Original Article

Preventive and curative effects of *Acalypha indica* on acetaminophen-induced hepatotoxicity

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Effect of ethanol extract of the leaves of *Acalypha indica* (Euphorbiaceae) was investigated against acetaminophen-induced hepatic damage. Acetaminophen (paracetamol) at the rate of 1 g/kg produced liver damage in rats as manifested by the significant (P<0.001) rise in serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), compared to respective control values. Treatment of rats with acetaminophen led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA). This was associated with a significant reduction in superoxide dismutase (SOD) and glutathione (GSH) contents. Pretreatment of animals with the plant extract (100 mg/kg) orally once daily for 5 days prevented (P<0.01) the acetaminophen-induced rise in serum transaminases (AST and ALT) and ALP. Post treatment with five successive doses of the extract (100 mg/kg) restricted the hepatic damage induced by the above said Paracetamol (P<0.001). Histological changes around the hepatic central vein were recovered by administration of the drug. Thus, it is evident that these biochemical and histological alterations resulting from acetaminophen administration were inhibited by pre and post treatment with *A. indica* leaf extract. One notable study of the study was the spontaneous recovery of liver damage within a week after stopping paracetamol. These results indicate that the crude ethanol extract of *A. indica* exhibits hepatoprotective action through antioxidant effect and validates the traditional use of the plant in hepatic dysfunction.

Key words: *Acalypha indica*, acetaminophen, hepatotoxins, transaminases

INTRODUCTION

Uncontrolled environmental pollution, poor sanitary conditions, alcohol intoxication and indiscriminate use of potent drugs predispose liver to a vast array of disorders. However, infections by viruses still remain as the major cause of liver diseases. According to global estimates,[1-3] there are about 796,000 deaths every year due to liver cirrhosis caused by hepatitis.

Many indigenous drugs are claimed to possess hepatoprotective activity. One such plant used by the tribals of South India, named *Acalypha indica* Linn. (Euphorbiaceae), is claimed to have control over hepatic dysfunction. Preliminary studies[4] have given positive results regarding the same. Hence, this work was undertaken to have a systematic study on the exact mechanism of the drug on male albino rats. Acetaminophen is a commonly used nonsteroidal analgesic and anti-inflammatory drug. Even though it is quite safe in human beings, massive hepatic necrosis is becoming a major problem, especially with higher dosage schedule. Hence, acetaminophen-induced liver toxicity was studied, with special emphasis given to the ability of liver to recover spontaneously after paracetamol challenge.

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MATERIALS AND METHODS

Plant Material

One kilogram of the whole plant of *A. indica* Linn. was collected from the suburbs of Thiruvananthapuram District of Kerala and identified by an eminent taxonomist of University Center, Karyavatom, Thiruvananthapuram. A voucher specimen was kept in the college of Pharmaceutical Sciences, Medical College, Thiruvananthapuram, where this work was carried out (MTCPS/Herb A 010/2001).

Preparation of the Extract/Drug

The freshly collected leaves of *A. indica* (AI) were shade dried and powdered. The powder was first defatted with petroleum ether (40–60°C).The marc was then subjected to cold maceration[5,6] using alcohol (70%) for

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6 days. The extract was concentrated using a rotary vacuum evaporator and then dried under reduced pressure[7] and kept in the refrigerator. The extract as well as paracetamol were suspended in 2% carboxymethyl cellulose (CMC) for the present study till the time of use.

Animals

Inbred male rats of Wistar strain (150–200 g) were used for the investigation. The animals were maintained at 12:12 hour dark/light cycle and they were allowed standard pellet diet (Hindustan Ltd., Bangalore, India) and water *ad libitum*. All animals were handled according to the guidelines for investigations of experimental pain in conscious animals.[8] The pharmacological experimental protocols were approved by the Institutional animal ethics committee (No.09/IAEC/MCT/02).

