Natural Product Sciences
9(3) : 186-191 (2003)

Hepatoprotective Effects and Antioxidant Role of *Caesalpinia bonducella* on Paracetamol-induced Hepatic Damage in Rats

Malaya Gupta*, Upal Kanti Mazumder, and Ramanathan Sambath Kumar

Department of Pharmaceutical Technology, Jadavpur University,Calcutta, India- 700 032.

Abstract – The hepatoprotective effect of methanol extract of leaves of *Caesalpinia bonducella* was studied by means of paracetamol induced liver damage in rats. The degree of protection was measured by using biochemical parameters such as serum transaminase (SGPT and SGOT), alkaline phosphatase (ALP), bilirubin, and total protein. Further, the effects of the extract on lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were estimated. The methanol extract of *C .bonducella* (MECB) (50,100 and 200 mg/kg) produced significant ($P<0.01$) hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin, and lipid peroxidation, while it significantly increased the levels of GSH, SOD, CAT, and protein in a dose dependent manner. The effects of MECB were comparable to that of standard drug Silymarin. However, at a lower dose (25 mg/kg) it could not restore the deleterious effect produced by paracetamol. The results indicate that *Caesalpinia bonducella* had antioxidant and hepatoprotective effects.

Key words – *Caesalpinia bonducella*, Hepatoprotective effects, Antioxidant role, Paracetamol.

Introduction

Aerobic organs, such as the liver generate reactive oxygen species that will induce oxidative tissue damage. These radicals, which react with cell membranes and thus induced lipid peroxidation or cause inflammation, have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes, gout and cancer (Slater *et al.*, 1984; Vuillaume *et al.*, 1987; Meneghini *et al.*, 1988). A major defence mechanism is the antioxidant enzymes which convert active oxygen molecules into non-toxic compounds (Halliwell and Gutteridge, 1984; Hochstein and Atallah, 1988).

Liver diseases remain one of the serious health problems. However, we do not have satisfactory liver protective drugs in allopathic medical practices for serious liver disorders. Herbal drugs play a role in the management of various liver disorders most of them speed up the natural healing processes of liver. Numerous medicinal plants and their formulations are used for liver disorder in ethnomedical practices as well as traditional system of medicine in India. More than 15 of these plants are evaluated for their hepatoprotective action in light of modern medicine (Subramoniam *et al.*, 1998).

*Author for correspondence
E-mail: Sambathju2002@yahoo.co.in

Caesalpinia bonducella F., (Family: Caesalpiniaceae) commonly known as Nata Karanja (Hindi), a prickly shrub found throughout the hotter parts of India, Myanmar, and Sri Lanka (Nadkarni, 1954). The leaves of this plant are traditionally used for the treatment of liver disorders (Kritikar and Basu, 1975). It has also been to posses multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic and antibacterial (Neogi and Nayak, 1958), anticonvulsant (Adesina, 1962), anti-anaphylactic and antidiarrheal (Iyenger and Pendse, 1965), antiviral (Dhar *et al.*, 1968), antiasthmatic (Gayaraja *et al.*, 1978),anti-inflammatory (Agarwal *et al.*, 1982; Vijaya sarathy *et al.*, 1981), antiamebic and, anti-estrogenic effects (Raghunathan and Mitra, 1982).

However, no work has been reported on the hepato-protective properties of this plant. Keeping this in view, the present study has been undertaken to investigate hepato-protective activity and antioxidant role of the methanol extract of *Caesalpinia bonducella* (MECB) leaves on paracetamol induced liver damage in rats.

Material and Methods

Plant materials – The plant *Caesalpinia bonducella* (Family: Caesalpiniaceae) was collected from Kolli Hills of Tamilnadu, India. The plant material was taxnomically identified by the Botanical Survey of India, Kolkata. A voucher specimen (No. GMS-2) has been preserved in

our laboratory for the future references.

