



RESEARCH PAPER

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Acute and subacute toxicity evaluation of methanolic extract of *Hodgsonia heteroclita* (Roxb.)

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Abstract

Hodgsonia heteroclita (Roxb.) is a medicinal plant used by the people of Assam as antihyperglycemic agent. However, very less scientific studies have been conducted to confirm its medicinal property. Therefore, present study investigates the toxicological effect of plant on mice model. Acute and sub-acute toxicity was carried out following OECD guidelines. Toxicity study was designed for 14 days period by intraperitoneal injection (i.p.) of different doses plant extract, 50, 100, 500, and 1000 mg/kg body weight (*b.w.*). Following i.p. injection the animals were continuously observed for 1 h, then frequently for 24 h, and thereafter once per day for 14 days to monitor any kind of behavioral changes. One-tenth of the highest dose of acute toxicity study (i.e., 100 mg/kg *b.w.*) was taken for sub-acute toxicity study and continued for 14 days with daily administration. On the 15th day, tissue samples, liver, kidney, heart, and spleen were collected and processed for histological and biochemical analysis. All the biochemical studies were done following standard protocols. Acute toxicity study revealed no toxicological effect on albino mice at the tested doses. Sub-acute toxicity study revealed slight changes in the various enzymes and molecules in control and treated mice. However, no significant differences were observed in the treated mice. Similarly, no such observable alterations were noticed in histological architecture of tissue sections of treated mice. The absence of any toxicological effects of extract of *H. heteroclita* suggests its suitability in medicinal systems. However, further investigations are needs to be done to see the mode of action.

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Introduction

Plants have been a source of medicine since time immemorial. With rich flora and fauna the use of plants against diseases is a traditional practice of rural India whose livelihood depends solely on agriculture and forest products. World Health Organization (WHO) defined traditional medicine (TM) as the sum total of all the indigenous knowledge, skills and practices used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2000). Plenty of studies have reported the medicinal property of several plants against different diseases. Despite of remarkable progress in the field of the synthetic medicines, more than half of the populations living in developing countries still rely on TM system for their daily healthcare needs because synthetic drugs have both positive and negative values. Approximately 80% population in Africa and majority of the populations in Asia and Latin America still use TM for their primary health care needs (WHO, 2002). In fact, both the old and modern drugs prescribed today by medical practitioners are directly or indirectly derived from plant source (Cragg and Newman, 2002).

People of this part of world, especially the northeastern region (NER) of India, use TM as one of the most common practices for daily health care needs. Recently studies have attempted to explore the vast resource of medicinal plants of this region (Namsa *et al.*, 2009; Syiem and Warjri, 2015). *Hodgsonia heteroclita* (Roxb.) belonging to the family Cucurbitaceae is an important medicinal, the fruit extract of which is consumed by Bodo tribe of Assam to control high blood sugar (Swargiary *et al.*, 2013). It is a perennial, climber plant that reaches up to 30 m in length and grows well in the hilly terrain of southern Asia such as Bangladesh, Bhutan, Cambodia, Laos, and north eastern states of India (Semwal *et al.*, 2014). Preliminary studies have shown that the alcoholic extract of *H. heteroclita* fruit is rich in phytochemical constituents and also possesses good antioxidant property (Basumatary *et al.*, 2015; Swargiary and Brahma, 2017). In a recent study by Usha *et al.* (2018) the crude alcoholic extract

of *H. heteroclita* fruit extract was found to show significant reduction in the serum glucose level of alloxan-induced diabetic rats. Although the plant is extensively used as an antihyperglycemic agent no detailed study has been done to study the biological activities of this plant.

Toxicity study and testing of any new drug or compound is an essential step for any drug development process. The preclinical toxicity testing on various biological systems reveals the species-, organ- and dose- specific toxic effects of an investigational product. The essence of toxicity testing is not only to check the safety profile of a test substance but also to characterize the possible toxic effects that a substance can produce on target organisms. Toxicity of a substance can be assessed by making direct exposure of the substance, in in-vitro cell culture tests or in in-vivo animal test (Parasuraman, 2011; Arome and Chinedu, 2013). Despite of wide use of *H. heteroclita* as antihyperglycemic agent, no studies have been carried out to investigate its toxicological effect. Hence, the present study was designed to carry out the acute and subacute toxicity of methanolic extract of *Hodgsonia heteroclita* (MEHh) in albino mice.

