

## A comparative study of the beneficial effects of *Osbeckia octandra* and *Osbeckia aspera* in liver dysfunction in rats

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### Summary

A study was conducted to compare the protective effects of aqueous extracts of *Osbeckia octandra* and *Osbeckia aspera* against carbon tetrachloride (CCl<sub>4</sub>)-mediated liver damage in Sprague Dawley rats by assessing their ability to protect livers against the toxin-mediated alterations in the liver histopathology and the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase.

Within 24 h of administering a sub-lethal dose (0.2 ml/100 g, i.p.) of CCl<sub>4</sub> to rats, the ALT, AST and alkaline phosphatase activities were found to be 380.8%, 101.4% and 222.2% higher respectively, than the corresponding base values in control animals untreated with the toxin. By pre-treatment of rats for 7 days with an aqueous extract of either the *O.octandra* or *O.aspera*, the CCl<sub>4</sub>-mediated changes in the serum enzyme activities could be considerably reduced. Thus, in rats pre-treated with an extract of *O.octandra* or *O.aspera*, the CCl<sub>4</sub> was able to cause only a 33.7% or 27.6% increase in ALT activity, a 9.2% or 4.2% increase in AST activity and a 16.6% or 17.6% increase in alkaline phosphatase activity respectively, above the corresponding values in control animals.

In post-treatment experiments also when serum enzyme levels in rats treated only with CCl<sub>4</sub> and left to recover for 4 days were compared with those in rats treated orally for 3 days with either plant extract starting 24 h after the toxin administration, it was found that both plant extracts were able to protect the livers against the toxin mediated changes, to a similar extent. Thus, on the 4<sup>th</sup> day after CCl<sub>4</sub> treatment, the serum ALT,

AST and alkaline phosphatase activities were still 162%, 76.5% and 90.1% respectively, higher than the corresponding values in control animals. In the *O.octandra* and *O.aspera* post-treated groups, the corresponding increases in the activities of ALT, AST and alkaline phosphatase respectively, were only 53.8% and 35.5% for ALT, 39.2% and 41.6% for AST and 29% and 18.6% for alkaline phosphatase. In both the pre-treatment and the post-treatment experiments it was also observed that, the CCl<sub>4</sub>-mediated alterations in liver histopathology could be prevented to a similar extent by both plant extracts. The overall results indicate that aqueous extracts of the leaves of both *O.octandra* and *O.aspera* possess very similar hepatoprotective abilities, thus rationalizing the use of both these plants in traditional medicine for the treatment of liver disease.

### Introduction

*Osbeckia octandra* and *Osbeckia aspera* are two very closely related plant species belonging to the family Melastomaceae, that are found in Sri Lanka (1). They are both referred to as "Heenbovitiya" by the local population and are both used in traditional medicine for the alleviation of jaundice related to liver disease (2,3), the species used depending on its local availability. Although many investigations have been carried out to assess the hepatoprotective ability of *O.octandra* (4-7), no information is available on the relative potencies of *O.octandra* and *O.aspera* as hepatoprotective agents. Since both plant species are being used by the local population for the therapy of liver disease, it was decided to conduct an investigation to determine if both plant species possess

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similar hepatoprotective abilities or whether one species is more effective than the other.

Carbon tetrachloride (CCl<sub>4</sub>) is an established hepatotoxin (8). A single sub-lethal dose of the toxin has been shown to cause marked alterations in liver function as observed by elevations in the serum levels of several enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase. In the present investigation therefore, due to convenience of obtaining the toxin, hepatic injury was induced in rats by administration of CCl<sub>4</sub>. The hepatoprotective potentials of *O.octandra* and *O.aspera* were assessed by comparison of the extent to which aqueous leaf extracts of the two plants could prevent the CCl<sub>4</sub>-mediated alterations in (a) serum levels of ALT, AST and alkaline phosphatase and (b) liver histopathology.

## Material and Methods

### Experiment animals

In all experiments, male Sprague-Dawley rats of 150 - 200 g body weight were used. The animals were maintained on a standard laboratory diet purchased from the Oils and Fats Corporation of Sri Lanka. All animals had access to food and water *ad libitum*.

