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HISTOPATHOLOGICAL AND BIOCHEMICAL EFFECTS OF PARACETAMOL-INDUCED TOXICITY IN FRESHWATER FISH *LABEO ROHITA*

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ABSTRACT

Recently, Chemical toxicity has become one of the major problems in aquaculture; many farms have been suffering from the "liver syndrome", with symptoms of liver enlargement (up to two to threefold of the original size) and colour change. The causes of this disease are not clear; pathogenic bacteria or viruses have not been identified. Xenobiotic challenge due to drug abuse and environmental pollution may be one of the most important causes of the diseases. Liver lesions have been reported in various fish species exposed to different xenobiotics. Biological compounds with antioxidant properties contribute to the protection of cells and tissues against the deleterious effects of reactive oxygen species and other free radicals. In spite of tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in the regeneration of hepatic cell. The present study aimed to evaluate the Histopathological and biochemical effects of paracetamol-induced toxicity in freshwater fish *Labeo rohita*. The following conclusion obtained after supplementation of paracetamol exposed fish. Increased the oxidative stress confirmed by the formation of MDA. Declined the antioxidant enzymes GSH, SOD, Catalase and GPx. Increased the liver markers ALT and AST. Decline the protein content in liver, gill and muscles. Histopathalogical observations confirmed its hepatotoxic activity. Over all, the protective effect of paracetamol-induced hepatotoxicity in fishes appears to be related to the increased of lipid peroxidation and decline of antioxidant enzyme levels in addition to free radical generation.

KEYWORDS: Histopathology, Biochemical, Antioxidant, liver markers, *Labeo rohita.*

1. INTRODUCTION

Fish is very important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged in developed or developing countries. According to nutritionists, fish is an excellent substitute for red meat and an excellent source of protein. Fish flesh contains all the essential amino acid and minerals viz., iodine, phosphorus, potassium, iron, copper and vitamin A and D in desirable concentrations. It serves as valuable ingredient to a healthy diet because of its low carbohydrate and unsaturated fat contents. So the inclusion of fish in our diet can make a valuable contribution to any diet that contain mainly of cereals, starchy roots and sugar for the healthy growth. Fish are the most at threat from aquatic pollution and together with their long-term exposure in natural habitat they are suitable bio monitors of environmental pollution (Padmini *et al.*, 2004). Fish is generally acknowledged as a worthy model for assessing aquatic contamination and is used as an environmental sentinel for water toxicants. Fish liver can be regarded as the body's detoxification organ and hence a target organ of various xenobiotic substances.

Several chemicals have been known to induce hepatotoxicity. Carbon tetrachloride (CCl_4), galactosamine, d-galactosamine/lipopolysachharide (GalN/LPS), thioacetamide, antitubercular drugs, paracetamol, arsenic etc., are used to induce experimental hepatotoxicity in laboratory animals (Ward and Daly, 1999). Paracetamol is a safe, effective, well tolerated and cheap analgesic and anti-pyretic drug with relatively few adverse effects when used at the recommended therapeutic dosage. It was first introduced in the year 1955 for its clinical application and since then, it is widely used almost throughout the world. In many countries the drug is readily available over the counter without the need of prescription. It’s easy availability and no need for prescription made it one of the commonest drugs used for suicidal or self harm purposes Its toxicity was noticed in the 1960s (Sheen *et al*., 2002). Since then number of case coming to the emergency department kept on increasing, especially in UK (Prescott *et al*., 2009, Bergen *et al*., 2010). It is also a frequent cause of poisoning in many of the countries, including North America, Australia and New Zealand and several other countries in Europe (Larson *et al*., 2005, Nourjah *et al*., 2006, Wei *et al*., 2007). Increasing number of the case brought the idea of legalization of drug from over the counter policy to prescription only status. Following legislation in 1998 to limit pack sizes, beneficial

effects on paracetamol related mortality and morbidity were reported in England. Although following legislations to limit pack sizes, morbidity and mortality reduced, however strict measures as required to reduce breaches of sales guidelines (Hawton *et al*., 2001). The present study aimed to evaluate the Histopathological and biochemical effects of paracetamol-induced toxicity in freshwater fish *Labeo rohita.*

3. MATERIAL AND METHODS

3.1 Collection and acclimation of experimental fishes

Labeo rohita (average weight 60g) were procured from Fish farm, Thittai, Thanjavur-district Tamil Nadu, India, using cast net and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water with continuous aeration for two weeks prior to experimentation. During this period, fishes were fed with a known amount of fish food.

