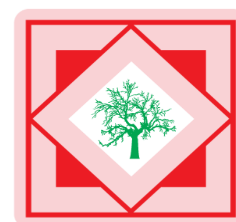
Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2011, 2 (3): 156-163



ISSN: 0976-8688
CODEN (USA): PSHIBD

Exploration of *in vivo* antioxidant potential of *Citrus maxima* leaves against paracetamol induced hepatotoxicity in rats

Sriparna KunduSen[1], Malaya Gupta[1], Upal K Mazumder [1], Pallab K Haldar[1*], Siva P Panda[1] and Sanjib Bhattacharya[2]

[1]Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India
[2]Bengal School of Technology, Sugandha, Hooghly, West Bengal, India

---

ABSTRACT

Pomelo or shaddock (Citrus maxima) is a widely cultivated and consumed citrus variety in India. In the present study, the methanol extract of Citrus maxima (J. Burm.) Merr. leaves (MECM) was evaluated for its in vivo antioxidant and hepatoprotective activity against paracetamol induced hepatotoxicity in Wistar albino rats. The results of the present study established the hepatoprotective and antioxidant activity of MECM at the dose levels of 200 and 400 mg kg^{-1} body weight and showed dose dependent decrease in the bilirubin, total protein and serum hepatic enzyme levels in comparison to the paracetamol control rats. By MECM treatment, the hepatic TBARS levels were decreased and GSH and catalase levels were increased as comparable to the silymarin treated group (25 mg kg^{-1} b.w.) which served as reference. The data were supplemented by histopathological studies of the rat liver sections.

Key words: Hepatotoxicity, paracetamol, silymarin, *Citrus maxima.*

INTRODUCTION

Liver damage is a widespread pathology which in most cases involves oxidative stress and is characterised by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [1]. Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies, have potential adverse side-effects, especially if administered chronically or sub-chronically. Therefore, herbal products and traditional medicines with better effectiveness and safe profiles are needed as a substitute for chemical therapeutics. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants have been proposed as therapeutic agents, as well as drug co-adjuvants, to counteract liver damage [1].

Production of free radicals or reactive oxygen species (ROS) was ascertained to play multiple important roles in tissue damage and loss of function in a number of tissues and organs [2].

Paracetamol is a widely used over-the-counter analgesic and antipyretic drug. It is commonly used in relief of fever, headaches, and other minor aches and pains. Paracetamol is primarily metabolised by the liver. Most of the metabolites are inactive, non-toxic and eventually excreted by the kidneys. But excessive use of paracetamol can damage multiple organs and the toxicity is from one of the metabolites, N-acetyl-*p*-benzoquinoneimine (NAPQI) [3]. Paracetamol hepatotoxicity is the most common cause of acute liver failure [4]. New restrictions should be placed to protect people from the potential toxic effects of paracetamol. Research and development of potential drugs used for treatment of liver damage caused by paracetamol is thus solicitated [5].

Hepatoprotective effects have been associated with plant extracts rich in antioxidants [1, 6]. Silymarin, one of these compounds, was used as a standard reference and exhibited significant hepatoprotective and antioxidant activity against paracetamol induced hepatotoxicity in rat models [7].

The plant, *Citrus maxima* (J. Burm.) Merr. (Rutaceae), is commonly known as shaddock or pomelo. The plant is indigenous to tropical parts of Asia. The plant is cited as antitoxic, appetizer, cardiac stimulant and stomach tonic in ancient and medieval literature [8]. *Citrus maxima* and *C. grandis* (family: Rutaceae) are both currently used for the shaddock or pomelo. Although *C. grandis* (L.) Osbeck is more frequently used, *C. maxima* (J. Burman) Merrill is correct under the International Code of Botanical Nomenclature (ICBN) [9].

