Osbeckia octandra (Heen Bovitiya) Leaf Extract Reduces the Thioacetamide Induced Liver Damage in Wistar Rats

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Abstract

Management of liver diseases with plant extracts has been in the traditional medicinal practice in Sri Lanka for centuries. However, scientific evidences on the beneficial effects of *Osbeckia octandra* are lacking. The present study was designed to determine the hepatoprotective activity of crude extracts of *O. octandra* against Thioacetamide (TAA) induced liver damage in Wistar rats. Five months old 15 male Wistar rats were treated in 5 groups (1-5; n=3 each group); 1) control group; distilled water (0.5mL/rat, orally)2) TAA in saline (100 mg/kg bw, intraperitoneally), 3) TAA with crude extract (CE) of *O. octandra* leaves (0.5 g DM/kg BW, orally), 4) CE (0.5 g DM/kg BW, orally), 5) saline (0.5mL/rat, intraperitoneally). Treatments were administered twice a week up to five weeks, after one month of adaptation period. Body weights were measured twice a week. Blood samples and liver tissues were collected at the end. TAA only treated group showed the lowest ($p<0.05$) body weight gain compared with the other four groups. Moreover, the TAA only treated group showed the significantly highest liver index ($p<0.05$). Both Alanine aminotransferase and Aspartate aminotransferase values were significant ($p<0.05$) in TAA only treated group than all other four groups. Furthermore, significantly low lymphocytes and high neutrophils percentages were observed in TAA only treated group ($p<0.05$). There was no any significant different between the control group and the TAA+ CE treated group for all the parameters. Livers from the TAA only treated group showed gross anatomical variations (surface roughness and discoloration) than the TAA + CE treated mice (Group 3). In conclusion, *O. octandra* crude extract demonstrated a possible hepatoprotective effects against TAA induced liver damage warranting further investigations.

Keywords: Crude extract, Hepatoprotective effect, *Osbeckia octandra*, Thioacetamide induced liver damage, Wistar rats,
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Introduction

Liver is one of the most vital and largest organs in the human body that carries out various functions including metabolism, detoxification of various metabolites, protein synthesis, production of biochemicals necessary for digestion and production of bile. Among various liver diseases, liver fibrosis is important. Because its marks the end stage of liver cirrhosis which causes major life loss in the world. Liver fibrosis is due to the disruption of hepatic architecture and subsequent development of nodules due to the excessive accumulation of extra cellular matrix proteins (Bataller and Brenner, 2005). This distorts the hepatic parenchyma and substitutes with collagen rich tissues. Hepatocellular dysfunction, hepatic insufficiency, hepatic resistant to blood flow and portal hypertension are major adverse results of the liver fibrosis. Hepatitis B and C viruses, alcohol abuse and non alcoholic steatohepatitis are the factors that affects to the liver fibrosis. Liver diseases are considered as a lethal disease and the health condition of the liver is a one factor which decide the well-being of human (Ahsan et al. 2009). Management of liver diseases with phytochemicals is not too costly and can have fewer or no adverse effects to the human body. *Osbeckia octandra* is the plant belongs to the family melostomacea, which is an indigenous herbal plant to Sri Lanka. It has been used in traditional medicine to treat various liver disorders and has positive curing effect (Thabrew and Jayatilaka, 1999). Extracts of *O.octandra* was believed to have hepatoprotective effect and therefore used as an effective treatment for liver damages by ancestors long years ago. Plant natural antioxidants can overcome oxidative damage by reactive oxygen species. So that herbal medicines can interfere to the further development of hepatic fibrosis. Many investigations have been conducted to identify efficacy of hepatoprotective effects of a

octandra aqueous extract, but not yet for the freeze dried powder of the leaves which will protect the phytochemical intact during the processing for treatments. The objective of present study was to determine the hepatoprotective activity of crude extract of *O. octandra* against thioacetamide induced liver damage using a Wistar rat model.

Materials and Methods

Fungus free *O. octandra* leaves were collected. Voucher sample was prepared and authenticated at the National Herbarium, Royal Botanic Gardens, Peradeniya. Washed and air dried leaves were freeze dried at -50°C for 6 hours. Freeze dried samples were powdered and sieved. Prepared samples were stored in 4°C in the refrigerator until use. Powdered samples were added into centrifuge tubes and dissolved in distilled water just before administration.