3.1Experimental design

In this experiment, fishes of uniform size (length, 10.3cm) and weight (60 g) were segregated from the stock and acclimated for 3 days to the lab conditions, temperature (28±2°C), pH 7.5–7.8, and an almost normal photoperiod (12:12-h L/D). The fishes were divided into three groups (one control and two experimental groups) of ten individuals each and introduced into the trough containing dechlorinated tap water. All the experimental fishes were administered orally with the respective material.

Group I Control fish with 0.09% saline,

Group II with a single dose of paracetamol (500 mg/kg body weight)

The paracetamol administration. Paracetamol was first dissolved in water at 70°C and then cooled to 37°C before administration. The fishes were killed at 24 h by decapitation. Tissue samples from the liver, gill, muscles were extracted and prepared for biochemical analysis. A duplicate was run simultaneously and the pooled samples were analysed.

3.2 Biochemical analysis

At the end of the experimental period, blood was from the caudal circulation with and without heparinised 2 ml disposable syringe fitted with a 21 gauge hypodermic needle. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000rpm for 10minutes, and then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

3.2.1 Tissue homogenate

Immediately after blood collecting, the fish was sacrificed and the muscletissue, liver and gill were dissected out, washed with ice-cold physiological saline. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M TrisHcl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

3.3 BIOCHEMICAL ESTIMATION

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Reduced glutathione was estimated by method of Moron *et al* (1979).The activity of mitochondrial glutathione peroxidase was assayed by the method of Rotruck *et al* (1973). Copper-zinc superoxide dismutase activity was determined by the procedure of Kakkar *et al.* (1984) in plasma. The activity of catalase was assayed by the method of Beers and Sizer (1952). The serum GOT was estimated by the method of Reitman and Frankel (1957). The serum GPT was estimated by the method of Reitman and Frankel *(*1957).Protein was estimated by the method of Lowry *et al.* (1951). The level of protein was expressed as mg/g tissue.

3.4 Statistical Analysis

The results were presented as Mean ± SD. Data was statistically analyzed using student "t" test. P.values set as lower than 0.05 were considered as statistically significant.

4. RESULTS AND DISCUSSION

The present study was carried out to analyze the various biochemical parameters in control, paracetamol treated *Labeo rohita* fish. The observations made on different groups of fishes were compared as follows.

Table 1 - Shows the levels of MDA, GSH, SOD, CAT and GPx in liver of control and experimental group fish. MDA content was increased in group II as compared with group I. GSH content was decreased in group II as compared with group I. SOD, Catalase and Glutathione peroxidase activity were increased in group II as compared with group I.

Table 1: The levels of liver antioxidant MDA, GSH, SOD, CAT and GPx in control and experimental fish.

Parameters	Group I	Group II
MDA (nmole of MDA/mg tissue)	1.33±0.10	2.58±0.15
GSH (µg/mg tissue)	1.71±0.11	2.59±0.20
SOD (U/mg tissue)	4.19±0.31	5.24±0.48
CAT (U/mg tissue)	0.±0.03	1.15±0.10
GP_X (U/mg tissue)	6.58±0.46	7.46±0.43

Values were expressed as mean ± SD for fishes.

Table 2 - Shows the levels of MDA, GSH, SOD, CAT and GPx in Gill of control and experimental group fish. MDA content was increased in group II as compared with group I. GSH content was decreased in group II as compared with group I. SOD, Catalase and Glutathione peroxidase activity were increased in group II as compared with group I.

Table 2: The levels of Gill antioxidant MDA, GSH, SOD, CAT and GPx in control and experimental fish.