Extraction – The leaves were dried under shade with occassional shifting and then powdered with a mechanical grinder and stored in an airtight container. The dried powder material of the leaf (500 g) was defatted with petroleum ether (60-80°) in a soxhlet apparatus. The defatted powder material thus obtained was further extracted with methanol for 72 hours in the soxhlet. The solvent was removed by distillation under suction and the resulting semisolid mass was vacuum dried using rotary flash evaporator to yield (8.78%) a solid residue (methanol extract). Phytochemical screening of the extract revealed the presence of alkaloids, saponins, flavonoids, triterpenes, tannins, and steroids.

Experimental animal – Wistar Albino rats (150-200 g) used in the present studies were procured from listed suppliers of Jadavpur University, Kolkata, India. The animals were fed with standard pellet diet supplied by Hindustan lever Ltd. Kolkata, and water ad *libitum*. All the animals were acclimatized for a week before use. MECB was dissolved in 10% propylene glycol.

Paracetamol induced liver damage in rats – Healthy albino rats were divided into 7 group of 6 animals in each. Group 1, which served as normal, received normal saline (0.9% w/v, NaCl), 5 ml/kg. Group 2 received paracetamol (500 mg/kg p.o) once daily for 7 days (control). Group 3,4,5, 6, and 7 received paracetamol (500 mg/kg, p.o.) and MECB (25,50,100 and 200 mg/kg p.o.) and standard drug silymarin (25 mg/kg) simultaneously for 7 days. The biochemical parameters were determined after 18 h fasting of the last dose.

Biochemical studies – The blood was obtained from all animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters namely, SGPT, SGOT (Bergmeyer *et al.*, 1978), SALP (King, 1965), Serum bilirubin (Malloy *et al.*, 1937), and Protein (Lowry *et al.*, 1951).

After collection of blood samples the rats were sacrificed and their livers excised, rinsed in ice cold normal saline followed by 0.15 M Tris-Hcl (pH 7.4) blotted dry and weighed. A 10% w/v of homogenate was prepared in 0.15 M Tris-Hcl buffer and processed for the estimation of lipid peroxidation (Ohkawa *et al.*, 1979). A part of homogenate after precipitating proteins with Trichloric acetic acid (TCA) was used for estimation of glutathione (Ellman, 1958). The rest of the homogenate was centrifuged at 15000 rpm for 15 minutes at 4°C. The supernatant thus obtained was used for the estimation of SOD (Kakkar *et al.*, 1984), CAT (Luck, 1971) activities, and protein content (Lowry *et al.*, 1951).

Statistical analysis – The experimental results were expressed as the mean±S.E.M. Data were assessed by the method of analysis of ANOVA, p value of < 0.05 was considered as statistically significant.

Results

The effect of MECB on serum transaminase, alkaline phosphatase, bilirubin, and total protein levels in paracetamol induced liver damage rats are summarized in Table 1. There was a significant ($P<0.01$) increase in the serum GPT, GOT, ALP and bilirubin levels and decrease in the total protein level ($P<0.01$) in paracetamol control group as compared with normal group, which was reversed on administration of (50,100 and 200 mg/kg) MECB. These effect were comparable to standard drug Silymarin (25 mg/kg). However, MECB at a dose of 25 mg/kg was unable to produce similar effects.

The effects of MECB on rat liver lipid peroxidation, glutathione, and antioxidant enzyme levels are shown in (Fig. 1 and 2). Lipid peroxidation levels (expressed in term of malondialdehyde {MDA} formation) are significantly high in paracetamol control rats compared with the normal

Table 1. Effect of MECB and Silymarin on serum enzymes (SGPT, SGOT and ALP), bilirubin and total protein on paracetamol induced liver damage in rats.