The major flavanones of Pomelo are neohesperidin and naringin, which are high in the seed case of unripe citrus fruits [10] and its extract showed antioxidant activity through free radical-scavenging *in vitro* and to reduce reactive oxygen species in H_2O_2-treated HepG2 cells [11]. *C. maxima* essential oil is composed of α-pinene, sabinine, 3-β-pinine, 4 methyl heptenone, 5-β-myrcene, hexanal, sabinine, DL-limonine, t-ocimine, linalool, 1-hexene, 4-methyl-1-hexene-3,3-dimethyl, geranyl formate, Z-citral, geranyl formate, E-citral, geranyl acetate, β-farnesene [12]. Hespiridin, naringin, caffeic, *p*-coumaric, ferulic and vanillic acid are present in the fruit juice [13].

In vitro free radical scavenging activity of MECM was explored and the extract was found to be having significant free radical scavenging activity when tested against different free radicals [14]. Antiinflammatory activity of MECM was explored against different acute models of inflammation [15]. The antitumor activity of MECM was investigated against Ehrlich's ascites carcinoma in Swiss albino mice [16]. The current study aims to explore the *in vivo* hepatoprotective and antioxidant activity of methanol extract of *Citrus maxima* Merr. leaves (MECM) in rats.

MATERIALS AND METHODS

Plant material and extraction

The fresh leaves of *C. maxima* were dried under shade and powdered by mechanical grinder. About 500 g of the plant material was successively extracted with petroleum ether and methanol in a Soxhlet apparatus. The methanol was then evaporated under reduced pressure to get the crude extract (MECM, yield: 18.1%).

Drugs and chemicals

Silymarin 70 mg capsules was purchased from Micro Labs, Hosur, Tamilnadu, India. Thiobarbituric acid, nitrobluetetrazolium chloride (NBT), were from Loba Chemie, Bombay, India; 5,5-dithio *bis*-2-nitrobenzoic acid (DTNB), carbon tetrachloride were from Sisco Research Laboratory, Bombay, India. All the other solvents and chemicals used were of analytical reagent grade obtained commercially.

Animals

Six to eight week old male Wistar albino rats (180 ± 20 g) were obtained. The animals were kept at 25 ± 2°C and a relative humidity of 40- 45% with alternative day and night cycles of 12 h each. All animals were acclimatized to identical laboratory conditions for seven days prior to the study. The animals had free access to pellet food (Hindustan Lever, Mumbai, India) and water *ad libitum*. All experimental procedures were reviewed and approved by the University Animal Ethics Committee, Jadavpur University (367001/C/ CPCSEA).

Acute toxicity

The acute toxicity of the extract was determined according to the OECD guideline No. 420 (15). Male albino mice weighing 27-30 g were used for this study. MECM was given to four groups (n = 5) of animals at 5, 50, 300 and 2000 mg kg^{-1} b.w. p.o. The treated animals were observed up to 14 days, for mortality and general behaviour.

Paracetamol induced liver damage in rats:

Male Wistar albino rats were divided into 5 groups (n = 6). The group I served as saline control. The group II-V received 650 mg/kg b.w. paracetamol in 1% sodium carboxy methylcellulose (CMC) p. o. [17]. Group II served as negative control. Three hours after the paracetamol induction the group III and IV received MECM at the dose of 200 and 400 mg/ kg b.w., p. o. and group V received standard drug silymarin (25 mg/kg b.w., p. o.) once in a day for 14 days [7]. 24-Hours after the last dose and 18 hours of fasting condition, the blood were collected by puncturing retro-orbital plexus for estimation of various biochemical parameters.

Biochemical studies

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, SALP and serum bilirubin [18-20].

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 (expressed as thiobarbituric acid reactive substances, TBARS) by the method of Ohkawa et al., 1979 [21]. A part of homogenate after precipitating proteins with trichloro acetic acid (TCA) was used for estimation of glutathione (expressed as reduced glutathione, GSH) by the method of Ellman, 1959 [22]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of catalase (CAT) using the method of Aebi, 1974 [23].