Parameters	Group I	Group II
MDA (nmole of MDA/mg tissue)	1.15±0.08	1.93±0.12
GSH (µg/mg tissue)	1.49±0.10	2.11±0.15
SOD (U/mg tissue)	3.28±0.21	3.63±0.25
CAT (U/mg tissue)	0.27±0.03	0.61±0.05
GP_X (U/mg tissue)	5.69±0.42	6.18±0.51

Values were expressed as mean ± SD for fishes.

Table 3 - Shows the levels of MDA, GSH, SOD, CAT and GPx in Muscles of control and experimental group fish. MDA content was increased in group II as compared with group I. GSH content was decreased in group II as compared with group I. SOD, Catalase and Glutathione peroxidase activity were increased in group II as compared with group I.

Table 3: The levels of Muscles antioxidant MDA, GSH, SOD, CAT and GPx in control and experimental fish.

Parameters	Group I	Group II
MDA (nmole of MDA/mg tissue	1.58±0.13	2.94±0.20
GSH (µg/mg tissue)	1.73±0.11	2.41±0.17
SOD (U/mg tissue)	3.01±0.21	3.95±0.24
CAT (U/mg tissue)	0.43±0.02	0.79±0.04
GP_X (U/mg tissue)	6.71±0.45	7.62±0.54

Values were expressed as mean ± SD for fishes.

Table 4 - Shows the levels of SGOT and SGPT in blood and liver of control and experimental group fish. SGOT and SGPT activity were increased in group II as compared with group I.

Table 4: The levels of blood and liver SGOT and SGPT in control and experimental fish.

Parameters	Group I	Group II
Blood		
SGOT (IU/L)	1.03±0.05	1.59±0.10
SGPT (IU/L)	1.07±0.06	1.71±0.15
Liver		
SGOT (µmole/mg tissue)	0.61±0.08	1.55±0.14
SGPT (µmole/mg tissue)	0.51±0.04	0.73±0.07

Values were expressed as mean ± SD for fishes.

Table 5 - Shows the levels of Proteinin Liver, Gill and Muscles of control and experimental group fish. Liver, Gill and Muscles Protein content were decreased in group II as compared with group I.

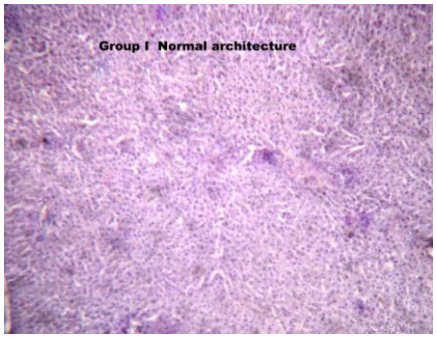
Table 5: The levels of Liver, Gill and Muscles Protein in control and experimental fish.

Parameter	Group I	Group II
Liver	2.21±0.18	1.79±0.13
Gill	0.45±0.06	0.20±0.03
Muscles	0.75±0.04	0.21±0.02

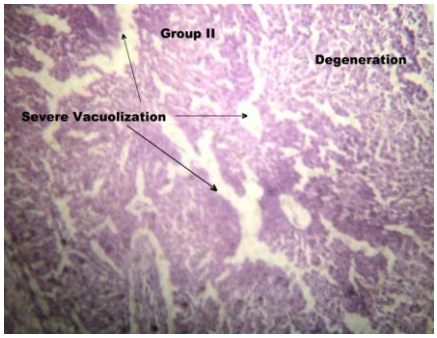
Values were expressed as mean ± SD for fishes.