### Preparation of plant extracts

Fresh leaves of *O.octandra* and *O.aspera* were collected from areas in the Gampaha district and the Galle district of Sri Lanka, respectively. The botanical identities of the plants were confirmed by the Curator of the Royal Botanical Gardens, Peradeniya, in comparison with authentic samples in the herbarium.

The leaves of each plant species were cut into small pieces and 200g of each type was homogenized in 1.5 L distilled water and refluxed for 4h. The mixtures were then strained through muslin and the final volume of each extract reduced to 100 ml *in vacuo*. The extracts were centrifuged at 3000 rpm to remove any plant debris and the supernatants then freeze dried and stored at -20°C until required. For each experiment, the

appropriate weight required was reconstituted in distilled water.

### Treatment of animals

The rats were randomly divided into 9 groups of 10 animals each. Group 1 served as the controls and were dosed orally by gavage with distilled water (10 mL/kg). Groups 2 and 3 were similarly dosed with 10 mL (equivalent to 20 g wet weight of leaves)/kg/day respectively of either *O.octandra* or *O.aspera* leaf extract, for 7 days. Groups 4 and 5 were treated with *O.octandra* and *O.aspera* extracts respectively, in a similar manner to Groups 2 and 3, but after 7 days pre-treatment, these animals were administered a single sub-lethal dose (intraperitoneally) of CCl<sub>4</sub> (CCl<sub>4</sub>: Olive oil (1 : 1) and 0.2 ml CCl<sub>4</sub> /100g/day), and killed after 24 h. Groups 6 and 7 were administered only the sub-lethal dose of CCl<sub>4</sub> and killed after 24 h and 4 days respectively. Groups 8 and 9 were administered the sub-lethal dose of CCl<sub>4</sub> and after 24 h, dosed for a further 3 days with 10 mL/kg/day of the *O.octandra* and *O.aspera* extracts respectively. All animals were sacrificed by cervical decapitation at the end of the prescribed regime of treatment. Livers of all animals were excised and fixed in buffered formalin for histopathological assessment of hepatic damage. The animals were dosed for only 3 days in the post-treatment experiments and for 7 days in the pre-treatment experiments because in a previous investigation with *O.octandra* (4) it was found that these post-treatment and pre-treatment time periods were sufficient for the plant extract to offer maximum protection against CCl<sub>4</sub> mediated liver damage in rats.

### Assessment of liver function

Liver function was assessed by a) the estimation of serum levels of alanine aminotransferase (ALT) aspartate aminotransferase (AST) and alkaline phosphatase and b) histopathological examination of liver slices.

### Blood collection for enzyme assays

Blood from rats subjected to cervical dislocation were collected into clean, dry, centrifuge tubes. The serum was allowed to separate and used in the enzyme assays.

### Enzyme assays

Serum ALT and AST levels were determined using a combination of the methods of Mohun and Cook (10) and Reitman and Frankel (11) as described by BDH Chemicals Ltd., (Poole, UK) in their assay kits for the respective enzymes. The method of King et al. (12) was used for the estimation of serum alkaline phosphatase.

### Statistics

Results are expressed as the mean  $\pm$  S.E.M. The level of significance was determined by the Student's t-test and analyses of significant differences between groups was carried out by one-way analysis of variance (ANOVA).

### Results

The effects of  $\text{CCl}_4$  and leaf extracts of *O.octandra* and *O.aspera* on the activities of serum ALT, AST and alkaline phosphatase in rats is summarized

in Tables 1 and 2. As expected from results of previous investigations (4,9), a significant increase in the activities of serum ALT, AST and alkaline phosphatase occurred within 24 h of exposure of rats to sub-lethal doses (0.2mL/100g) of  $\text{CCl}_4$ .