Chemicals and Instruments

Paracetamol (Kerala State Drugs and Pharmaceuticals, Alleppy, India), thiobarbituric acid (TBA) and trichloro acetic acid (SD chemicals limited, Mumbai, India) were used for the study. All other chemicals and reagents used were of analytical grade. UV spectra were recorded in Shimadzu 1601 UV-Visible spectrophotometer**.**

Hepatoprotective Study

Induction of liver damage

Animals were divided into seven groups of six each. Group I served as negative control receiving only 2% CMC (10 ml/kg). Groups II and III received paracetamol (1 g/kg) for Induction of liver damage[9] Group IV was given the drug extract first (100 mg/kg) followed by paracetamol as in the previous cases. Groups V and VI received paracetamol and the test extract simultaneously. But group VII received paracetamol first and later the drug extract. Depending upon the groups of animals under study, blood samples were withdrawn on 1st, 2nd, 6th and 8th days and serum enzyme activity was estimated using standard procedures.[10-12] A few animals from each group were sacrificed on the above days and liver was isolated and subjected to histopathological studies.

Antioxidant Studies

Inhibition of lipid peroxidation

This was determined by the TBA method. Different concentrations of the plant extract were incubated at 37°C with rat liver homogenate (25%) (0.1 ml) containing 30 mM KCl, Tris-HCl buffer (0.04 M; pH 7), ascorbic acid (0.06 mM) and ferrous iron (0.16 mM) (total volume was 0.5 ml) for 1 hour. At the end of 1 hour, thiobarbituric acid reactive substance (TBARS) was measured by the method of Okhawa *et al.*,[13] and percentage of inhibition calculated from the control in which no test extract was added.

Superoxide dismutase activity

This was determined by the method of Kakkar *et al*.[14] Assay mixture contained 1.2 ml sodium pyro phosphate buffer (0.052 M, P.h 8.3), 0.1 ml of 186 μM phenazine metho sulphate (PMS), 0.3 ml 300 μM nitro blue tetrazolium (NBT) 0.2 ml 780 μM NADH appropriately diluted liver homogenate and water in a total volume of 3ml. A system devoid of enzyme served as control.

Glutathione content

This was estimated by the method of Benke *et al*.[15] The absorbance of a mixture containing 20% tissue homogenate (0.2 ml), 4.75 ml of 0.1 M sodium phosphate buffer (pH 8), and 0.05 ml of 0.01 m 5,5, di thiobis- 2-nitro benzoic acid (DTNB) was read at 412 nm within 4 minutes

Statistical Analysis

The results were expressed as mean±SEM. The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's "*t*" test. The values of $P<0.01$ and $P<0.001$ were considered to indicate a significant difference between the groups.[16]

RESULTS AND DISCUSSION

Paracetamol intoxication with a toxic dose produced a significant increase in the values of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) within 24 hours of its administration. But on keeping the animals in the same condition for a period of another 5 days, as observed in the group III animals, there was a reduction in the levels of the above said parameters denoting spontaneous recovery. When the drug was administered prior to the toxicant (group IV), there was reduction in the levels of AST (53.64%), ALT (92.39%) and ALP (78.54%). In group V where the drug was given along with paracetamol a better result could be observed as evidenced by the AST (43.96%), ALT (93.49%) and ALP (95.05%) levels. In group VI, there was a rise observed in the ALT and ALP levels after stopping the drug. In the curative group (group VII), there was reduction in the activities of AST (91.93%), ALT (81.67%) and ALP (88.68%). But the control group (group 1) did not show any change in the biochemical parametres as evidencrd in table [Table 1, Figures 1-4].

There was a threefold increase in the activity of malonedialdehyde (MDA) in the toxin-treated group compared to the control group. But this was spontaneously reduced after 5 days. In the drug-treated groups also, reduction in the activity was observed [Table 2].

When albino rats were intoxicated with paracetamol, there was reduction in reduced glutathione (GSH) content

(73.80%), whereas superoxide dismutase (SOD) was reduced to 28.90%. After spontaneous recovery, much effect was not observed. But in the preventive, protective and curative groups, significant results (P<0.001) were obtained [Table 2, Figures 5–7].