Five months old 15 male Wistar rats were divided into 5 groups; Group 1 rats received distilled water (0.5mL/rat) only, Group 2 received Thioacetamide (TAA) dissolved in saline only (100 mg/kg BW, intraperitoneally), Group 3 received TAA (100mg/kg BW, intraperitoneally) and crude extract (CE) of *O. octandra* leaves preparation (0.5 g DM/kg BW, orally), Group 4 rats received CE of leaves preparation only (0.5 g DM/kg BW, orally) and Group 5 rats received saline only (0.5mL/rat).*O. octandra* crude extract was given using a gavage twice a week for 5 weeks. Rats were injected by TAA (2% W/V TAA solution) solution with the dose of 100 mg/kg BW twice a week for 5 weeks. The initial body weight (BW) and BW after each week of each rat from every group were recorded in grams. After 5 weeks of treatment rats from each group were sacrificed with ethically approved protocol using chloroform and blood samples were collected through cardiac puncture. Liver samples were taken from the dissected rats and weighed. Liver index was calculated and gross pathological changes and histopathological (H & E) were observed. Following liver index formula was used to calculate liver index (Yogalakshmi et al. 2010). Liver index = (liver mass (g)/individual body mass (g))*100.The differential count was done following an established protocol.

Alanine aminotransferase (ALT/SGPT) and Aspartate aminotransferase (AST/SGOT) were measured using readily available test kits (Randox UK) following the manufacturer's protocols.

All the data were statistically analyzed by one way ANOVA followed by Tukey's test using Minitab 17. Group mean comparisons were done by using the Tukey's test.

Ethical clearance for this study was obtained from the Ethical Clearance Committee of the Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya (Ethical clearance certificate No. VER-16-001)

Results and Discussion

Thioacetamide administration caused severe liver damage by production of fibrous septa and alterations caused to hepatocytes structure. Thus TAA intoxification leads to alteration of hepatic function. Thioacetamide only treated group showed the lowest ($p<0.05$) body weight gain compared with the other four groups (Figure 1). Significant difference of body weight gain between TAA only treated groups with other groups may be due to TAA intoxification. According to the previous research findings, body weight reduction is associates with increment in free fatty acid, inflammation and oxidative stress. Thioacetamide produces reactive oxygen species (ROS) and thus leads to oxidative stress (Yogalakshmi et al. 2010).

The data from the group treated with TAA + CE indicated a significant retardation of TAA action on hepatic tissues. This may represent possible antioxidant activity present in the leaves preparation and the phenolic compounds may contribute to the antioxidant activity (Thabrew et al. 1995). The groups treated with CE only and saline only showed slightly higher body weight gain compared to control group without any significant difference. This is justifying the fact that there is no any adverse effect by both CE oral gavage and saline injection intraperitoneally.

Thioacetamide only treated group showed the highest ($p<0.05$) liver index compared with the other four groups (Figure 1). This may be due to excessive accumulation of extra cellular matrix proteins or accumulation of lipid(Yogalakshmi et al. 2010). So that significant increment in the liver index of TAA only treated group clearly indicated that the total fiber in the liver was increased by TAA. This indicates the CE of *O. octandra* suppressed the formation of liver fibrosis when administrated with hepatotoxic chemical TAA. In an early study, leaf preparation

has shown ability to inhibit both protein and glycogen synthesis (Thabrew et al. 1995).

cellular membrane degradation and increment of cell membrane permeability and finally