4.2 Histopathological observation of control and experimental fish

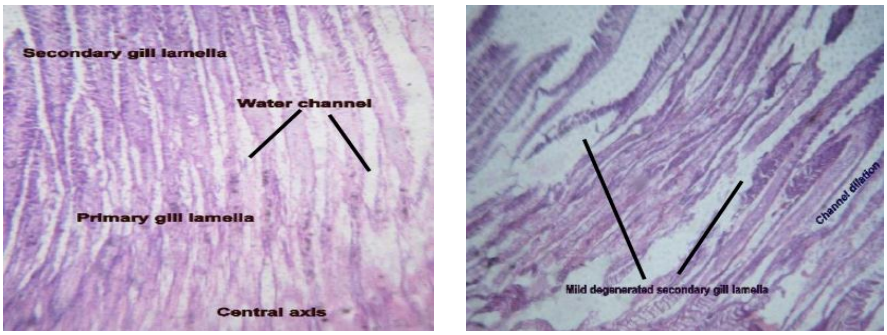
Histopathological studies showed that Group II paracetamol caused the derangement of liver to become irregular and the gap to widen. The liver and gill cells show hepatocellular vacuolization and degeneration. Control group (I) shows the normal architecture of liver and gill. (Fig 5).



Liver (Group I)



Liver (Group II)



Gill (Group I) **Gill (Group II)**

Fig 5: Histopathological observation of control and experimental fish.

5. DISCUSSION

Paracetamol-induced hepatic failure is the second leading cause of liver transplantation and accounts for considerable levels of morbidity and mortality (Lee 2004). Paracetamol is a widely used analgesic and antipyretic drug. It is selected as a hepatotoxicant in inducing injury tothe liver as it is known to cause hepatotoxicity in man and experimental animals when taken overdose, which leads to the elevation of liver enzymes (Ahmed and Khater, 2001; Kumar *et al.* 2004). Leakage of cellular enzymes in to plasma indicates a hallmark sign of hepatic injury or damage (Kumar *et al.* 2004; Ramaiah 2007). In addition, the extent and type of liver injury or damage can be assessed based on the presence or absence of specific enzymes in the bloodstream (Kumar *et al.* 2004). Generally, measurements of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are commonly used as marker enzymes in assessing Paracetamol-induced hepatotoxicity (Asha *et al*., 2004).

We found that the activities of the tissue-damaging enzymes AST and ALT markedly increased in *Labeo rohita* 24 h after exposure to paracetamol. AST and ALT are enzymes involvedin the metabolism of amino acids. An increase in the levels of ALT and AST has been shown to reflect liver damage (Bhattacharya *et al.*, 2005). In the assessment of liver damage by Paracetamol, determination of enzyme levels such as AST and ALT is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence can be measured in the serum. AST is an enzyme found in the mitochondrion and cytoplasm of all cells. High levels of AST indicate liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury. AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. ALT is a cytoplasmic hepatocellular enzyme whose increase in blood is highly indicative of liver damage, e.g. hepatitis, cirrhosis or hepatic tumors. Therefore, ALT is more specific to the liver and is thus a better parameter

for detecting liver injury. Elevated levels of enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver (Kumar *et al.* 2004). The elevated activity of AST and ALT were significantly increase by treatment with Paracetamol. The present results showed that Paracetamol, deplete the hepatic levels of proteins. There is a close relationship between the rate of protein synthesis in the liver tissue and total protein concentrations in the body tissues. The depleted level of total protein (composed of albumin and globulin) in liver reflects the decrease of protein synthesis in gill and muscles. Supplementation of Paracetamol showed significant decreased on gill, muscle and liver tissue proteins.

In this study, a single dose of Paracetamol (500 mg/kg body weight) treatment caused a significant elevation of enzyme levels such as SOD, CAT, GPx and increased content of MDA, decreased content of GSH when compared with the control group I. The increased enzyme levels in Paracetamol-induced liver damage and antioxidant activities. All the identified enzymes were brought back to normal or near-normal levels. This was also supported by the limited extent of histological changes. Free radicals play an important role in the physiopathological situations involving lipid peroxidation reactions. It is established that a fraction of paracetamol is converted via the cytochrome P450 pathway to a highly toxic metabolite, Nacetyl-p-benzoquinone imine which is normally conjugated with glutathione and excreted in urine. An overdose of paracetamol depletes glutathione stores, leading to the accumulation of NAPQI mitochondrial dysfunction (Parmar and Kandakar 1995) and the development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in Paracetamol bio activation to NAPQI. P450 2E1 have been suggested to be primary enzymes for Paracetamol bio activation in liver microsomes (Raucy *et al.*, 1989). Studies demonstrated that Paracetamol-induced hepatotoxicity can be modulated by substances that influence P450 activity (Mitchell *et al.*, 1973). Wendel *et al.*, (1982) reported that paracetamol metabolism triggers lipid peroxidation, which is responsible for liver injury.