Histopathological results were as follows. In the paracetamol-treated group, after 24 hours of administration, there was perivenular ballooning and degeneration as well as sinusoidal congestion. But after 5 days, this was reduced to mild inflammation, but degenerative changes were not fully corrected. In the preventive group, the same result could be seen. But when the drug was given along with paracetamol, it was reduced to minimum degenerative changes as observed on the following day. But on the 8th day, normal architecture could be regained. In the curative group of animals when drug was given after paracetamol administration, normal architecture could be obtained.

Table 1: Effect of *A. indica* extract on serum enzyme levels in paracetamol-treated albino rats

Group	Drug and dose	Duration of treatment (days)	Biochemical parameters (Mean±SEM)		
			AST (IU/l)	ALT (IU/l)	ALP (IU/l)
I	Control	–	123.83±4.74	102.67±1.83	133.83±4.21
II	Paracetamol (1 g/kg)	2	638.66±4.40^{a*}	451.00±5.84^{a*}	586.00±12.42^{a*}
III	Paracetamol (1 g/kg)	2	372.67±5.57^{b**}	165.17±3.09^{b**}	309.33±8.51^{b**}
IV	*A. indica* ext. (100 mg/kg) +	1–5	362.50±3.77^{b}	129.17±3.80^{b}	223.00±9.47^{b}
	Paracetamol	6–7			
V	Paracetamol +	1–5	412.33±4.35^{b}	125.33±3.32^{b}	146.67±3.81^{b}
	A. indica ext. (100 mg/kg)	1–5			
VI	Paracetamol +	1–5	233.67±2.22^{b}	152.33±2.44^{b}	203.67±3.12^{b}
	A. indica ext. (100 mg/kg)	1–5			
VII	Paracetamol +	1–7	165.33±2.00^{b}	166.50±1.75^{b}	185.00±3.99^{b}
	A. indica ext. (100 mg/kg)	3–7			

ALP – Alkaline phosphatase; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; Means in the same column scored by the same alphabet are not significantly different at 1% level; Number of animals per group is six; ^{a}P<0.001 compared to group I; ^{b}P<0.001 compared to group II; *Blood sample collected on 3rd day; **Blood sample collected on 8th day

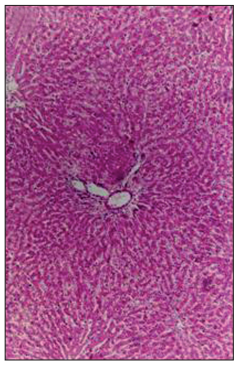

Figure 1: Liver of control rat showing normal architecture of hepatocytes (H and E, ×10)

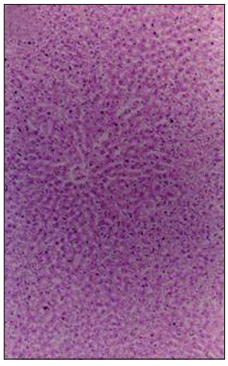

Figure 2: Liver section after paracetamol treatment, demonstrating cloudy swelling, pervenular ballooning and degeneration; lymphoplasmocytic infiltration also seen (H and E, ×40)

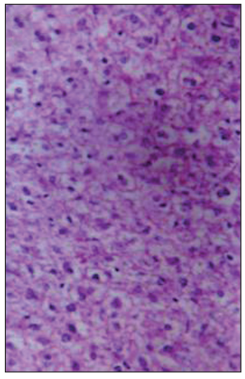

Figure 3: Liver section after paracetamol treatment (6th day), showing degeneration of hepatocytes with minimum inflammation (H and E, ×40)

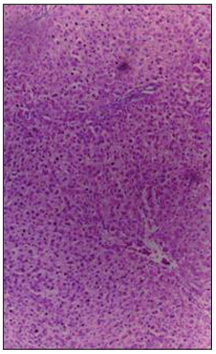

Figure 4: Liver section after preventive treatment (*A. indica* extract + paracetamol), showing degenerative changes; minimum inflammation also seen (H and E, ×40)

Table 2: Effect of *A. indica* on the activity of MDA, GSH and SOD in paracetamol-induced liver toxicity (curative effect)

