 28 days) alone administered orally using an intragastric tube.

At the end of the experimental period in 24 h after last treatment the animals were killed by cervical decapitation. Blood was collected without anticoagulant for the separation of serum for evaluating serum maker enzymes such as AST, ALT, ALP, Bilirubin, cholesterol and protein.

Biochemical analysis

Blood samples were taken into centrifuge tube with rubber caps, labeled and centrifuged at 3000 rpm for 15 minutes. Serum biochemical parameter such as Transaminases (AST and ALT), ALP, Bilirubin, cholesterol and protein levels were estimated according to standard methods (Reitman and Frankel, 1957; King and Armstrong, 1980; Malloy and Evelyn 1937; Zlatkis *et al*., 1953; Lowry *et al*., 1951).

Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Duncan's multiple range test (DMRT). The level of statistical significance was set at $p \leq 0.05$(Duncan, 1957).

RESULT

Levels of serum hepatic serum marker enzymes

The levels of serum AST, ALT and ALP were estimated in normal and experimental rats. Significant elevation in serum AST, ALT and ALP in rats treated with acetaminophen when compared with the corresponding control rats. Oral administration of aqueous flower extract of *Nymphaea pubescens* (100, 200 and 400 mg/kg body weight) and silymarin (25 mg/kg body weight) to acetaminophen induced hepatic damaged rats caused a marked reduction in the activities of these enzymes. The extract alone treated rats did not show any significant alterations when compared with control group (Table 1).

Levels of serum bilirubin, cholesterol and protein

The levels of serum bilirubin, cholesterol and protein were analysed in normal and experimental rats. There was a significant increase in bilirubin and cholesterol while the level of protein decreased in rats treated with acetaminophen when compared with the corresponding control group. Oral administration of aqueous flower extract of *Nymphaea pubescens* (100, 200 and 400 mg/kg body weight) and silymarin (25 mg/kg body weight) to acetaminophen treated rats exhibited a remarkable decrease in bilirubin and cholesterol while there was a significant increase in protein level when compared to acetaminophen alone treated rats. The extract alone treated rats did not show any alterations when compared with control group (Table 2).

Table 1. Serum hepatic marker enzyme activities in control and experimental groups

Group	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	98.73 ± 7.65^{ab}	51.49 ± 4.36^{ab}	168.96 ± 11.34^{a}
Acetaminophen (3g/kg)	214.06 ± 10.12^{f}	170.11 ± 11.24^{f}	584.33 ± 15.28^{e}
Acetaminophen + *Nymphaea pubescens* (100 mg/kg)	135.89 ± 9.44^{e}	86.20 ± 5.02^{e}	255.28 ± 18.44^{d}
Acetaminophen + *Nymphaea pubescens* (200 mg/kg)	128.34 ± 6.82^{de}	73.05 ± 4.82^{d}	222.50 ± 10.82^{c}
Acetaminophen + *Nymphaea pubescens* (400 mg/kg)	110.44 ± 8.25^{bc}	59.35 ± 4.68^{bc}	194.86 ± 10.65^{b}
Acetaminophen + Silymarin(25 mg/kg)	119.12 ± 7.18^{cd}	65.33 ± 3.74^{cd}	216.60 ± 8.72^{bc}
Nymphaea pubescens (400 mg/kg) alone	96.34 ± 6.82^{a}	50.77 ± 4.82^{a}	165.59 ± 10.82^{a}

All the values are mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% level (P < 0.05).
Duncan Multiple Range Test (DMRT).

Table 2. Serum bilirubin, cholesterol and protein levels in control and experimental groups

Group	Bilirubin (mg/dL)	Cholesterol (mg/dL)	Protein (mg/dL)
Control	0.95 ± 0.02^{a}	89.69 ± 5.24^{a}	7.78 ± 0.18^{d}
Acetaminophen (3g/kg)	3.61 ± 0.20^{e}	186.59 ± 9.12^{e}	5.26 ± 0.23^{a}
Acetaminophen + *Nymphaea pubescens* (100 mg/kg)	1.62 ± 0.01^{d}	157.45 ± 7.42^{d}	6.12 ± 0.19^{b}
Acetaminophen + *Nymphaea pubescens* (200 mg/kg)	1.45 ± 0.02^{c}	142.38 ± 5.18^{c}	6.75 ± 0.39^{bc}
Acetaminophen + *Nymphaea pubescens* (400 mg/kg)	1.12 ± 0.02^{b}	109.78 ± 6.74^{b}	7.28 ± 0.21^{cd}
Acetaminophen + Silymarin(25 mg/kg)	1.30 ± 0.01^{c}	118.64 ± 4.66^{b}	6.52 ± 0.52^{cb}
Nymphaea pubescens (400 mg/kg) alone	0.94 ± 0.02^{a}	88.30 ± 5.18^{a}	7.80 ± 0.39^{d}

All the values are mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% level (P < 0.05).
Duncan Multiple Range Test (DMRT).