SOD catalyses the transformation of superoxide radicals to H_2O_2 and O_2, and is the first enzyme to deal with oxyradicals (Kappus 1985). As a result, SOD activity made a counteractive response under oxidative stress. In this work, SOD activity was induced significantly under Paracetamol treatment, which indicated the increase of superoxide radical anion. SOD responded to the oxidative stress of Paracetamol treatment at 500 mg/kg, which suggested that its activity was very sensitive. Thus, the SOD and CAT induction observed in

this study could be related to increased ROS production. Elevated antioxidant status attempts to neutralize the impact of ROS. The peroxidases are enzymic antioxidants widely distributed in all animal tissues which decompose hydrogen peroxide and protect the tissue from highly reactive hydroxyl radicals. 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 (Murugesh *et al.*, 2005).

Oxidative stress occurs when the critical balance between oxidants and antioxidants is disrupted due to the depletion of antioxidants or excessive accumulation of ROS, or both, leading to damage (Scandalios 2005). Ainecrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in live injury (Curtis and Mortiz 1972). SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defence system. It scavenges the superoxide anion to form hydrogen peroxide, thus diminishing the toxic effect caused by this radical. In Paracetamol, it causes a significant decline in hepatic, gill and muscle SOD activity and thus reduces reactive free radical-induced oxidative damage to liver.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidants present in the liver. It removes free radical species such as hydrogen peroxide and superoxide radicals and maintains membrane protein thiols. Also, it is substrate for GPx (Prakash *et al.*, 2001). GSH plays an essential role in the detoxification of paracetamol and protects hepatocytes by uniting with the reactive metabolites of paracetamol. Thus, it prevents them from binding covalently with liver proteins. Intracellular decrease of GSH exposes the cell to the destructive effects of oxidative stress (Lauterburg and Velez 1988). The increased level of GSH is associated with an enhanced lipid peroxidation in Paracetamol-treated fishes.

Histopathological studies showed in Paracetamol treated liver tissue an explicit disarrangement of muscle bundles, becoming irregular with a widened gap. The liver and gill cells showed hepatocellular vacuolization and degeneration.

The present study concluded that the protective effect of paracetamol-induced hepatotoxicity in fishes appears to be related to the increased of lipid peroxidation and decline of antioxidant enzyme levels in addition to free radical generation.

7. REFERENCES

1. Ahmed and Khater., 2001. Effect of inorganic fertilizer (Nitrophos) applied at 0.2g/100g of fish body weight daily on the growth performance of major carps (*Labeo rohita, Catla catla* and *Cirrhinus mrigala*). *PakistanVet. J.,* 21(3): 128-131.
2. Beers, R and Sizer, I., 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, 195: 133.
3. Bergen, H., Hawton, K., Waters, K., Cooper, J and Kapur, N., 2010. Epidemiology and trends in non-fatal self-harm in three centres in England: 2000-2007. *Br J Psychiatry*, 197: 493-498.
4. Beuge, JA and Aust, SD., 1978. The thiobarbituric acid assay. Methods in Enzymology, 52: 306-307.
5. Curtis, J. and Mortiz, M.J. 1972. Serumenzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. *Gastroenterol,* 62: 84–92.
6. Hawton, K., Townsend, E., Deeks, J., Appleby, L and Gunnell, D., et al. 2001. Effects of legislation restricting pack sizes of paracetamol and salicylate on self poisoning in the United Kingdom: before and after study. BMJ, 322: 1203-1207.
7. Kakkar, P., Das, B and Viswanathan PN., 1984. A modified spectrophotometric assay of SOD. *Ind J Biochem Biophy*, 21: 130-132.
8. Kappus H. 1985. Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. In: Sies, H. (ed), Oxidative Stress. Academic Press, Inc., London, 273–310.
9. Kumar, SM., Burgess, SN and Luu, LT., 2004. Review of nutrients management in freshwater polyculture. *Journal of Applied Biology*, 16 3/4: 17-44.
10. Larson, AM, Polson, J., Fontana, RJ., Davern, TJ and Lalani, E., et al. 2005. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology, 42: 1364-1372.
11. Lauterburg, B.H and Velez, M.E. 1988. Glutathione deficiency in alcoholics: risk factor for paracetamol hepatotoxicity. *Gut,* 29(1): 153–1157.
12. Lee, WM., 2004. Acetaminophen and the U.S. Acute Liver Failure Study Group: lowering the risks of hepatic failure. Hepatology, 40: 6-9.
13. Lowry, OH., Rosenbrough, NJ., Farr, AL and Randall RJ., 1951. Protein measurement with the Folin's reagent. *Journal of Biological Chemistry*, 193: 265-276.
14. Mitchell R. H., Karnovsky M. J. and Karnovsky M. L. 1970. The distributions of some granule-associated enzymes in guinea pig polymorph nuclear leucocytes. *Biochem. J,* 116: 207–216.

