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Hepatoprotective Activity of *Rauwolfia serpentina* Rhizome in Paracetamol Intoxicated Rats*

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Abstract: Hepatoprotective activity of Aqueous Ethanolic Extract (AET) of rhizome of mature *Rauwolfia serpentina* against paracetamol induced hepatic damage in rats was investigated. The effect of AET of rhizome of *R. serpentina* on blood and liver glutathione, Na^+K^+-ATPase activity, serum marker enzymes, serum bilirubin and thiobarbutiric acid reactive substances, liver glutathione peroxidase, glutathione-S-tranferase, glutathione reductase, superoxide dismutase, catalase activity and glycogen against paracetamol induced damage in rats have been studied with a view to elucidate possible mechanism behind its hepatoprotective action. It was interesting to observe that AET of rhizome of *Rauwolfia* has reversal effects on the levels of above-mentioned parameters in paracetamol hepatotoxicity. It was observed that AET extract of *Rauwolfia* is a promising hepatoprotective agent and this hepatoprotective activity of *Rauwolfia* may be due to its antioxidant and normalization of impaired membrane function activity. A possible mechanism behind the results is discussed.

Key words: Antioxidant, glutathione, glutathione peroxidase, glutathione reductase, glutathione-s-tranferase, hepatoprotection, lipid peroxidation, paracetamol, *Rauwolfia serpentina*, serum marker enzymes

Introduction

Paracetamol is a well known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Boyd and Bereczky, 1966). Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome (Dahlin *et al*., 1984) or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity (Moron *et al*., 1979).

Paracetamol hepatotoxicity is caused by the unstable toxic reactive metabolite N-acetyl-p-benzo quininemine (NAPQI), generated by microsomal cytochrome P-450-II-E1, which causes oxidative

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stress and glutathione (GSH) depletion. Free radicals play a crucial role in a complex interplay of different mechanisms in both aging and liver diseases, are generated continuously via normal physiological processes, more so in pathological conditions. They are simultaneously degraded to non-reactive forms by enzymatic and non-enzymes antioxidant defense mechanisms. However, deleterious effects of free radicals remain a problem.

Liver is the most important and first organ to encounter ingested nutrients, drugs and environmental toxicants that enter the hepatic portal blood so regulate important metabolic functions. Hepatic damage is associated with distortion of many metabolic function (Wolf, 1999). The number of cases of liver disease remains one of the serious health problems. Inspite of tremendous strides in the modern medicine, there are not much drugs available for the treatment of liver disorders (Chaterjee, 2000). However, there are member of drugs employed in traditional system of medicine for liver affections. Many formulation containing herbal extracts are sold in Indian market for liver disorders (Chattopadhyay, 2003).

Rauwolfia serpentina (family *Apocynacea*) has great reputation, as Miracle medicinal plant reported in various texts of indigenous system of medicine Ayurveda, Siddha and Unani. *Rauwolfia* has been used in Africa for hundreds of years and in India for at least 3000 years. It was used as an antidote to snake bite, to remove white spots in the eyes, against stomach pains, fever, vomiting and headache and to treat insanity (Dewick, 2002). The roots of the plant contains alkaloids; ajmalicine, ajmaline, ajmalinine, chandrine, rauwolfine, renoxidine, rescin-namine, reserpiline, reserpinine, sarpagine, serpentine, serpentinine, tetraphyllicine, yohimbine, 3-epi-a-yohimbine and reserpine. Serpentine is not used as such but first it converts into ajmalicine through hydrogenation. Ajmalicine is used in hypertension and obstricles of cerebral blood flow, used for treatment of liver disorders. Reserpine is the most important alkaloid present in root. But still no scientific and methodical investigation has so far been reported in literature regarding its action on liver. Therefore, the present investigation has been designed to study the possible mechanism of aqueous ethanolic extract of rhizome of *R. serpentina* on the biochemical parameter against paracetamol induced hepatic damage in rats.

Materials and Methods

Plant Material and Extraction

Rhizomes of *R. serpentina* (Serpgandha; family *Apocynacea*) were locally collected from Regional Research Institute (Ayurveda), Jhansi, India. Dr. R.S. Dikshit from this Institute authenticated the plant and dried in a shade.