Treatment	Dose (mg/kg)	SGPT (IU/L)	SGOT (IU/L)	SALP (IU/L)	Bilirubin (mg/dl)	Total protein (mg/dl)
Normal saline	5 ml/kg	65.5 ± 3.62	76.66 ± 3.31	31.75 ± 2.12	0.98 ± 0.01	7.01 ± 0.42
Paracetamol (control)	500	114.44 ± 5.24[a]	152.45 ± 7.42[a]	61.0 ± 3.55[a]	1.94 ± 0.01[a]	5.58 ± 0.34[a]
Paracetamol+Silymarin	500 + 25	65.78 ± 2.33[b]	74.23 ± 5.34[b]	34.73 ± 2.32[b]	1.07 ± 0.01[b]	7.18 ± 0.53[b]
Paracetamol+MECB	500 + 25	109.55 ± 5.31[b]	146.66 ± 8.42[b]	58.43 ± 3.54[b]	1.87 ± 0.01[b]	5.48 ± 0.36[b]
Paracetamol+MECB	500 + 50	98.45 ± 5.35[b]	124.32 ± 7.27[b]	52.56 ± 3.17[b]	1.71 ± 0.01[b]	5.93 ± 0.33[b]
Paracetamol+MECB	500 + 100	82.74 ± 3.45[b]	108.54 ± 6.73[b]	46.78 ± 3.58[b]	1.47 ± 0.01[b]	6.56 ± 0.43[b]
Paracetamol+MECB	500 + 200	67.38 ± 4.31[b]	84.33 ± 4.81[b]	35.25 ± 2.42[b]	1.22 ± 0.01[b]	7.10 ± 0.35[b]

Values are mean±S.E.M: n = 6 animals in each group.
[a] $P<0.01$ Control group Compared with normal group.
[b] $P<0.01$ Experimental groups Compared with control.

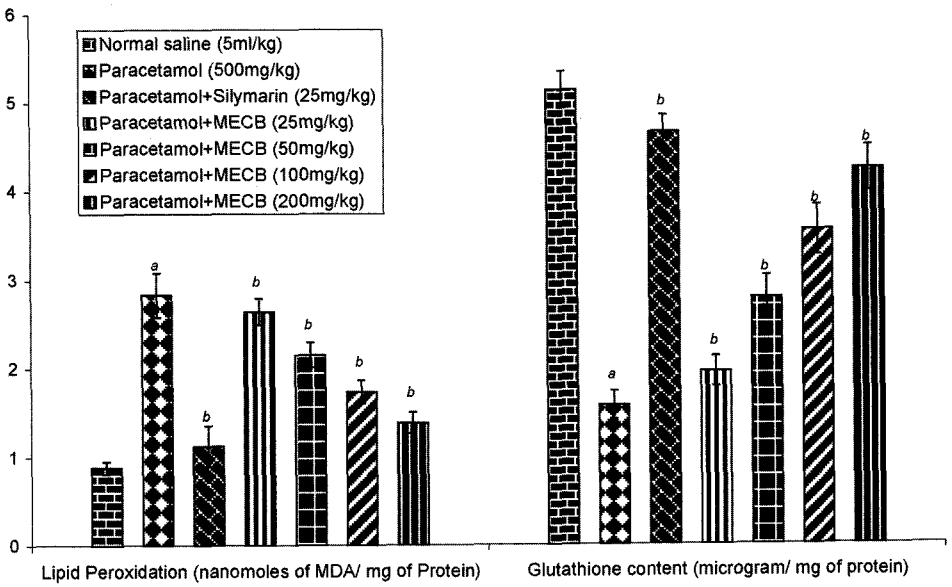

Fig. 1. Effect of MECB and silymarin on liver lipid peroxidation and glutathione levels on paracetamol induced liver damage in rats. Values are mean±SEM.
n = 6 animals in each group.
[a]P<0.01, Control group compared with normal group.
[b]P<0.01, Experimental group compared with control group.
MDA = Malondialdehyde.