Histopathological observation in the liver

The rat liver tissues were fixed with 10% formalin buffer solution (pH 7.4) for 24 h and dehydrated with a sequence of ethanol solution and embedded in paraffin. The serial sections

were cut 5 µm thick and stained with haematoxylin-eosin (HE), and then observed for the changes of liver injury by photomicroscope.

Statistical analysis

All results are expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way (ANOVA) followed by Dunnett's test using Graph Pad Prism ver. 5.0, Graph pad software, USA. The p values of $p<0.05$ and $p<0.001$ were considered to be significant and highly significant respectively.

RESULTS AND DISCUSSION

The effect of MECM and silymarin on different serum biochemical parameters in paracetamol treated rats is given in figure 1. MECM at the dose level of 400 mg kg^{-1} body weight was successful in restoration of the serum biochemical parameters as compared to the paracetamol control group.

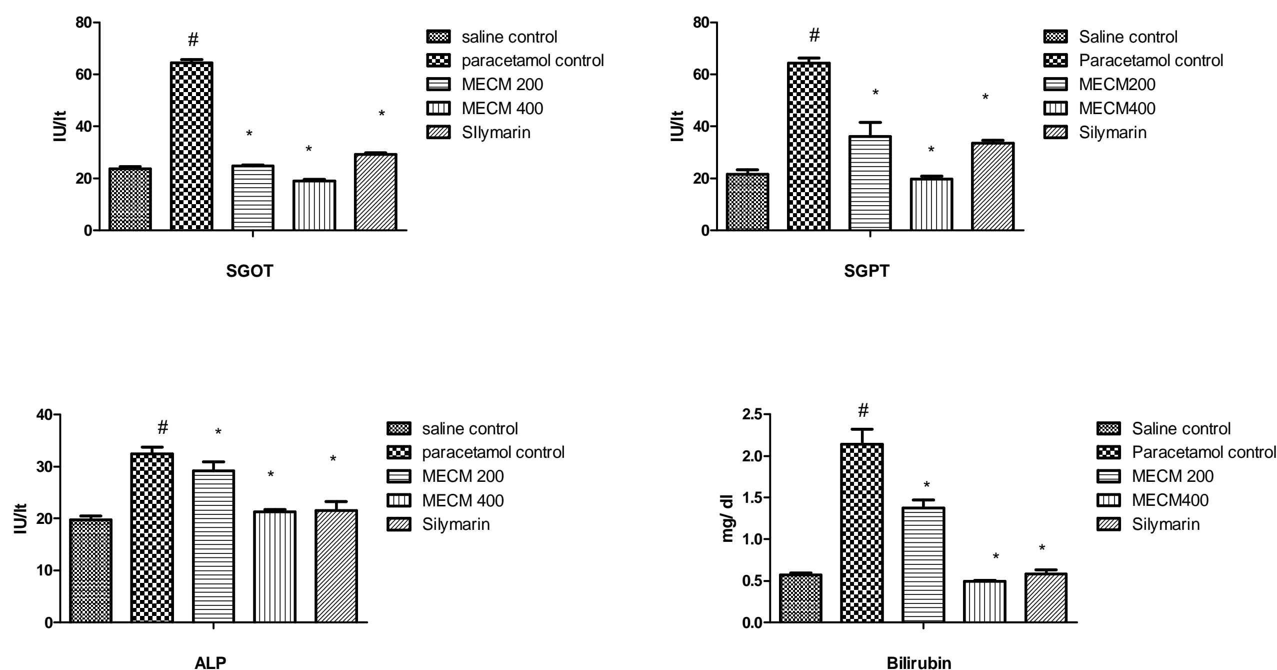


Fig. 1. Effect of MECM on serum SGPT, SGOT, ALP and bilirubin in paracetamol treated rats. (*n* = 6).
*Values expressed as mean ± SEM, #p < 0.001 when tested against saline control. *p < 0.001 when tested against paracetamol control.*

