SCREENING OF ANTI-OXIDANT POTENTIAL OF AQUEOUS EXTRACT OF *CRATAEVA RELIGIOSA* AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT

The present study was taken up to evaluate the antioxidant potential of aqueous extracts of *Crataeva religiosa* in paracetamol induced hepatotoxicity in rats and also to compare their effects with the standard drug, silymarin. Twenty four female Wistar rats were used for the study. Toxicity was induced on day one in all the animals with an oral acute toxic dose of paracetamol at the rate of 3 g/kg bw. Treatments with standard drug (silymarin at 100 mg/kg bw.) and test drug (aqueous extract of *C. religiosa* at 200 and 400 mg/kg bw.) were given from day 2 to day 8. Both the standard and test drugs exhibited a significant effect in restoring the altered antioxidant parameters in the liver. The aqueous extract at 400 mg/kg bw. excelled better than the dose of 200 mg/kg bw. in restoring the antioxidant parameters.

Keywords: *Crataeva religiosa*, antioxidants, paracetamol, hepatotoxicity, lipid peroxidation.

INTRODUCTION

Liver, being an important organ in the body plays a major role in the maintenance of internal environment through its multiple and diverse functions (Talwar, 1989). It is rich in antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) that can metabolize reactive oxygen species (ROS), produced mainly due to peroxidation of cellular membranes (Bulger *et al*., 2001).

Crataeva religiosa commonly known as Maralingam in Tamil is a common medicinal plant distributed throughout India is known to have versatile medicinal values. Hence, the antioxidant potential of its aqueous extract at the dose rate of 200 and 400 mg/kg body weight was evaluated against paracetamol induced hepatotoxicity in Wistar rats and its effect was compared with the standard drug Silymarin.

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MATERIALS AND METHODS

Bark of the plant *C. religiosa* was procured, dried in shade and made into fine powder. Aqueous extract of the plant material was prepared from the dried bark powder by maceration method and the extract obtained was stored at a temperature of 4°celcius. Qualitative analysis of the extract was performed to find the presence of phytochemicals in it.

Twenty four Wistar rats of same age and size were divided into four groups randomly. On the first day of trial, all the 24 animals were given paracetamol orally with selected acute toxic dose of 3 g/kg bw. Group I served as paracetamol control and received only water. From second day till eighth day, Group II rats were treated with standard drug Silymarin at a dose rate of 100 mg/kg bw., Group III rats with aqueous extract of *C. religiosa* at a dose rate of 200 mg/kg bw., Group IV rats with aqueous extract of *C. religiosa* at a dose rate of 400 mg/kg bw. (as indicated in Table- 1)

On day 9, animals were sacrificed and liver tissues of all the animals were excised, weighed and rinsed in ice-cold saline for estimation of lipid peroxidation and antioxidant profiles.

Lipid peroxidation was estimated by the formation of thiobarbituric acid reactive substances (TBARs) by the method of Yagi (1976). Superoxide dismutase (SOD) was measured by the method of Marklund and Marklund (1974). Glutathione peroxidase (GPX) was measured by the method of Rotruck *et al.* (1973). Catalase (CAT) was assayed by the method of Caliborne (1985). Reduced glutathione (GSH) was estimated by the method of Meron *et al.* (1979). Total protein (TP) estimation was carried out by the method of Lowry *et al.* (1951).

RESULTS

The phytochemical analysis of the extract showed the presence of flavonoids, saponins, terpenoids and quinones.

The effect of aqueous extracts of *C. religiosa* on non-enzymatic and enzymatic antioxidants, total protein and lipid peroxidation are given in table – 2.

The group treated with paracetamol alone showed a drastic change in their anti-oxidant levels. The group treated with the standard drug silymarin, presented a significant restoration of antioxidant parameters compared to paracetamol treated group.

Groups treated with aqueous extracts of *C. religiosa* at the dose rate of 200 and 400 mg/kg bw. showed a dose dependent increase in GSH, SOD, GPX, CAT levels and TP when compared to paracetamol treated group. Both the treatments also produced a dose dependent decrease in the MDA levels when compared to paracetamol treated group. Aqueous extract of *C. religiosa* at the dose rate of 400 mg/kg

bw. exhibited a significant ($P<0.05$) increase in enzymatic and non-enzymatic antioxidants when compared to the paracetamol group.

DISCUSSION

Paracetamol at toxic doses results in the saturation of sulfation and glucuronidation pathways, thus shifts the primary metabolic process to the cytochrome P-450 isoenzymes and results in the large production of N- acetyl para benzoquinone imine (NAPQI) in liver (Peter and Edward, 1999). This NAPQI rapidly reacts with glutathione (GSH) and leads to depletion of hepatic GSH in cells and mitochondria, which further results in hepatocellular death and mitochondrial dysfunction (Mitchell *et al.*, 1973). In addition, NAPQI increases the formation of ROS and reactive nitrogen species (RNS) such as superoxide anion, hydroxyl radical, hydrogen peroxide, nitrous oxide and peroxynitrite respectively. Excess levels of ROS and RNS attacks biological molecules such as DNA, protein and phospholipids which leads to lipid peroxidation, nitration of tyrosine and depletion of the antioxidant enzymes which eventually results in oxidative stress (Michael *et al.*, 1999).