Shade-dried powder (1 kg of rhizome of *Rauwolfia*) was extracted by percolation at r.t. with 70% EtOH. AET of rhizome was concentrated under reduced pressure and dried in a vacuum desiccator (50°C). The residue (46.4 g) was dissolved in 70% EtOH and filtered.

Animals

Albino rats of either sex (120-180 g) were maintained under control conditions of light and temperature (25±1°C) in animal house of Institute of Pharmacy, Bundelkhand University, Jhansi. Standard food pellets (DRDE, Gwalior) and tap water were provided *ad labitum*. For experimental, animals were kept fasting overnight but were allowed free access to water. The animal experiments were performed as per the rules and regulation of Animal Ethical Committee, Govt. of India.

Paracetamol Toxicity

The rhizome extract/paracetamol/saline were given with the help of feeding cannels. Rats were divided in three groups (I, II and III) and each group had six rats. The rhizome extract at a fixed dose (425 mg kg^{-1}, p.o.) that was daily fed for 7 days to one group (Group III) of rats and paracetamol (200 mg kg^{-1} p.o.) was administered on 5th day after 5th administration of the extract. The paracetamol treated group (Group II) received normal saline in place of rhizome extract. After 48 h of paracetamol feeding rats were sacrificed by cervical dislocation for estimation of blood glutathione, reduced liver glutathione, liver Na^+ K^+-ATPase activity and liver thiobarbutiric acid reactive substances using standard methods.

Blood was collected, allowed to clot and serum separated. Liver was dissected out and used for biochemical studies. Glutathione peroxidase (GPX) (Rotruck *et al*., 1973), glutathione reductase (GRD) (Dubler and Anderson, 1981), glutathione-S-transferase (GST) (Habig *et al*., 1974), superoxide dismutase (SOD) (Roos *et al*., 1959), catalase (CAT) (Aebi, 1983), glycogen (GLY) (Hassid and Abraham, 1957) and protein (Bradford, 1976) were estimated in liver homogenate.

Assay of Liver Glutathione and Blood Glutathione

Freshly collected livers were washed with 0.9% NaCl, weighed and homogenates were made in a ratio of 1 g of wet tissue to 9 mL of 1.25% KCl by using motor driven Teflon-pestle. Reduced glutathione (GSH) was estimated using DTNB (Sedlak and Lindsay, 1968). The blood glutathione was estimated by the method of Beutler (1963). The absorbance was read at 412 nm.

Liver Na^+, K^+-ATPase Activity

To measure the liver Na^+K^+ -ATPase activity the liver was dissected out quickly, rinsed with cold phosphate buffer, liver plasma membranes were isolated and subjected for the estimation of Na^+K^+-ATPase activity (Corcoram *et al*., 1987).

Thiobarbutiric Acid Reactive Substances (TBARS)

The concentration of TBARS was measured in liver using the method of Ohkawa *et al*. (1979). The concentration of TBARS was expressed as "moles of malondialdehyde per mg of protein using 1,1,3,3, -tetra-ethoxypropane as the standard.

Serum Marker Enzymes

The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) (Reitman and Frankel, 1957; Kind and King, 1954), Total Serum Protein (TSP) (Bradford, 1976) and bilirubin (Jendrassik and Grof, 1938) were measured.

Statistical Analysis

The data were expressed as mean±SE and statistically assessed by student's t-test to statistical significance.

Results and Discussion

Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses (Mitchell *et al*., 1973; Kuma and Rex, 1991; Eriksson *et al*., 1992). Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations (Ahmad and Khatar, 2001; Asha *et al*., 2004; Kumar *et al*., 2004; Singh and Handa, 1995; Visen *et al*., 1993).

Table 1: Effect of AET of rhizome of *Rauwolfia serpentina* on blood and liver glutathione (GSH), liver Na^+K^+-ATPase, serum marker enzymes (ALT, AST, ALP) and liver thiobabutiric acid reactive substances (TBARS) in paracetamol intoxicated rats