The effect of MECM on thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and catalase activities in the liver tissues of the paracetamol treated rats is given in figure 2. The reduced level of GSH and catalase in paracetamol control rats are almost restored to the normal level by MECM in a dose-dependent manner. The increased TBARS level in paracetamol control rats is also restored to normal level by MECM. The results of the present study showed that the methanol extract of *Citrus maxima* leaves at the dose of 400mg kg^{-1} have some degree of hepatoprotective ability as seen in fig. 3D, where there was fewer necrotic cells and wider sinusoidal spaces when compared with the negative control group (Fig. 3B) that showed marked

distorted hepatic cords, necrotic cells and obliterated sinusoids. Paracetamol was used in this study to induce the liver damage (fig. 3B) and it was reported to be hepatotoxic [7,17]. Based on the results obtained, we therefore inferred that *Citrus maxima* leaf extract has some protective effect on the liver as shown by the reduced damage in group III (fig. 3).

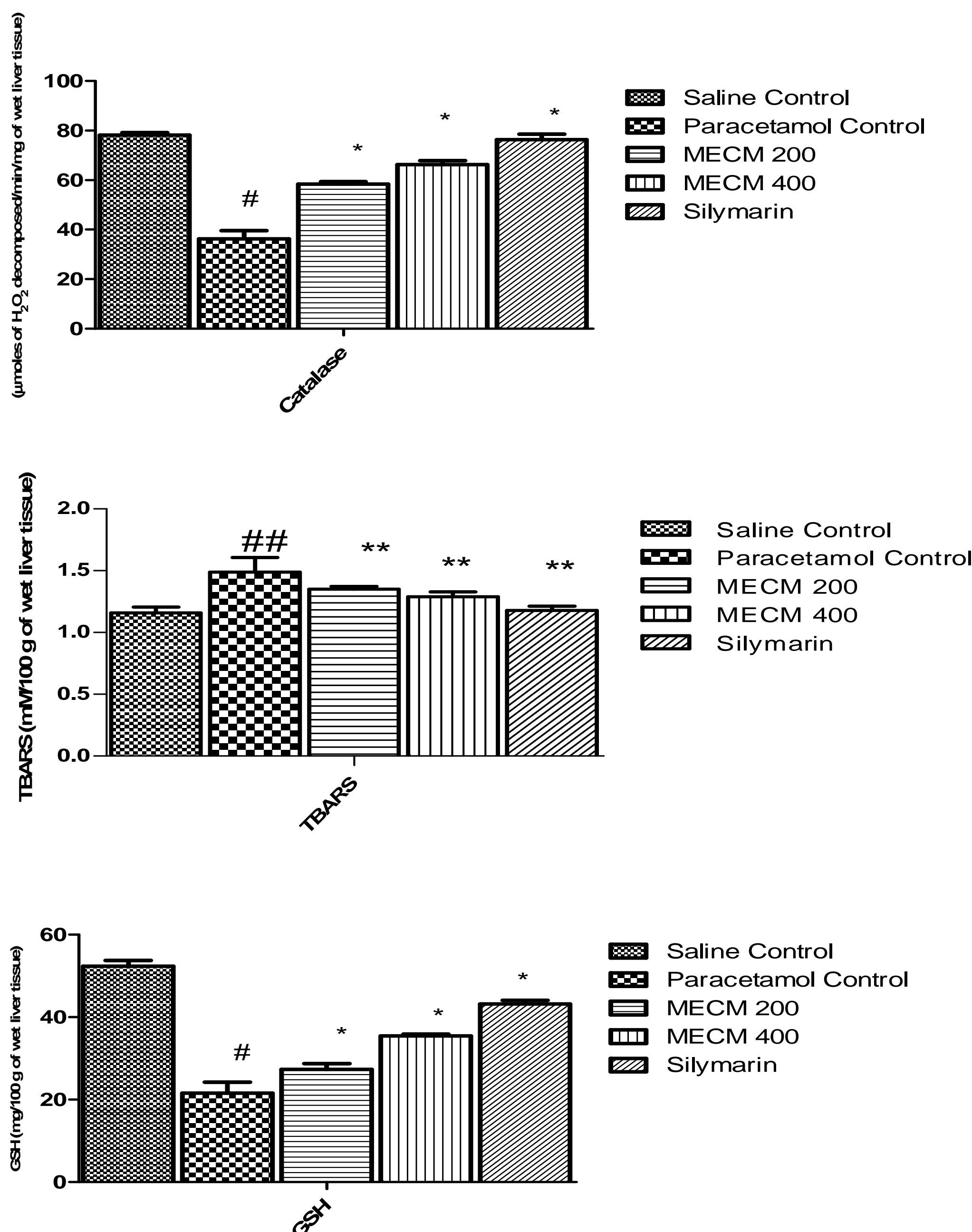


Fig. 2. Effect of MECM on catalase, TBARS and GSH levels in liver tissues of paracetamol treated rats.
*Values expressed as mean ± SEM. #p < 0.001 when tested against saline control. *p < 0.001 when tested against paracetamol control. ##p < 0.05 when tested against saline control. **p < 0.05 when tested against paracetamol control.*

Liver is an organ involved in many metabolic functions and is prone to xenobiotic injury because of its central role in xenobiotic metabolism. Hepatotoxic drugs like paracetamol cause damage to the liver [24].