DISCUSSION

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits (Rajesh *et al*., 2009).

Liver disease is a worldwide problem. Conventional drugs used in the treatment of liver diseases are some times inadequate and can have serious adverse effects. It is therefore necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety. Liver damage induced by paracetamol was commonly used model for the screening of hepatoprotective drugs (Slater *et al*., 1965; Plaa *et al*., 1982). The rise in serum levels AST and ALT has been attributed to the damaged structural integrity of the liver (Chenoweth *et al*., 1962), because these are cytoplasmic in location and are released into circulation after cellular damage (Sallie *et al*., 1991). An obvious sign of hepatic injury is leakage of cellular enzyme into plasma (Wilkinson, 1962; Schmidt *et al*., 1975). Liver cell plasma membrane is damaged a variety of enzymes normally located in the cytosol are released into blood stream. Their estimation in the serum is a useful quantitative marker for the extent and type of hepatocellular damage (Ansari *et al*., 1991). The activities

of enzyme AST, ALT and ALP in serum and used routinely to access the functional status of the liver in both clinical and experimental settings. They are used as serum markers of hepatic damage. Assay of serum ALP activity has been recognized as a suitable marker of skeletal and hepatobiliary disorder. Moreover, an elevated serum level of ALP activity is frequently associated with various pathological conditions (Simko, 1991; Moss, 1989). Alkaline phosphate is a non-specific tissue enzyme widely spread, mainly in the ostaoblasts, liver and biliary canaliculi (Poole and Lesile, 1989; Ringler and Dabich, 1979).

In the present study acetaminophen treated rats showed an increase in the levels of AST, ALT and ALP when compared with control rats. Oral administration of aqueous flower extract of *N. pubescens* (100, 200 and 400 mg/kg body wt.) and silymarin to acetaminophen treated rats showed an inhibition in the elevated levels of serum AST, ALT and ALP levels were increased than acetaminophen alone treated rats. Similar changes observed concomitant administration of petroleum ether and ethyl acetate extract of the aerial parts of *Flacourtia indica* with paracetamol significantly attenuated the serum concentration of AST and ALT in comparison to that of paracetamol alone treated rats (Nazneen *et al*., 2009). The abnormal high level of serum ALT, AST, ALP and bilirubin observed in paracetamol induced liver toxicity. Treatment with aqueous ethanolic extract of *Chamomile recutita* reduced the enhanced level of serum ALT, AST, ALP and bilirubin (Gupta and Misra, 2006). Oral administration of alcoholic, aqueous and chloroform extract of *Baliospermum montanum* showed significant reduction in AST, ALT and ALP elevated by paracetamol (Wadekar *et al*., 2008). Treatment with *G. manshurica* to acetaminophen administration lowered markedly both serum ALT and AST levels (Ai-yan wang, *et al*., 2010).

Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hypatocyte (Manokaran *et al*., 2008). Lipids are the most important cellular entities which are not only the constituents of cell membrane but also involved in many cellular functions, metabolic processes and are vital for energy production. Liver is the organ and involved in the synthesis of lipoproteins and metabolism of cholesterol. The changes in the level of plasma lipids could be sensitive and serve as a simple marker of assessing liver disorders (Honma and Sudha, 1997). Protein plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants, which enter into the animal body (Ramasamy, 1987; Muthulingam, 2008).

In the present study acetaminophen treated rats showed an increase in the levels of bilirubin and cholesterol where as protein level was decreased when compared with control rats. Oral administration of aqueous flower extract of *N. pubescens* (100, 200 and 400 mg/kg body wt.) and silymarin to acetaminophen treated rats showed decrease in the elevated levels of serum bilirubin and cholesterol where as protein level was increased than acetaminophen alone treated rats. Simillarly administration of methanolic extract of *Psidium guajava* leaves to paracetamol treated rats showed the bilirubin levels were significantly lowered than the paracetamol alone treated group of rats (Roy and Das, 2010). Administration of 3-bromo-6-(4-chlorophenyl)-4-methylthio-2H-pyran-2-one against paracetamol treated group of rats showed bilirubin and cholesterol levels were decreased when compared with paracetamol alone treated rats (Tripathi *et al*., 2003). *Alchornea cordifolia* extracts treated with acetaminophen alone treated rats showed that decreased the levels of bilirubin and cholesterol where as protein level was increased (Olaleye *et al*., 2006). Oral administration of *Arctium lappa* to paracetamol treated rats showed protein, cholesterol and bilirubin levels were attenuated near normalcy when compared with paracetamol alone treated rats (Predes *et al*., 2009).