Parameters	Group I	Group II	Group III
Blood GSH (mg%)	1.47±0.06[a]	0.46±0.05	1.20±0.04[c]
Liver GSH (μ moles g^{-1} liver)	12.04±0.32[c]	7.42±0.34	10.42±.21[b]
Na^+K^+-ATPase (U mg^{-1} protein)	8.76±0.54[a]	6.90±0.19	10.06±0.26[c]
TBARS (nmol of MDA g^{-1} of wet tissue h^{-1})	298.70±10.3[a]	612.40±9.5	299.70±7.21[c]
ALT (U mg^{-1} protein)	65.60±1.09[c]	252.60±12.9	42.98±2.89[c]
AST (U mg^{-1} protein)	76.98±2.09[a]	182.80±23.9	41.45±1.01[b]
ALP (KA unit)	65.30±7.6[b]	101.23±9.34	61.79±3.24
Bilirubin (mg%) (Total)	1.34±0.16[a]	2.67±0.09	1.26±0.11[b]
TSP (mg protein mL^{-1} serum)	63.94±2.36[a]	56.12±2.16	65.31±2.16[c]

Results are mean of six observations±SE, [a]$p<0.05$ when compared with Group II, [b]$p<0.01$ when compared with Group II, [c]$p<0.001$ when compared with Group II

The glutathione level in liver homogenate and in blood, liver Na^+ K^+ -ATPase, serum marker enzymes and liver thiobarbutiric acid reactive substances are given in Table 1. The concentration of GSH in animals treated with paracetamol was significantly ($p<0.001$) reduced in homogenate of liver and so was the level of glutathione in blood ($p<0.05$) and Na^+K^+-ATPase level ($p<0.05$) as compared with saline control rats (Table 1). While thiobarbutiric acid reactive substances (TBARS) of paracetamol treated rats was significantly higher than the saline treated control animals (Group I). Administration of rhizome of *R. serpentina* extract increased the concentration of GSH in liver ($p<0.01$) and glutathione in blood ($p<0.001$) and liver Na^+ K^+-ATPase activity significantly ($p<0.001$) when compared to its paracetamol treated control group. On the other hand, the increased level of liver thiobarbutiric acid reactive substances of paracetamol treated animals was significantly reduced ($p<0.001$) in Group III of animals receiving both rhizome extract and paracetamol.

The abnormal high level of serum ALT, AST, ALP and bilirubin observed in this study (Table 1) are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Feeding of *R. serpentina* extract to such paracetamol treated rats reduced the enhanced level of serum ALT, AST, ALP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells.

Reduced activities of superoxide dismutase (SOD) and catalase (CAT) in paracetamol treated rats (Table 2) confirm the hepatic damage to the rats (Kalpowitz *et al*., 1986). Treatment with AET of *Rauwolfia* to the rats in which hepatic damage was induced paracetamol treatment caused significant increase in SOD and CAT activities ($p<0.001$). Hence, antilipid peroxidative and/or adaptive nature of the system as brought about by the *Rauwolfia* extract against the damaging effects of free radical produced by the paracetamol.

In nonenzymatic system, glutathione (GSH) constitutes the first line of defense against free radicals. Reduction in liver GSH and decrease in GPX, GST, GRD activity in paracetamol treated rats as observed in this study (Table 2), indicates the damage to the hepatic cells, which is confirmed by the earlier reports (Singh *et al*., 1999). Administration of AET of *Rauwolfia* promoted the conversion of GSSG (oxidized glutathione) into GSH by the reactivation of hepatic glutathione reductase enzyme in paracetamol intoxicated rats. The availability of sufficient amount of GSH thus increased the detoxification of active metabolites of paracetamol through the involvement of GPX. But the restoration of GSH level after the enduration of the *Rauwolfia* extract to such paracetamol treated rats account for the protective efficacy of the extract.

Table 2: Effect of AET of rhizome of *Rauwolfia serpentina* on the levels of liver SOD, CAT, GPX, GRD, GST, GLY, body weight (before and after treatment) and liver weight in paracetamol intoxicated rats

Parameters	Group I	Group II	Group III
SOD (Units of activity mg^{-1} protein)	1.24±0.06[a]	0.62±0.05	1.22±0.04[c]
CAT (μmoles of H_2O_2 decomposed mg^{-1} protein min^{-1}	29.56±2.12[b]	6.34±0.29	28.19±.52[c]
GPX (nmol of GSH oxidized min^{-1} mg^{-1} protein)	317.5±26.6[b]	174.50±19.4	296.00±16.3[a]
GRD (nmol of GSSG utilized min^{-1} mg^{-1} protein)	26.5±1.2[a]	9.30±0.6	23.50±1.5[c]
GST (nmol of CDNB conjugate formed min^{-1} mg^{-1} protein)	279.3±21.5[b]	151.90±11.2	263.40±15[a]
GLY (mg g^{-1} wet tissue) Body weight	29.14±2.94[a]	21.45±1.11	28.49[c]±2.74[c]
Before treatment (g)	136.00±3[a]	138.60±2	138.00±4[c]
After treatment (g)	150.00±5[b]	144.30±3	154.00±7[b]
Liver weight (g)	7.19±0.3	6.11±0.6	7.15±0.5[b]