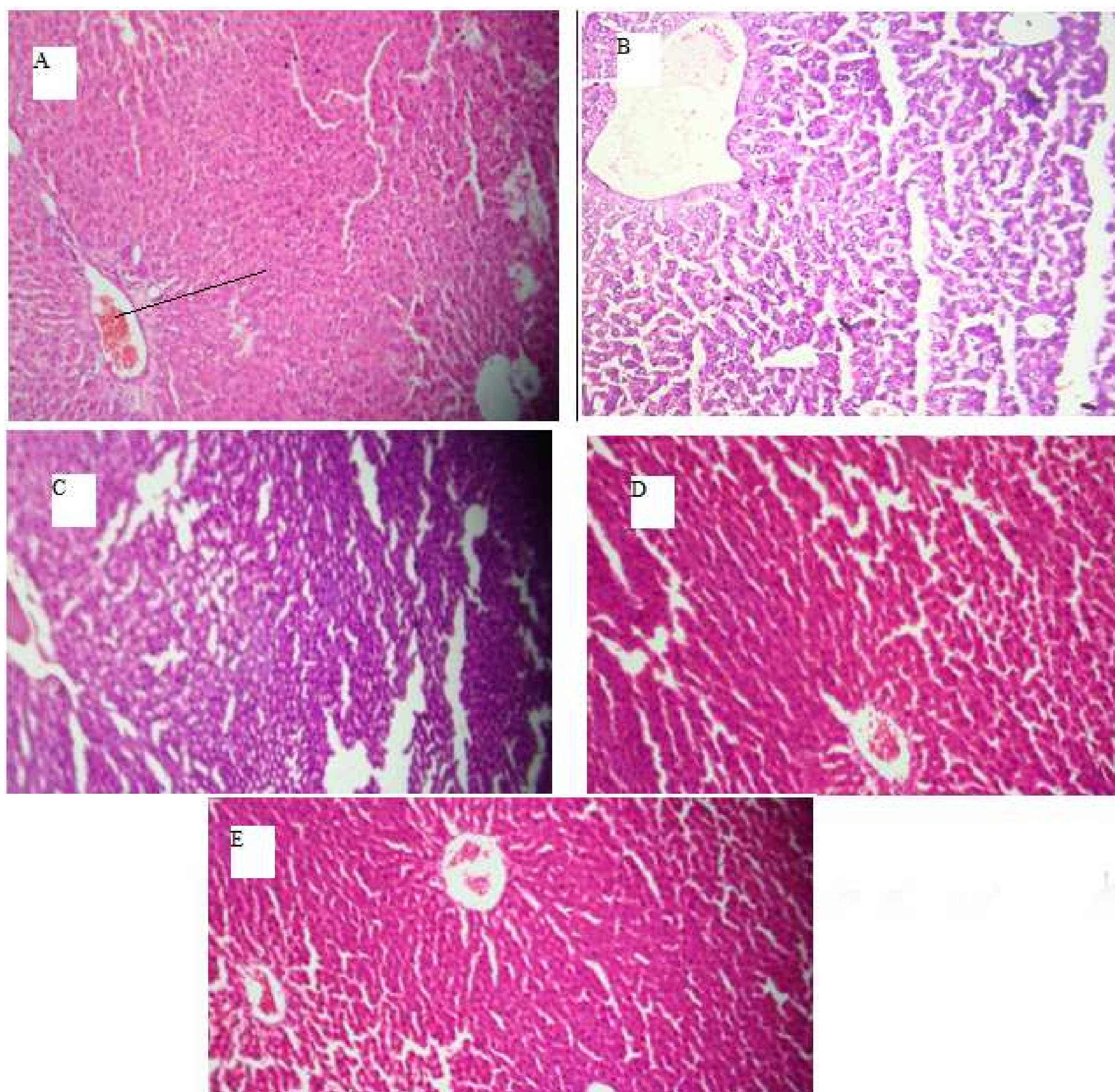


Fig. 3. Histopathological sections of liver. A: Normal liver having normal histological structures of hepatic lobules. B: Toxicant treated animal liver showing hepatocellular vacuolization and focal hepatic necrosis. C: MECM 200 mg kg^{-1} treated liver showing mild vacuolization. D: MECM 400 mg kg^{-1} treated liver showing normal hepatocytes. E: Silymarin treated liver showing normal hepatocytes.

When the liver cell membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. In the present study, the activity of SGOT, SGPT and ALP was found to be significantly decreased in MECM administered rats when compared to paracetamol control. The activity of SGOT and SGPT was found to be significantly increased in paracetamol induced animals. The serum bilirubin level was also restored as compared to the paracetamol control animals. These findings are in accordance with the findings of Gupta et al., 2004 [7].

High dose of paracetamol and its metabolized product N-acetyl-*p*-benzoquinoneimine (NAPQI) can alkylate, and oxidise intracellular GSH and protein thiol groups, which results in the depletion of GSH and subsequently leads to the increase of lipid peroxidation and hepatic damage [5].

Catalase is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and liver. It decomposes H_2O_2 and protects the tissue from highly reactive hydroxyl and is thought to be the first line of defence against oxidative damage caused by H_2O_2 and other radicals induced by carcinogen. Inhibition of these protective mechanism results in enhanced sensitivity to free radical induced cellular damage [25].