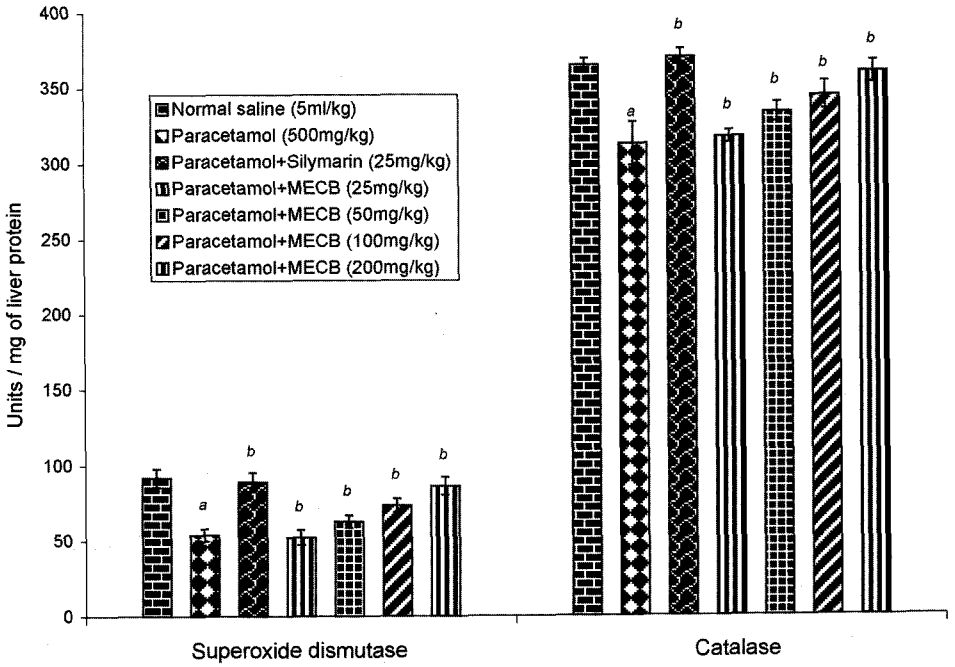

Fig. 2. Effect of MECB and silymarin on superoxide dismutase and catalse enzyme specific activity on paracetamol induced liver damage in rats values are mean±SEM
n = 6 animals in each group.
[a]P<0.01, Control group compared with normal group.
[b]P<0.01, Experimental group compared with control group.

rats ($P<0.01$). Treatment with MECB (50,100 and 200 mg/kg) significantly prevented the increase in MDA levels ($P<0.01$) and bring them near to normal level. Liver enzymatic and non enzymatic antioxidant levels were significantly altered in paracetamol treated rats compared with normal group ($P<0.01$). GSH, SOD, and CAT levels were significantly increased ($P<0.01$) in MECB (50,100 and 200 mg/kg) treated group. The effects of MECB were comparable to that of silymarin. However, lower dose (25 mg/kg) of MECB could not restore the deleterious effect produced by paracetamol.

Discussion

Paracetamol (Acetaminophen) is a widely used antipyretic analgesic produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates (Jollow *et al.*, 1974; Wong *et al.*, 1981). However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 (Savides and Oehme, 1983) to a highly reactive metabolites N-acetyl-P-benzoquinoneimine (NAPQI) (Vermeulen *et al.*, 1992). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid (Moore *et al.*, 1985). However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of protein and alters the homeostasis of calcium after depleting GSH.

The elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotmann and Lowhorn, 1978). Serum ALP and bilirubin level on other hand, are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure (Moss and Butterworth, 1974). Treatment with MECB decreases the serum levels of GPT, GOT towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell.

Lipid peroxidation has been postulated to the destructive process of liver injury due to acetaminophen administration (Muriel *et al.*, 1992). In our study, elevations in the levels of end products of lipid peroxidation in liver of rat treated with paracetamol were observed. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with MECB significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of MECB is due to its antioxidant effect.

Glutathione is one of the most abundant tripeptide non-enzymetic biological antioxidant present in the liver. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, maintenance of membrane protein thiols and as a substrate for glutathion peroxidase and GST (Jai Prakash *et al.*, 2001). In our present study the decreased level of GSH has been associated with an enhanced lipid peroxidation in paracetamol treated rats. Administration of MECB significantly increases the level of glutathione in a dose dependent manner.