CONCLUSION

The results of the present investigation clearly indicate that the aqueous flower extract of *N. pubescens* have a lowering effect of serum hepatic marker enzymes on acetaminophen induced liver damage in rats. It was also found to be highly effective in reducing serum cholesterol and bilirubin and enhancing the levels of serum protein associated hepatotoxicity. Hence *N. pubescens* of aqueous flower extract shows the curative role against acetaminophen induced hepatotoxicity in rats. Further studies are in progress to isolate the active principle and elucidate the exact mechanism of action of *N. pubescens* flowers.

Acknowledgement

The author thankful to Dr. N. Indra, Professor of Zoology and Dr. S. Sethupathy, Professor and Head, Department of Biochemistry, Rajah Muthiah Medical College and Hospital, Annamalai University for providing necessary facility.

REFERENCES

Aghel, N., Rashidi, I and Mombeini, A., 2007. Hepatoprotective Activity of *Capparis spinosa* Root Bark Against CCl4 Induced Hepatic Damage in Mice. Iranian Journal of Pharmaceutical Research. 6 (4): 285-290.

Ai-Yan wang, Li-hua lian, Ying-zi jiang, Yan-ling wu, Ji-xing Nan. 2010. *Gentiana manshurica* kitagawa prevents acetaminophen induced acute hepatic injury in mice via inhibiting jnk/erk mapk pathway. World J. Gastroenterol., 21; 16(3): 384-391.

Ansari, R.A., Tripathi, S.C., Patnaik G. K and Dhawan, B.N. 1991. Anti hepatotoxic properties of picroliv an active function from rhizome of picrorhiza kurroa. J. Ethnopharmacol., 34: 61-68.

Chenoweth, M. B and Hake, C.L. 1962. The smaller halogenated aliphatic hydrocarbons. J. Ann. Rev. Pharmacol., 2: 363-98.

Duncan, B.D., 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics, 13: 359 – 364.

Garba, S.H., Sambo, N and Bala, U. 2009. The effect of the aqueous extract of *Kohautia grandiflora* on paracetamol induced liver damage in albino rats. *Nigerian* Journal of Physiological Sciences. 24 (1): 17 –23.

Gupta, A and Misra, N., 2006. Hepatoprotective Activity of Aqueous Ethanolic Extract of *Chamomile capitula*

in Paracetamol Intoxicated Albino Rats. American Journal of Pharmacology and Toxicology, 1 (1): 17-20.

Hazai, E.,Vereczkey,L and Monostory, K., 2002. Reduction of toxic metabolite formation of acetaminophen. Biochemistry Biophysics Research Communication 291, 1089-1094.

Honma, T. and M. Sudha, 1997. Changes in plasma lipoprotein as toxicity markers for carbon tetrachloride, chloroform and dichloromethane. Indian Health, 34(4): 519-531.

King, E. J and A.R. Armstrong, 1980 . Determination of serum and bile phosphate activity. Ganad. Med. Assoc. J., 31: 376.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and R.J. Randall, 1951. Protein measurement with the folic phenol reagent. J. Biochem., 193: 163.

Malloy, H. T and E. Evelyn, 1937. The determination of bilirubin with photoelectric colormeter. Journal of Biochem., 119: 484-485.

Manokaran, S., Jaswanth, A., Sengottuvelu, S., Nandhakumar, J., Duraisamy, R., Karthikeyan, D., and Mallegaswari, R., 2008. Hepatoprotective Activity of *Aerva lanata* Linn. Against Paracetamol Induced Hepatotoxicity in Rats. Research J. Pharm. and Tech. 1(4): 398-400.

Masubuchi, Y., Suda, C., Horie, T., 2005. Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. J. Hepatology.42, 110–116.

Moss DW, Butterworth PJ. Enzymology and medicine. London: Pitman Medical; 1974.

Muthulingam, M., 2008. Antihepatotoxic effects of *Boerhaavia diffusa* L. on anti- tuberculosis drug, Rifampicin induced liver injury in rats. Journal of Pharmacology and Toxicology 3 (2): 75 – 83.

Nazneen, M., Abdul Mazid, Md., Kundu, J.K., Bachar, S.C., Begum, F., and Datta, B.K., 2009. Protective effects of *Flacourtia indica* aerial parts extracts against paracetamol-induced hepatotoxiciy in rats. Journal of Taibah University for Science, 2: 1-6.