Results in Tables 1 and 2 demonstrate that prior or subsequent administration of either of the leaf extracts under investigation, can moderate to a similar extent,  $\text{CCl}_4$ -mediated alterations in the serum levels of ALT, AST and alkaline phosphatase, even though the plant extracts by themselves have no effect on their activities. Within 24 h of  $\text{CCl}_4$  administration, the ALT, AST and alkaline phosphatase activities were 380.8%, 101.4% and 222.2% higher than the corresponding base values in the control group of rats. In rats pre-treated for 7 days with *O.octandra* or *O.aspera*, and then administered  $\text{CCl}_4$ , the percentage increase in the activities of a) ALT were 33.7% and 27.6% respectively, b) AST were 9.2% and 4.2% respectively and c) alkaline phosphatase were 16.6% and 17.7% respectively.

Table 1

Effect of *O.octandra* and *O.aspera* pre-treatment on  $\text{CCl}_4$ -induced hepatotoxicity

Animal group	Serum Enzyme Activities		
	ALT (I.U./L)	AST (I.U./L)	Alkaline phosphatase (K.A. Units/dL)
Controls	20.8 $\pm$ 3.5	42.5 $\pm$ 4.7	34.4 $\pm$ 2.4
$\text{CCl}_4$ treated	100.1 $\pm$ 4.9**	85.6 $\pm$ 4.5**	110.5 $\pm$ 2.3**
<u><i>O.octandra</i> pre-treated</u>			
<i>O.octandra</i> only (7 days)	20.9 $\pm$ 1.2 <sup>NS</sup>	43.7 $\pm$ 3.1 <sup>NS</sup>	39.2 $\pm$ 5.1 <sup>NS</sup>
<i>O.octandra</i> (7 days) + $\text{CCl}_4$ (24 h)	27.8 $\pm$ 4.4 <sup>NS a</sup>	46.4 $\pm$ 2.9 <sup>NS a</sup>	40.1 $\pm$ 4.4 <sup>NS a</sup>
<u><i>O.aspera</i> pre-treated</u>			
<i>O.aspera</i> only (7 days)	21.1 $\pm$ 2.1 <sup>NS</sup>	41.7 $\pm$ 1.2 <sup>NS</sup>	32.2 $\pm$ 5.5 <sup>NS</sup>
<i>O.aspera</i> (7 days) + $\text{CCl}_4$ (24 h)	25.5 $\pm$ 1.2 <sup>NS a</sup>	44.3 $\pm$ 2.3 <sup>NS a</sup>	40.5 $\pm$ 1.9 <sup>NS a</sup>

mean  $\pm$  SEM; n=10

ALT = alanine aminotransferase; AST = aspartate aminotransferase

IU = International Units; K.A. Units = King Armstrong Units

Results statistically different from controls are indicated by asterisks - \*\*  $p < 0.001$ ; NS - Not significantly different; a - results significantly different from group treated with only  $\text{CCl}_4$  at  $p < 0.001$ .

Table 2  
Effect *O.octandra* and *O.aspera* post-treatment on CCl<sub>4</sub>-induced hepatotoxicity

Animal group	Serum Enzyme Activities		
	ALT (I.U./L)	AST (I.U./L)	Alkaline phosphatase (K.A. Units/dL)
Controls	20.8 ± 3.5	42.5 ± 4.7	34.4 ± 2.4
CCl <sub>4</sub> treated (single dose)			
After 24 h	100.0 ± 4.9*	85.6 ± 4.5*	110.5 ± 2.3*
After 4 days	54.5 ± 0.8*	75.0 ± 1.4*	65.4 ± 2.9*
CCl <sub>4</sub> (24 h) + <i>O.octandra</i> (3 days)	32.0 ± 3.8***	59.2 ± 1.4***	35.4 ± 2.4 <sup>NSa</sup>
CCl <sub>4</sub> (24 h) + <i>O.aspera</i> (3 days)	28.2 ± 1.2***	60.2 ± 4.2**	40.8 ± 4.1 <sup>NSa</sup>

mean ± SEM; n=10

ALT = alanine aminotransferase; AST = aspartate aminotransferase

IU = International Units; K.A. Units = King Armstrong Units

Results statistically different from controls are indicated by asterisks - \* p<0.001; \*\* p<0.01;

NS - Not significantly different from corresponding CCl<sub>4</sub> control group at p<0.001.