Materials and methods

Collection, identification, and preparation of crude extract of plants

Fresh fruits of *Hodgsonia heteroclita* (Roxb.) (Family Cucurbitaceae) was collected from Kokrajhar area, India with proper permission from the village head and the University. The collected plants were identified in the Department of Botany, Bodoland University (CN0102, BUBH). The methanolic fruit extract was prepared following the process as described in our earlier publication (Swargiary and Brahma, 2017). The semi-solid extract obtained was kept in deep freeze (-20°C) until further use.

Experimental animal

Healthy albino mice of both sexes (weight, 14-20g) were procured from Pasteur Institute, Shillong. They were acclimatized to the laboratory conditions of temperature (25±2°C), humidity (30-70%), and 12 h

light/dark cycles prior to experiments. During acclimatization, the animals were housed in polycarbonate cages and provided standard food pellet and tap water *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee of the University (BU/REG/316/2016/993). Acute and sub-acute toxicity study was carried out in accordance with OECD guidelines (OECD, 2001) and Mangathayaru *et al.* (2015).

Single dose acute toxicity study

A total of 16 mice were taken for acute toxicity study. Mice were divided into 4 groups, each containing 4 mice (2 male, 2 female). Acute toxicity study was designed for 14 days period. For acute toxicity, different doses of plant extract, 50, 100, 500, and 1000mg MEHh/kg b.w. were given i.p. injection once to each group of mice. The animals were continuously observed for 1 h, then frequently for 24 h, and thereafter once per day for 14 days to monitor any kind of behavioral changes and mortality. One-tenth of the highest dose of acute toxicity study (i.e., 100 mg MEHh/ kg *b.w.*) was taken for sub-acute toxicity study.

Sub-acute toxicity study

For sub-acute toxicity study, mice were divided into two groups of four mice each. One group acted as a control group and the other group as treated group. The latter group was given a dose of 100 mg MEHh/ kg *b.w.* i.p. injection once a day daily for 14 days. On 15th day, both the control and plant treated mice were sacrificed and major tissue samples were collected and processed for histological and biochemical analysis.

Histological analysis

Soon after washing with distilled water, major tissue samples (liver, kidney, heart, and spleen) were fixed in Bouin's fixative for 72 h and processed for paraffin method of tissue sectioning. The tissues were cut at 5 - 8 μm thin sections and stained with eosin and hematoxylin and viewed under a light microscope (Suvarna *et al.*, 2013).

Biochemical analysis

A 10% tissue homogenate was made for various biochemical analyses. Tissue protein content was

estimated following Lowry's folin-phenol reagent method (Lowry *et al.*, 1951) and carbohydrate by anthrone method (Ludwing and Goldberg, 1956). All the four tissue samples were processed for the estimation of the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) (King, 1965), alkaline phosphatase (ALP) (Plummer, 1987), glutathione S-transferase (GST) (Habig *et al.*, 1974), catalase (CAT) (Bergmeyer *et al.*, 1974) and reduced glutathione (GSH) (Moron *et al.*, 1979).

Statistical analysis

All the statistical calculation was carried out in excel. All data are presented as mean \pm standard deviation (SD) for at least three replications for each experiment. A correlation study was carried out by one-way analysis of variance followed by Tukey's multiple comparison tests using Origin software (OriginLab Corp., USA) at $P < 0.05$ significance level.

Results

Acute and sub-acute toxicity study

In the present study, methanolic extract of *H. heteroclita* was tested for its acute and sub-acute toxicity. During 14 days of acute toxicity study, no mortality was recorded in control as well as plant extract treated mice groups of mice. Even at its highest dose 1000 mg MEHh/ kg *b.w.*, mice did not suffer any kind of observable changes in the general and other physiological behaviour or any kind of toxicity symptoms. Similarly, daily administration of MEHh at 100 mg/kg *b.w.* for 14 days, mice did not suffer from any kind of toxicological symptoms. All kinds of external behaviour such as movement, feeding behaviour etc. were seen to be normal during the period of experiment.

Biochemical studies

The effect of plant extract on protein and carbohydrate content of all major tissues - liver, kidney, heart, and spleen is shown in Fig. 1. Biochemical studies revealed slight alterations in protein and carbohydrate content between control and plant treated mice groups. Highest concentration of tissue protein was observed in spleen (22.91 ± 1.15 mg/g wet tissue) followed by kidney (20.61 ± 1.75

mg/g tissue), liver (15.61 ± 0.58 mg/g tissue) and heart (14.26 ± 1.17 mg/g tissue), respectively. Statistical analysis revealed significant differences ($P < 0.05$) between all the tissue protein contents, except between spleen and kidney. On administration with the plant extract the protein content was found to be slightly increased in liver and spleen, whereas kidney and heart protein was slightly decreased. The percentage increase or decrease in protein content of liver and spleen was about 22% and 18%, respectively.