Group	Treatment	MDA (nmol/g wet tissue) Mean±SEM	GSH (mg/g) Mean±SEM	SOD (Units/mg) Mean±SEM
I	Control	0.74±0.01	3.93±0.01	7.30±0.01
II	Paracetamol (1 g/kg)	2.57±0.01*	2.90±0.01*	2.11±0.02*
III	Paracetamol (1 g/kg)	2.33±0.01**	2.91±0.02**	2.26±0.01**
IV	*A. indica* ext. (100 mg/kg) + Paracetamol	1.31±0.01[b]	2.70±0.01[b]	2.92±0.01[b]
V	Paracetamol + *A. indica* ext. (100 mg/kg)	1.21±0.02[b]	3.05±0.02[b]	4.13±0.03[b]
VI	Paracetamol + *A. indica* ext. (100 mg/kg)	1.19±0.01[b]	3.26±0.01[b]	4.43±0.02[b]
VII	Paracetamol + *A. indica* ext. (100 mg/kg)	1.18±0.02[b]	3.01±0.01[b]	4.31±0.02[b]

GSH – Glutathione; MDA – Malondialdehyde; SOD - Superoxide dismutase; Means in the same column scored by the same alphabet are not significantly different at 1% level; Number of animals per group is six; [a]*P*<0.001 compared to group I; [b]*P*<0.001 compared to group II; *Blood sample collected on 3rd day; **Blood sample collected on 8th day

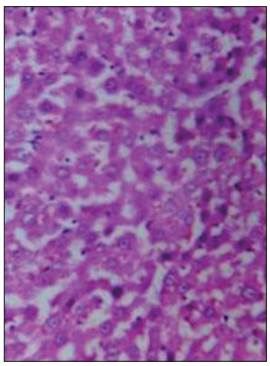

Figure 5: Liver section of rat treated with paracetamol and *A. indica* extract (on 6th day), showing minimum degenerative changes (H and E, ×40)

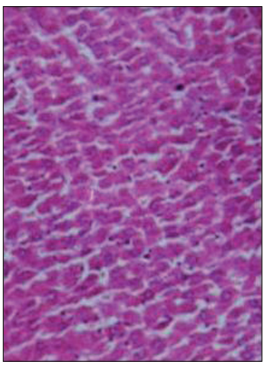

Figure 6: Liver section of rat treated with paracetamol and *A. indica* extract (on 8th day), showing normal architecture of hepatocytes (H and E, ×40)

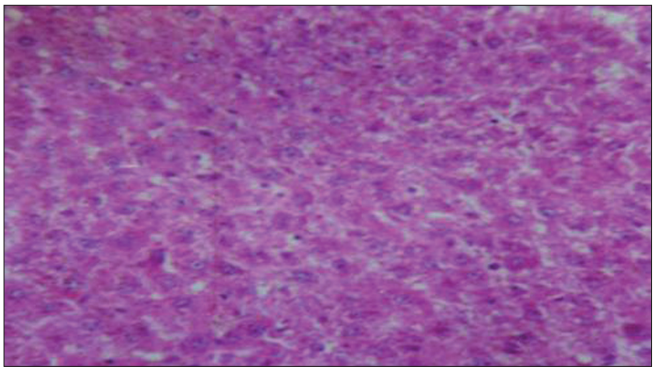

Figure 7: Liver section of rat treated with paracetamol and *A. indica* extract (curative), showing normal architecture and regenerative changes (H and E, ×40)

Paracetamol or acetaminophen is a commonly used analgesic drug, which is known to cause centrilobular hepatic necrosis upon overdose.[17] Its toxicity also accounts for many emergency hospital admissions and continues to be associated with high mortality.[17] Its hepatotoxicity has been related to the production of a highly reactive intermediate metabolite, N-acetyl-*p*-benzoquinone imine, formed by the cytochrome P_{450} mediated oxidation.[18] Following an overdose, hepatic glucuronide and sulphate become depleted with a consequent increase in P_{450} catalysed oxidation. The increased production of NAPQI coupled with a decreased capacity to render the substance non-toxic, results in its intracellular accumulation.[18] NAPQI with electrophilic and oxidant characteristics consequently can deplete intracellular GSH and protein thiol groups by alkylation and oxidation and lead to the formation of mixed disulphide. These events subsequently give rise to changes in the cellular calcium homeostasis, lipid peroxidation and loss of mitochondrial respiratory function.[19-21] The damaged hepatocytes release factors that both attract and activate hepatic macrophages, causing cell necrosis by release of proteolytic lysosomal enzymes and reactive oxygen species.[22]