Administration of *O.octandra* or *O.aspera* extract 24 h after exposure to CCl<sub>4</sub> also resulted in a faster recovery of the liver as can be judged by comparing the serum enzyme activities in these animals with those treated with the toxin and left to recover for 4 days on their own, without the plant extracts (Table 2). In these post-treatment experiments also, both plant extracts appear to be equally effective in modulating the toxin-mediated alterations in serum enzyme activities. Thus, on the 4<sup>th</sup> day after CCl<sub>4</sub> treatment, the serum ALT, AST and alkaline phosphatase activities were still 162.0%, 76.5% and 90.1% higher than the corresponding values in control animals. In the *O.octandra* and *O.aspera* post-treated groups respectively, activities of ALT were 53.8% and 35.5% higher; AST were 39.2% and 41.6% higher while

alkaline phosphatase were 29% and 18.6% higher than the corresponding values in control animals.

Histopathological evidence also provided supportive evidence for the enzyme results (Table 3). Centrilobular necrosis accompanied by mononuclear infiltration in the portal tract, fatty deposition and loss of cell boundaries were observed in livers of rats challenged with CCl<sub>4</sub> alone. The toxin-mediated changes in livers of rats pre- or post-treated with either *O.octandra* or *O.aspera* extracts were of much less intensity than those observed in livers of CCl<sub>4</sub> controls untreated with the plant extracts. The extent of protection against the toxin-mediated liver injury in the groups of rats pre- or post-treated with either *O.octandra* or *O.aspera* extract appeared to be very similar.

Table 3

Liver histology scores in rats (a) treated with *O.octandra* and *O.aspera* extract prior to  $\text{CCl}_4$  administration, or (b) post-treated with either plant extract subsequent to the toxin administration.

Animal group (10 rats/group)	Histology Score			
	0	1	2	3
Controls	10	-	-	-
$\text{CCl}_4$ only (24 h after single dose)	-	-	3	7
<i>O.octandra</i> (7 days) + $\text{CCl}_4$ (24 h)	6	4	-	-
<i>O.aspera</i> (7 days) + $\text{CCl}_4$ (24 h)	7	2	1	-
$\text{CCl}_4$ (4 days after a single dose)	-	3	6	1
$\text{CCl}_4$ (24 h) + <i>O.octandra</i> (3 days)	-	7	2	1
$\text{CCl}_4$ (24 h) + <i>O.aspera</i> (3 days)	-	6	3	1

Liver damage was graded on an arbitrary scale 0 - 3 according to the following criteria: 0 - normal histology, 1 - minimal changes involving the centrilobular zone, in the form of partial degranulation and/or changes in staining of the hepatocyte cytoplasm; 2 - extensive loss of cell boundaries and mononuclear infiltration in centrilobular area; 3 - gross fatty changes accompanying the necrotic changes observed in group with a score of 2.

## Discussion

Both *Osbeckia octandra* and *Osbeckia aspera* are used in Sri Lanka for the alleviation of jaundice associated with liver disease (2,3). Results of the present investigation confirms the presence of hepatoprotective principles in both these plant species. Thus aqueous extracts of the leaves of both plant species were not only able to hasten the rate of recovery of  $\text{CCl}_4$ -injured rat livers, but also on pre-treatment, protect against  $\text{CCl}_4$ -mediated hepatocellular damage. The magnitude of protection offered in the present investigation by *O.octandra* and *O.aspera* against  $\text{CCl}_4$ -mediated alterations in the levels of serum enzymes and liver histology are comparable with that observed by Thabrew *et al.* in a previous investigation (4) with *O.octandra*. The overall results of the present study indicate that under the present experimental conditions, leaf extracts of both *O.octandra* and

*O.aspera* possess comparable hepatoprotective abilities, thus rationalizing the ethnopharmacological uses of both these plant species for the treatment of patients with liver disease. The similarity in hepatoprotective activities of the two plant species under investigation may be due to the presence of a common active principle/s as has been demonstrated for some other closely related plant species such as *Hypericum perforatum* and *Hypericum japonicum* (13).

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