15. Moron, MS., DsePierre, JW and Manerwik, KB., 1979. Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochimica et Biophysica Acta, 582: 67-68.
16. Murugesh K. 2005. Veerendra channabasappa yeligar; Bhim charan maiti; Tapan kumar maity. Hepatoprotective and antioxidant role of *Berberis tinctoria Lesch* leaves on paracetamol induced hepatic damage in rats. *Iranian J Pharmacol Therapeut*, 4: 64–69.
17. Nourjah, P., Ahmad, SR., Karwoski, C and Willy, M., 2006. Estimates of acetaminophen (Paracetomal)-associated overdoses in the United States. Pharmacoepidemiol Drug Saf, 15: 398-405.
18. Padmini, E., Thendral Hepsibha, B and Shanthalin Shellomith, AS., 2004. Lipid alteration as stress markers in grey mullets (Magi! cephalus L.) caused by industrial eflluents in Ennore estuary (oxidative stress in fish). Aquaeult, 5: 1l5-118.
19. Parmar, D. and Kandakar, M. 1995. Mitochondrial ATPase: a target for paracetamol-induced hepatotoxicity. *Eur. J. Pharmacol*, 293: 225–229.
20. Prakash, J., Gupta, S.K. and Singh, N. 2001. Chemo-preventive activity of *Withania somnifera* in experimentally induced fibro sarcoma tumors in Swiss albino rats. *Phytother. Res*, 15: 200–204.
21. Prescott, K., Stratton, R., Freyer, A., Hall, I and Le Jeune, I., 2009. Detailed analyses of self-poisoning episodes presenting to a large regional teaching hospital in the UK. Br J Clin Pharmacol, 68: 260-268.
22. Reitman, S and Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Path, 25: 56.
23. Rotruck, JT., Pope, AL., Ganther, HE., Swanson, AB., Hafeman, DG and Hoekstra WG., 1973. Selenium biochemical roles as component of glutathione peroxidase. *Science*, 179: 588-590.
24. Sheen, CL., Dillon, JF., Bateman, DN., Simpson, KJ and Macdonald, TM., 2002. Paracetamol toxicity: epidemiology, prevention and costs to the health-caresystem. QJM, 95: 609-619.
25. Ward, FM and Daly, MJ., 1999. Hepatic Disease. In: Walker R, Edwards C, editors. Clinical Pharmacy and Therapeutics. Churchill Livingstone, New York, 195-212.
26. Wei, G, Bergquist, A, Broomé, U., Lindgren, S and Wallerstedt, S., et al. 2007. Acute liver failure in Sweden: etiology and outcome. *J Intern Med*, 262: 393-401.
27. Wendel, A., Jaeschke, H. and Gloger, M. 1982. Drug induced lipid peroxidation in mice. II. Protection against paracetamol-induced liver necrosis by intravenous liposomally entrapped glutathione. *Biochem Pharmacol*, 31: 3601–3605.