As described in figure 2, MECM at the dose level of 400 mg/kg body weight was able to decrease the increased lipid peroxidation in paracetamol treated rat liver to the normal level comparable to that of saline control. It was also able to restore the depleted catalase and reduced glutathione levels in paracetamol intoxicated rat liver to the normal levels indicating the *in vivo* antioxidant potential of MECM in paracetamol challenged rats.

General mechanisms of hepatotoxicity induced by drug may include reactive metabolite formation, antioxidant depletion, and protein alkylation. For paracetamol, the intrinsic toxicity mainly depends on the expression of genetic variants and hepatocyte death typically follows an apoptotic or necrotic pathway mainly depending on predisposing factors [5].

The histopathological studies have also shown that the hepatocellular vacuolization and focal hepatic necrosis in paracetomol control animals is significantly reduced in the MECM 400 mg/kg treated animals and silymarin treated animals.

The present study, therefore demonstrated that the methanol extract of *Citrus maxima* leaves showed favourable *in vivo* antioxidant potency to protect the paracetamol induced liver damage in rats, at the dose of 400 mg/kg body weight, which suggested that MECM could be used as a potential hepatoprotective agent.

Acknowledgement
The authors are thankful to the authority of Jadavpur University, Kolkata 700032, India, for providing the necessary facilities for the present study.