Increase in the serum activity of superoxide dismutase is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury (Curtis *et al.*, 1972; Korsrud *et al.*, 1973). 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, hence diminishing the toxic effect caused by this radical. In the present study, it was observed that the MECB caused a significantly increased in the hepatic SOD activity of the paracetamol induced liver damage rats. It means that the MECB can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Catalase is an enzymatic antioxidant widely distributed in all animal tissue and the highest activity is found in the red cells and in liver. Catalases decompose hydrogen peroxide and protect the tissue from highly reactive hydroxyl radicals (Chance *et al.*, 1952). Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of MECB increases the activities of catalase in paracetamol induced liver damage rats to prevent the accumulation of excessive free radicals and protects the liver from paracetamol intoxication.

These altered biochemical profiles due to paracetamol treatment were significantly reversed towards normalization by MECB. The maximum protection against paracetamol induced hepatic injury was afforded by the 200 mg/kg dose, which reversed the elevated levels of liver markers

enzyme near to normal values.

It may be concluded that the MECB exhibits significant hepatoprotective property as it reduces cell membrane disturbances induced *in vivo* by paracetamol. Therefore, the hepatoprotective effect of MECB as claimed by the traditional system has a sound scientific basis. Further investigation is underway to determine the exact phyto-constituents that are responsible for its hepatoprotective effect.

Acknowledgement

One of the author Ramanthan Sambath Kumar (Senior Research Fellow), grateful to AICTE, New Delhi, India, for providing financial support to this work.

Reference

Adesina, S.K., Studies on some plants used as anticonvulsants in amerindian and african traditional medicine. *Fitoterapia.*, **53,** 147-162 (1982).

Agrawal, R.C. and Kapadia, L.A., Treatment of piles with indigenous drug-Pilex tablets and ointment alone with styplone. *Probe.*, **21,** 201-204 (1982).

Bergmeyer, H.U., Scheibe, P. and Wahlefeld, A.W., Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clin. Chem.*, **24,** 58-61 (1978).

Chance, B., Green Stein, D. S. and Roughton, R. J. W., The mechanism of catalase action 1-steady state analysis. *Arch. Biochem. Biophys.*, **37,** 301-339 (1952).

Curtis, S. J., Moritz, M. and Snodgrass, P. J., Serum enzymes derived from liver cell fraction and the response to carbon tetrachloride intoxication in rats. *Gastroenterology.*, **62,** 84-92 (1972).

Dhar, M. L., Dhar, M. M., Dhawan, B. N., Mehrotra, B. N. and Roy, C., Screening of Indian medicinal plant for biological activity. *Ind. J. Expt. Biol.*, **6,** 232-247 (1968).

Drotman, R. B. and Lawhorn, G. T, Serum enzymes are indicators of chemical induced liver damage. *Drug. Chem. Toxicol.*, **1,** 163-171 (1978).

Ellman, G. L., Tissue sulphydryl groups. *Arch. Biochem. Biophys.*, **82,** 70-77 (1959).

Gayaraja. S., Shinde, S. and Agarwal, S.L., Antiasthmatic properties of *Caesalpinia bonduc* leaves. *Ind. J. Pharmacol.*, **10,** 86-88 (1978).

Halliwell, B. and Gutteridge, J. M. C., Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. *Lancet.*, **23,** 1396-1397 (1984).

Hochstein. P. and Atallah, A. S., The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. *Mutat. Res.*, **202,** 363-375 (1988).

Iyengar, M. A. and Pendse, G. S., Anti-diarrhoeal activity of the nut of *Caesalpinia bonducella* F. *Ind. J. Pharmacol.*, **27,** 307-308 (1965).

Jollow, D. J.,Thorgeirsson, S. S., Potter, W. Z., Hashimoto, M. and Mitchell, J. R., Acetaminophen induced hepatic necrosis VI. Metabolic disposition of toxic and non- toxic doses of acetaminophen. *Pharmacology.*, **12,** 251-271 (1974).

Kakkar, P., Das, B. and Viswanathan, P. N., A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys.*, **21,** 131-132 (1984).

King. J., The hydrolases-acid and alkaline phospatase, *Practical Clinical Enzymology*, Van, D (ed.), Nostrand company Ltd, London, pp. 191-208, (1965).

Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants,* Vol II, Dehradum, India, Bishen mahendra pal singh. pp. 842-844, (1975).

Korsrud, G. O., Grice, H. G., Goodman, R. K., Knipfel, S. H. and Mc Laughlan, J. M., Sensitivity of several enzymes for the detection of thioacetamide, nitrosamine-and diethanolamine-induced liver damage rats. *Toxicol. Appl. Pharmacol.*, **26,** 299-313 (1973).

Luck, H., Catalase, In *Methods of enzymatic analysis*, edited by. Bergmeyer, H.U., Vol III, Academic Press, New York, p. 885. (1971).

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the folin- phenol reagent. *J. Biol. Chem.*, **193**, 265-275 (1951).

Meneghini, R., Genotoxicity of active oxygen species in mammalian cells. *Mutat. Res.*, **195,** 215-230 (1988).

Malloy, H. J. and Evelyn K. A., *J. Biol. Chem.*, **119,** 481 (1937).

Moore, M., Thor, H., Moore, G., Nelson, S., Moldeus, P. and Orrenius, S., The toxicity of acetaminophen and N-acetyl P-benzoquinone-imine in isolated hepatocytes is associated with thio depletion and increased cystosolic Ca^{2+}. *J. Biol.Chem.*, **260,** 13035-13040 (1985).

Moss, D. W. and Butterworth, P. J., *Enzymology and Medicine.* Pitman Medical, London, p. 139 (1974).

Muriel, P., Garciapina, T., Perez-Alvarez, V. and Murelle, M., Silymarin protect against paracetamol-induced lipid peroxidation and liver damage. *J. Appl. Toxicol.*, **12,** 439-442 (1992).

Nadkarni, A. K., In: *Indian Materia Medica.* Popular Prakashan. Bombay, India, p. 229. (1954).

Neogi, N. C. and Nayak, K. P., Biological investigation of *Caesalpinia bonducella* F, *Ind. J. Pharmacol.*, **20,** 95-100 (1958).

Ohkawa, H., Onishi, N. and Yagi, K., Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.*, **95,** 351-358 (1979).

Prakash, J., Gupta, S. K., Kochupillai, V., Singh, N., Gupta, Y. K. and Joshi. S., Chemopreventive activity of *Withania somnifera* in experimentally induced fibrosarcoma tumours in swiss albino mice. *Phytother. Res.*, **15,** 240 - 244 (2001).

Raghunathan, K., Mitra, R. and Karanja. S., In: Raghunathan, K. and Mitra. R., Editors, *Pharmacognosy of Indogenous Drugs.*

Part-1, Central Council for Research in Ayurveda and Siddha, New Delhi, India. pp. 484-510 (1982).

Savides, M. C. and Oehme, F. W., Acetaminophen and its toxicity. *J. Appl. Toxicol.*, **3,** 95-111 (1983).

Subramoniam, A., Evans, D.A. and Rajasakharan, S., Pushpangadan., Hepatoprotective activity of *Trichopus Zeylanicus* extracts against paracetamol induced damage in rats. *Ind. J. Expt. Biol.*, **36,** 385-389 (1998).

Slater, T. F., Free-radical mechanisms in tissue injury. *Biochem J.*, **222,** 1-15 (1984)

Vermeulen, N. P. E., Bessems, J. G. M. and Van de streat, R., Molecular aspects of paracetamol induced hepatotoxicity and it mechanism based prevention. *Drug Metab. Rev.,* **24,** 367-407 (1992).

Vijayasarathy, V., Sharma, L. K. and Prakash, A., Indigenos drug treatment for hemorrhoids. *Probe.*, **20,** 285-287 (1981).

Vuillaume, M., Reduced oxygen species, mutation, induction and cancer initiation. *Mutat. Res.*, **186,** 43-72 (1987).

Wong, L. T., Whitehouse, L. W., Solemonraj, G. and Paul, C. J., Pathways of acetaminophen conjugate in the mouse. *Toxicity. Lett.*, **9,** 145-151 (1981).

(Accepted September 1, 2003)