CAT, SOD and GPX are free radical scavenging enzymes and are the first line of cellular defense against oxidative stress. SOD catalyzes the conversion of superoxide (O_2^-) to H_2O_2 which further can be rapidly converted to water by CAT and GPX (Bulger *et al.*, 2001). Aqueous extracts of *C. religiosa* at 200 and 400 mg/kg bw. showed a dose dependent restoration of CAT, SOD and GPX activity when compared to the paracetamol treated group, indicating their free radical scavenging potential.

Depletion of hepatic GSH is the primary factor for the formation of lipid peroxides (Ross, 1988). These lipid peroxides attack phospholipids of biological membranes which in turn results in loss of functional and structural integrity of cell membrane. The increase in MDA levels in liver suggests enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Aqueous extracts of *C. religiosa* at 200 and 400 mg/kg bw. produced dose dependent increase in the levels of GSH and TP levels. Both the doses further decreased the MDA levels in the liver homogenates indicating their ability to neutralize the lipid peroxides, thus showing their potential to replenish the anti-oxidants in the liver tissue.

Aqueous extract at 400 mg/kg bw. produced maximal effect in restoring the antioxidant parameters. The effect of these extracts may be due to the free radical scavenging activity of the flavonoids, saponins and terpenoids contained in the extract. Our findings are similar with the findings of Patel *et al.* (2010) in which they stated that saponins, flavonoids, tannins and triterpenoids content of the plant *Tecomella undulate* was responsible for its antioxidant and hepatoprotective potential. These active principles such as flavonoids and terpenoids act as antioxidants by donating

electrons to the electron-poor free radicals. This prevents the free radicals from taking electrons from the cellular machinery and thereby reduces damage to the cells. These phytochemicals also might have increased the glutathione levels in the liver thereby further decreased the drastic effects of ROS on antioxidant enzymes.

SUMMARY

The antioxidant potential of aqueous extract of *C. religiosa* at two different doses viz., 200 and 400 mg/kg bw. for 7 days was studied in paracetamol induced hepatotoxicity model in Wistar rats. Both the doses exhibited effect in restoring the anti-oxidants in the liver. Aqueous extract at 400 mg/kg bw. excelled better than the dose of 200 mg/kg bw. in restoring the antioxidant parameters. Hence, *C. religiosa* can be used for future studies with different extracts and different doses to further strengthen its antioxidant potential.

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Table- 1

Experimental design

Group	**Number of animals**	**Treatments (2nd day to 8th day)**
I	6	Paracetamol 3g/kg bw. p.o. on the first day + water.
II	6	Paracetamol 3g/kg bw. p.o. on the first day + Silymarin 100 mg/kg bw. p.o.
III	6	Paracetamol 3g/kg bw. p.o. on the first day + 200mg/kg bw. of aqueous extract of *C. religiosa* p.o.
IV	6	Paracetamol 3g/kg bw. p.o. on the first day + 400mg/kg bw. of aqueous extract of *C. religiosa* p.o.

Table – 2
Effects of ethanolic extracts of *C. religiosa* on the antioxidant levels in liver homogenates in paracetamol induced hepatotoxicity in Wistar rats

n=6

Groups	GSH	CAT	SOD	GPX	MDA	TP
1.Paracetamol control	2,625.37^{a}±12	0.32^{a}±0.06	0.53^{a}±0.11	62.14^{a} ±8.38	7.49^{e} ±0.14	4.95^{a}±0.26
2.Silymarin control	8,973.76^{c} ± 63	0.73^{b}±0.05	2.24^{b}±0.18	111.46^{b}±7.42	2.49^{a} ±0.19	10.14^{c}±0.07
3. Aq C. rel 200	3,866.46^{ab} ± 28	0.35^{a}±0.07	0.58^{a}±0.21	80.28^{a}±4.08	6.52^{d} ±0.18	9.10^{b} ±0.58
4. Aq C. rel 400	5,740.92^{abc} ± 48	0.52^{ab}±0.09	0.78^{a}±0.12	81.60^{a}±6.95	4.92^{c} ±0.26	9.64^{bc} ±0.13

GSH- mg/g of tissue, **CAT**-ìm of H_2O_2 decomposed/ min/ mg protein, **SOD**- enzyme required to inhibit 50% pyrogallol autoxidation/min/mg protein, **GPX**-ìm of glutathione utilized/min/mg protein, **TBARs**-nm of MDA/g of tissue, **TP**-mg/g tissue.