Olaleye, T.M., Adegboye, O.O., and Akindahunsi, A.A., 2006. *Alchornea cordifolia* extract protects wistar albino rats against acetaminophen-induced liver damage. African Journal of Biotechnology., 5 (24): 2439-2445.

Plaa, G.L. and H.R. Hewitt, 1982. Quantitative evaluation of indices of hepatotoxicity. In: toxicology of the liver. Raven Press, New York, 103-120.

Poole, A. and G.B. Leslie, 1989. A practical approach to toxicological investigation, Cambridge, 44-86.

Prajapathi N.D., Purohit S.S., Sharma A., Kumar T.: A handbook of medicinal plants: A complete source book. Agrobios, India, 2004.

Predes, F.S., Pinto, S.L., Matta, D., Monteiro, J.C., and Oliveira, T.T.D., 2009. Investigation of Liver Tissue and Biochemical Parameters of Adult Wistar Rats treated with *Arctium lappa* L., Braz. Arch. Biol. Technol. 52 (2): 335-340.

Rajesh, S.V., Rajkapoor, B., Senthil kumar, R., and Raju, K., 2009. Effect of *Clausena dentata* (Willd.) M. Roem. against paracetamol induced hepatotoxicity in rats. Pak. J. Pharm. Sci., .22(1) : 90-93.

Rajkapoor, B., Venugopal, Y., Anbu, J., Harikrishnan, N., Gobinath, M. and Ravichandran, V., 2008. Protective effect of *Phyllanthus polyphyllus* on acetaminophen induced hepatotoxicity in rats. Pak. J. Pharm. Sci. 21(1): 57-62.

Ramasamy,M., 1987. Effects of sevin on blood free amino acids level of the fish *Sarotherodon mossambicus*. Environment and Ecology 5(4), 633-637.

Reitman, S. and S. Frankel, 1975. *In vitro* determination of transamination activity in serum. Am. J. Clin. Pathol., 28: 56.

Ringler, D.H. and L. Dabich, 1979. Haematology and clinical biochemistry in the laboratory Rat, Vol. I, ed. By H.J. Barker, J.R. Lindsey and S.H. Weisbroth, Academic Press, London, 105-118.

Roy, C.K., and Das, A.K., 2010. Comparative evaluation of different extracts of leaves of *Psidium guajava* Linn. for Hepatoprotective activity. Pak. J. Pharm. Sci., .23 (1): 15-20.

Sallie, R., J. Tredger and R. William, 1991. Drugs and the liver. Biopharmaceutics and drug disposition. 12: 251-259.

Schmidt, E., F.W. Schmidt, J. Mohr, P. Otto, I. Vido, K. Wrogeman and C. Herfarth, 1975. Liver morphology and enzyme release. Further studies in the isolated perfused rat liver. In Keppler (Ed) Parthinogenesis and mecahism of liver cell necrosis. Medical and Technical Publishing Co.Ltd., Lancaster. Pp. 147.

Simko V., 1991. Alkaline phosphatase in biology and medicine. Dig. Dis. Sci., 9: 189-209.

Slater, T.F., 1965. Biochemical studies on liver injury. In: Biochemical mechanism of liver injury. London: Academic Press, 1-44.

Song, Z., McClain, C.J., Chen, T., 2004. S-adenosylmethionine protects against acetaminophen-induced hepatotoxicity in mice. Pharmacology.71, 199–208.

Thomas, S.H.L, 1993. Paracetomol poisoning. Pharmacology and Therapeutics, 60, 91-120.

Tripathi, B., Srivastava, S., Rastogi, R., Raina, D., Jiram, V., and Srivastava, A., 2003. Hepatoprotection by 3-bromo-6-(4-chlorophenyl)-4-methylthio-2*H*-pyran-2-one against experimentally induced liver injury in rats. *Acta Pharm.* 53: 91–100.

Visen, P.K.S., Shukula, B., Patriak, N.,Dhawan,1993. Adrographloide protects rat hepatocytes against paracetomol kinduced damage. *J. Ethanopharmacol.* 40: 131-136.

Wadekar, R.R., Supale, R.S., Tiwari, K.M., Patil, K.S., and Jalalpuri, S.S., 2008. Screening of roots of *Baliospermum montanum* for hepatoprotective activity against paracetamol induced liver damage in albino rats. *International Journal of Green Pharmacy*. 220- 223.

Willianson, E.M., Okpako, D.T., Evans, F.J., 1962. Selection, preparation and pharmacological evaluation of plant material. England: John Wiley.

Zlatkis, A., Zak, B. and G.J. Boyle, 1953. A method for the determination of serum cholesterol. *J. Clin. Med.,* 41: 486-492.

****