An obvious sign of hepatic injury is the leakage of cellular enzymes into the plasma[23] Under oxidative stress, the hepatocytic membrane appears to be the critical locus of damage and oxidative alterations responsible for membrane damage in rats.[19,21,24] It is established that serum enzymes such as ALT and AST levels are elevated in paracetamol-induced hepatotoxicity.[21,25,26] In the present study, elevation in the levels of AST, ALT and ALP were found in paracetamol-treated animals. But *A. indica* has significantly reduced these enzyme levels. The observed reversal produced by the drug in serum biochemical parameters may be a manifestation of the reduction in the cell membrane disturbances.[27]

In the present study, paracetamol intoxication resulted in an increased production of MDA, which is a product of lipid peroxidation, suggesting enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms and production of excessive free radicals, and also depletion of GSH and SOD leading to hepatic necrosis which is in concurrence with published reports.[28-31] Hence, it can be postulated that covalent bonding of paracetamol and its metabolites to cellular proteins may induce a series of events, which produce hepatocellular necrosis. This observation is well correlated with histological changes in hepatic parenchyma described as cloudy swelling which is an early indicator of degenerative changes and later necrosis. As administration of *A. indica* increased GSH and superoxide contents along with reduction in the amount of MDA, there is every possibility for reduced hepatic necrosis. Hence, it can be assumed that the hepatoprotective activity of the drug is due to inhibition of the activity of cytochrome P_{450} and consequent glucuronidation along with its antioxidant effect. Further investigations regarding chemical constituents contained in the extract and their possible mechanism of action are in progress.

CONCLUSION

The present study concludes that the ethanol extract of the leaves of *A. indica* possesses significant hepatoprotective activity in acetaminophen-induced male rats at a dose of 100 mg/kg/p.o. Thus, it has been scientifically proved that the traditional knowledge obtained from the tribal people of South India is true and the extract has enough potential as a hepatoprotective agent and hence worth investigative.