Results are mean of six observations±SE, [a]$p<0.05$ when compared with Group II, [b]$p<0.01$ when compared with Group II, [c]$p<0.001$ when compared with Group II

GPX plays a pivotal role in H_2O_2 catabolism (Eaton, 1991) and the detoxification of endogenous metabolic peroxides and hydroperoxides, which catalyzes GSH (Floka, 1971). GPX activity was significantly reduced ($p<0.05$) after paracetamol treatment as compared to control (Group I). The reversal of the GPX activity was found to normal after treatment with AET.

GST plays a physiological role in initiating the detoxification of potential alkylating agents GST level was significantly ($p<0.05$) reduced in paracetamol intoxicated rats and upward reversal was observed after the treatment with AET. This may be attributed to a direct action of the extract on the hepatic GST activation. The mechanism of which is not known.

Liver is the most important and main part of the animal body. It is highly affected primarily by toxic agents and thatswhy the above-mentioned parameters have been found to be of great importance in the assessment of liver damage. From our results, it can be speculated that (i) decreasing effect of liver and blood GSH, liver Na^+ K^+ -ATPase activity and increasing effect of liver thiobarbutiric acid reactive substance level in paracetamol treated rats were due to hepatocellular damage and (ii) AET of rhizome afforded protection from such paracetamol induced liver damage. Possible mechanism that may be responsible for the protection of paracetamol induced the following AET of rhizome by itself could act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. (a) Thus, by trapping oxygen related free radicals rhizome extract could hinder their interaction with polyunsaturated fatty acids and would abolish the enhancement of lipids peroxidation. (b) Rhizome extract significantly increases the hepatic content of GSH and blood glutathione. These results suggests that a significantly higher content of glutathione in blood and liver would afford the tissue a better protection against an oxidative stress, thus contributing to the abolishment of paracetamol induced hepatotoxicity. The activity of Na^+K^+-ATPase is decreased in paracetamol-induced rats, rhizome extract prevented this effect of paracetamol. Therefore, the AET of rhizome may be useful agent for the normalization of paracetamol induced impaired membrane function.

A reduction in total serum protein (TSP) (Table 1) and liver glycogen (GLY) (Table 2) observed in the paracetamol treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and GLY and consequently decrease in the liver weight (Table 2). But, when the AET of rhizome was given along with paracetamol, the significant increase in TSP and liver GLY was observed indicating the antihepatotoxic activity of extract and also accounting for the increase in the liver weight most probably through the hepatic cell regeneration. Now, further it has been confirmed that AET of rhizome of *Rauwolfia serpentina* is the most useful for hepatoprotection. The possible mechanism of AET of rhizome of *R. Serpentina* may be its protective action against paracetamol induced hepatocellular metabolic alteration could be by the stimulation of hepatic regeneration through an important synthesis of protein or accelerated detoxification and excretion.

Conclusions

The above observation lead to the conclusion that AET of *Rauwolfia* in affording the hepatoprotective activity against paracetamol may be due to the cell membrane stabilization, hepatic cell regeneration and activation of antioxidative enzymes such as GPX, GST, GRD, SOD and CAT. Therefore, rhizome extract is a promising hepatoprotective agent and this hepatoprotective activity may be due to its antioxidant and normalization of impaired membrane function activity. Thus, the hepatoprotective action combined with antioxidant activity has a synergistic effect to prevent the process of initiation and progress of hepatocellular diseases (Wilkinson, 1962). Further studies both on the extract and/or its chemical constitutes are needed to pinpoint the findings. This report may serve as a footstep on this aspect. The isolation of active component involved in hepatoprotection from rhizome of *Rauwolfia* and their mechanism of action is under progress in the laboratory.

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