REFERENCES

[1] A. Srivastava, T. Shivanandappa. *Food Chem*., **2010**,118, 411–417.
[2] A.R. Verma, M.Vijaykumar, C.S. Mathela, C.V. Rao. *Food Chem. Toxicol*., **2009**, 47, 2196-2201.
[3] J.R. Mitchell, D.J. Jollow, W.Z. Potter, J.R. Gillette, B.B. Brodie. *J. Pharmacol. Exp Ther.*, **1973**, 187, 211–217.
[4] A.M. Larson, J. Polson, R.J. Fontana, T.J. Davern, E. Lalani, L.S. Hynan. *Hepatol*., **2005**, 42, 1364–1372.
[5] Y.T. Liu, B.N. Lu, J.Y. Peng. *Food Chem*., **2011**, 125, 719–725.
[6] P. Shanmugasundaram, S. Venkataraman. *J. Ethnopharmacol*., **2006**, 104, 124–128
[7] M. Gupta, U.K. Mazumder, T.S. Kumar, P. Gomathi, R.S. Kumar. *Iranian J. Pharmacol. Ther*., **2004**, 3,12-20.
[8] B.A. Arias, L. Ramon-Laca. *J. Ethnopharmacol*., 2005, 97, 89 – 95.
[9] R.W. Scora, D.H. Nicolson. *Taxon*, **1986**, 35, 592-595.
[10] S.K. Chung, S.H. Kim, Y.H. Choi, E.Y. Song, S.H. Kim. *Food Ind. Nutr*., **2000**, 5, 42–52.
[11] H.K. Lim, E.S. Yoo, J.Y. Moon, Y.J. Jeon, S.K. Cho. *Food Sci. Biotech*., **2006**, 15, 312–316.

[12] P. Singh , R. Shukla , B. Prakash, A. Kumar , S. Singh , P.K. Mishra , N.K. Dubey . *Food Chem. Toxicol*., **2010**, 48 , 1734–1740.
[13] G. Xu, D. Liu, J. Chen, X. Ye, Y. Ma, J Shi. *Food. Chem*., **2008**, 106, 545–551.
[14] S. KunduSen, P. Saha, S. Bhattacharya, A. Bala, U.K. Mazumder, M. Gupta, P.K. Haldar. *Pharmacologyonline*, **2010**, 6, 850-857.
[15] S. KunduSen, M. Gupta, U.K. Mazumder, P.K. Haldar, P. Saha. S. Bhattacharya, S. Naskar, S.P. Panda. *Pharmacologyonline*, **2011**, 7, 702-709.
[16] S. KunduSen, M. Gupta, U.K. Mazumder, P.K. Haldar, P. Saha. *ISRN Pharmacol*., **2011**, doi:10.5402/2011/138737.
[17] S.P. Lim, F.J. Andrews, P.E. O'Brien. *Hepatol*., **1995**, 22, 1776-81.
[18] H.U. Bergmeyer, P. Scheibe, A.W. Wahlefeld. *Clin. Chem*., **1978**, 24, 58-61.
[19] J. King. The hydrolases-acid and alkaline phospatase, In: D. Van, (Ed.) Practical Clinical Enzymology (Nostrand Company Ltd., London, 1965) 191.
[20] H.J. Malloy, K.A. Evelyn. *J. Biol. Chem*., **1937**, 119, 481.
[21] H. Ohkawa, N. Onishi, K. Yagi. *Anal. Biochem*., **1979**, 95, 351-358.
[22] G.L. Ellman. *Arch. Biochem. Biophys*., **1959**, 82, 70-77.
[23] K.A. Sinha. *Annal. Biochem*., **1972**, 47, 389-394.
[24] A.A. Buraimoh, I.G.Bako, F.B. Ibrahim. *Int. J. Animal Vetern. Adv*., **2011**, 3, 10-13.
[25] M.G.P. Alanivel, B.R. Ajkapoor, R. Senthilkumar, J.K. Einstein, E.P. Kumar. *Sci. Pharm*., **2008**, 76, 203–215.