REFERENCES

1. Mathers CD, Stein C, Ma Fat D, Rao C. Global burden of diseases – 2000. Version 2, methods and results. GPE discussion paper No:50. Geneva world Health Organization; 2002. p. 1-2.
2. Salomon JA, Murray CJ. The epidemiological transition revisited: Compositional models for causes of deaths by age and sex. Popul Dev Rev 2002;28:205-28.
3. WHO World Health Report 2002. Reducing risks promoting healthy life. Geneva: WHO; 2002. p. 1-13.
4. Mathew M, Shenoy KT, Gopi TV. Hepatoprotective activity of *Acalypha indica*: A comparative evaluation. Drug Lines 2002-2003;5:5-7.
5. Anbu Jeba Sunilson J, Anita GK, Jisha J, Varatharajan R, Jayaraj P, John T. Formulating and screening of herbal wound healing ointment. Adv Pharmacol Toxicol 2008;2:1-7.
6. Anbu Jeba Sunilson J, Mathu MS, Syam MM, Anita GK, John T, Jayaraj P. Antidiabetic activity of *Momordica tuberose* Roxb COGN tuber extracts. Int J Pharmacol Biol Sci 2008;9:41-5.
7. Suma V, Swamy MM, Vijayalakshmi S, Reddy YS, Suresh B. Pharmacological observations on Sida rhomboidea: A report. Indian Drugs 1994;31:421-5.
8. Zimmerman M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109-10.
9. Rao GM, Rao G, Ramanarayanan K, Sreenivasan KK. Effect of hepatogard on paracetamol induced liver injury in male albino rats. Indian Drugs 1993;30:40-4.
10. Bergmeyer HU, Bowers S Jr, Horder M, Moss DW. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes: Part II. Clin Chem 1977; 23:887-99.
11. Berg HU, Horder M. IFCC method for the measurement of catalytic concentrations of enzymes: Part -3. IFCC method for alanine amino transferase. J Clin Chem Clin Biochem 1980;18:521-34.
12. Bowers GN Jr, McComb RB. Measurement of total alkaline phosphatase activity in human serum. Clin Chem 1975;21: 1988-95.
13. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
14. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay for superoxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
15. Benke GM, Cheever KL, Mirer FE, Murphy SP. Comparative toxicity, anticholinesterase action and metabolism of methyl parathione and parathione in sunfish and mice. Toxicol Appl Pharmacol 1974;28:97-109.
16. Kulkarni SK. Hand book of experimental pharmacology. New Delhi; India. Vallabh Prakashan; 1999. p. 172.
17. Kiso Y, Tohkin M, Hikino H, Hattori M, Sakamoto T, Namba T. Mechanism of antihepatotoxic activity of Glycyrrhizin, I: Effect on free radical generation and lipid peroxidation. Planta Med 1984;50:298-302.
18. Robin MW, Racz WJ, McElligott TF. Scanning electron microscopic examination of Acetaminophen-induced Hepatotoxicity and congestion in mice. Am J Pathol 1983;113:321-30.
19. Hong RW, Rounds JD, Helton WS, Robinson MK, Wilmore DW. Glutamine preserves liver glutathione after lethal hepatic injury. Ann Surg 1992;215:114-9.
20. Lee E, Miki Y, Ichi FY, Simizu H, Karia K. Selective release of Glutathion transferase subunits from primary cultured rat hepatocytes by carbon tetrachloride and deoxycholic acid. Toxicology 1991;67:237-48.
21. Dixon MF, Nimmo J, Prescott LF. Experimental paracetamol - induced hepatic necrosis: A histopathological study. J Pathol 1971;103:225-7.
22. Weishen B, Kamendulis LM, Ray SD, Corcoran GB. Acetaminophen-induced cytotoxicity in cultured mouse hepatocytes: Correlation of nuclear Ca^{2+} accumulation and early DNA fragmentation with cell death. Toxicol Appl Pharmacol 1991;111:242-54.
23. Keppler D. Liver morphology and enzyme release: Further studies in the isolated perfused rat liver. In: Schmidt E, Schmidt FW, Mohr J, Otto P, Vido I, Wrogeman K, *et al*, editors. Pathogenesis and mechanism of liver cell necrosis. 4th ed. Lancester: Medical and Technical publications; 1975. p. 147.
24. Koul IB, Kapil A. Effect of diterpenes from *Andrographis paniculata* on antioxidant defense system and lipid peroxidation. Indian J Pharmacol 1994;26:296-300.
25. Singh B, Saxena AK, Chandran BK, Anand KK, Suri OP, Suri KA, *et al*. Hepato protective activity of Verbenalin on experimental liver damage in rodents. Fitoterapia 1998;69:135-41.
26. Pauline T, Dipti P, Anju B, Kavimani S, Sharma SK, Kain AK, *et al*. Studies on toxicity, anti stress and hepato-protective properties of Kombucha *tea*. Biomed Environ Sci 2001;14:207-13.
27. Cederbaum AI, Cohen G. Microsomal metabolism of hydroxyl radical scavenging agents: Relationship to the microsomal oxidation of alcohol. Arch Biochem Biophys 1980;19:438-47.
28. Ashbum LL, MeQueency AJ, Faulkner RR. "Scarring and precirrhosis" of the liver in chronic phosphorus poisoning of

Guinea pig. Proc Soc Exp Biol Med 1948;67:351-8.
29. Mitchell JR, Zimmerman HJ, Ishak KG, Thorgeirsson UP, Timbrell JA, Snodgrass WR, *et al*. Isoniazid liver injury: Clinical spectrum, pathology and probable pathogenesis. Ann Intern Med 1976;84:181-92.
30. Savides MC, Oehme FW. Acetaminophen and its toxicity. J Appl Toxicol 1983;3:95-111.
31. Zhou L, Erickson RR, Holtzman JL. Studies comparing the kinetics of cysteine conjugation and protein binding of paracetamol by hepatic microsomes from male mice. Biochim Biophys Acta 1997;1335:153-60.

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