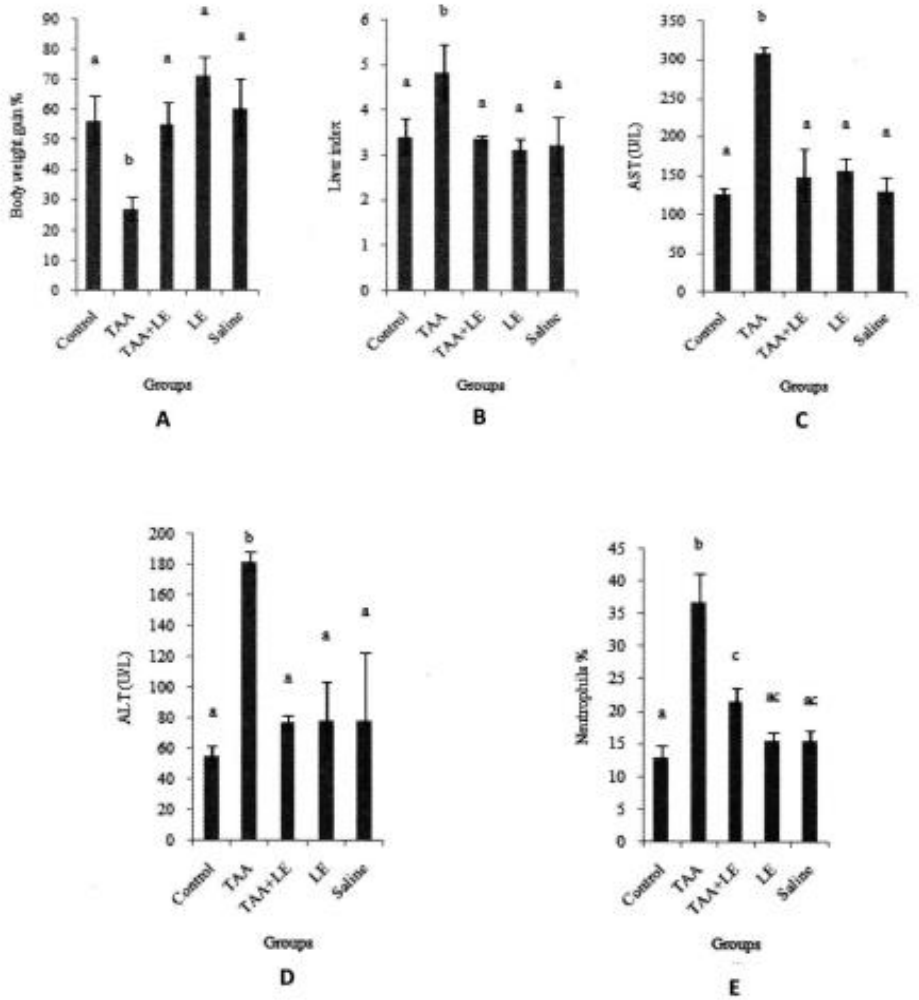

Figure 1: *Results of the different parameters tested during the experiment. A) Body weight gain in different groups, B) Liver indexes in different groups, C) AST values in different groups, D) ALT values in different groups E) Neutrophil percentage in different groups. Different values are expressed as Mean ± SEM; p<.05; n=3. a-c Different superscripts indicate the significant difference (p<.05). ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; LE, Leaf Extract; TAA, Thioacetamide*

Thioacetamide only treated group showed the highest ($p<0.05$) AST value and ALT value compared with the other four groups (Figure 1). Aspartate aminotransferase and ALT are different kinds of serum enzymes that mark liver injury (Yogalakshmi et al. 2010).Oxidative stress followed by TAA intoxification leads to peroxidation of cellular membrane resulting leakage of serum enzymes in to blood circulation.

Accordingly, the present data showed no significant difference in both AST and ALT values of distilled water only received group and TAA+CE treated group. This may be due to recovery and protective effects of leaves

preparation. The recovery and protective effects may be due to presence of phenolic compounds in the CE of *O.octandra* leaves but this need to be investigated further.

Thioacetamide only treated group showed the highest ($p<0.05$) neutrophils percentage compared with other four groups (Figure 1). Neutrophils are the first immune cells that defend against in an inflammation. Therefore, significant elevation of neutrophils percentage in TAA only treated group represents the liver damage induced by TAA. Activation of HSC is the result of liver fibrosis and thus increases the production of cytokines. These cytokines are responsible for the activation of neutrophils. Neutrophils percentage of TAA + CE treated animals showed significantly higher value than that distilled water only treated animal group and significantly lower than TAA only treated animal group. This effect may be due to the incomplete recovery induced by CE of *O. octandra* preparation and the significantly lower value than TAA only treated group may be due to the recovery up to certain degree owing to the possible hepatoprotective effect of the leaf extract.

Liver of TAA only treated animals showed anatomical abnormalities like rough surface with pale red (discoloration) surface color. Thioacetamide + CE treated animals also showed similar changes but in less intensity. Such changes were completely absent in livers from distilled water only received group, CE only and saline only treated animal groups and showed shiny smooth surface with meaty red color. Since the gross pathology changes present in TAA + CE group was mild, it represents the recovery and possible protective effects of CE confirming the hepatoprotective effects of CE of *O. octrandra.*

As this was performed as pilot research, present study was conducted only up to 5 weeks, but it is worthwhile to continue the treatments further to find out whether a complete recovery can be obtained with the treatment of *O. octndra* crude leaf extract. In addition to the above changes, with the disease progression, some other changes like enlargement of liver, internal damage and nodule formation can occur and these characteristics can also be assessed during the prolong treatments to identify the recovery.

Conclusion

Osbeckia octandra crude extract from freeze-dried leaves demonstrated a possible hepatoprotective effects against TAA induced liver damage warranting further investigations.

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