Of all the tissues, only spleen protein showed significant differences between control and the treated mice ($P < 0.05$). On the contrary, the carbohydrate (glucose) content was found to be reduced in all the tissues (Fig. 1) in plant extract treated mice compared to control. Highest reduction ($\approx 38\%$) was noticed in spleen followed by kidney ($\approx 27\%$) and heart ($\approx 15\%$). The decrease in total glucose concentration of the various tissues indicates the hypoglycaemic property of the plant.

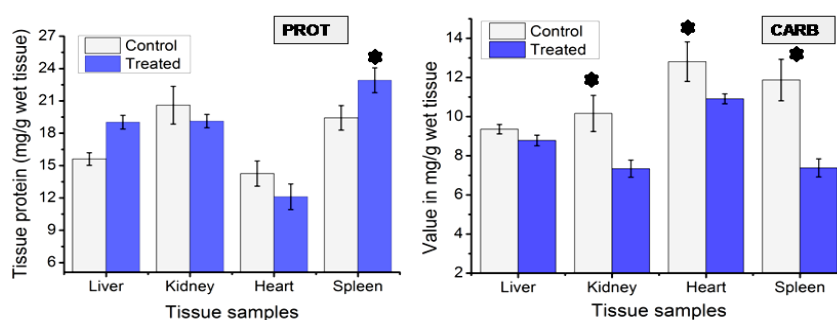


Fig. 1. Protein (PROT) and carbohydrate (CARB) content of different tissues in control and *H. heteroclit* treated mice. Star mark indicates the significant differences between control and treated group at $P < 0.05$.

The toxicological effects of MEHh have also been investigated by observing the changes in the toxicity related enzymes such as ALP, ALT and AST. These enzymes are regarded as the markers of toxicity, especially, liver toxicity. Fig. 2 showed the alterations of ALP, ALT, and AST enzymes between

control and treated mice. After 14 days of continuous administration of plant extract at 100 mg/kg *b.w.*, the mice showed slight changes in the enzyme activities. Liver and heart showed decreased ALP activity, while the kidney and spleen showed increased activity.

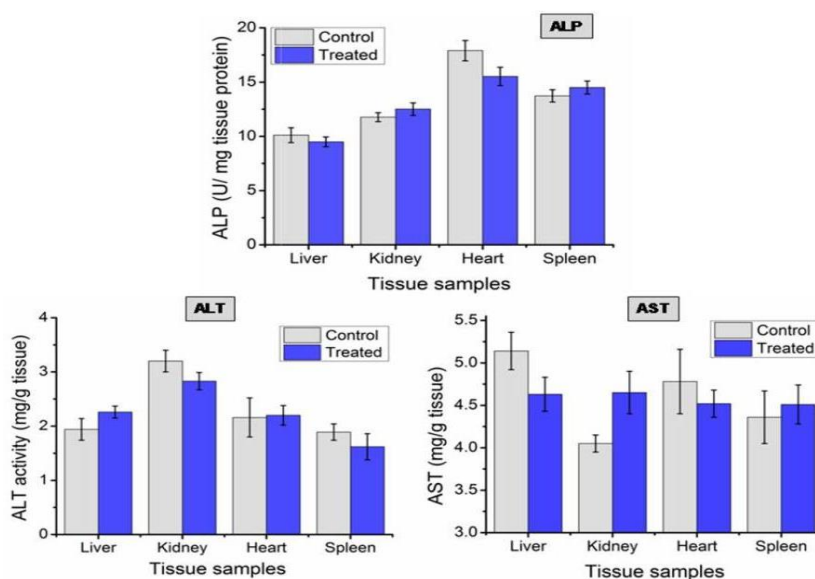


Fig. 2. Graphical representation of ALP, ALT and AST activities in control and *H. heteroclit* treated mice. ALP = one unit (U) of enzyme activity has been defined as the micromole of product formed per minute per mg tissue protein.

Compared to control group, plant extract-treated group showed no significant differences in the ALP activities in all the tissues. Similarly, changes in the ALT and AST activities between control and treated group revealed no significant difference (at $P < 0.05$ level). Insignificant changes in the activities of ALP, ALT, and AST suggest that MEHh possesses very less toxicological effect of plant extract. To see whether MEHh has any effect on the antioxidant

capacity, CAT, GST, and GSH activities were also studied in control and treated mice groups. Fig. 3 represents the changes in the activities of CAT, GST, and GSH. Statistically, no significant changes were observed between control and treated mice group in all the tissues. The alterations of antioxidant status as well as oxidative stress enzymes partially explain the mechanism of slight toxicological effect induced by MEHh.

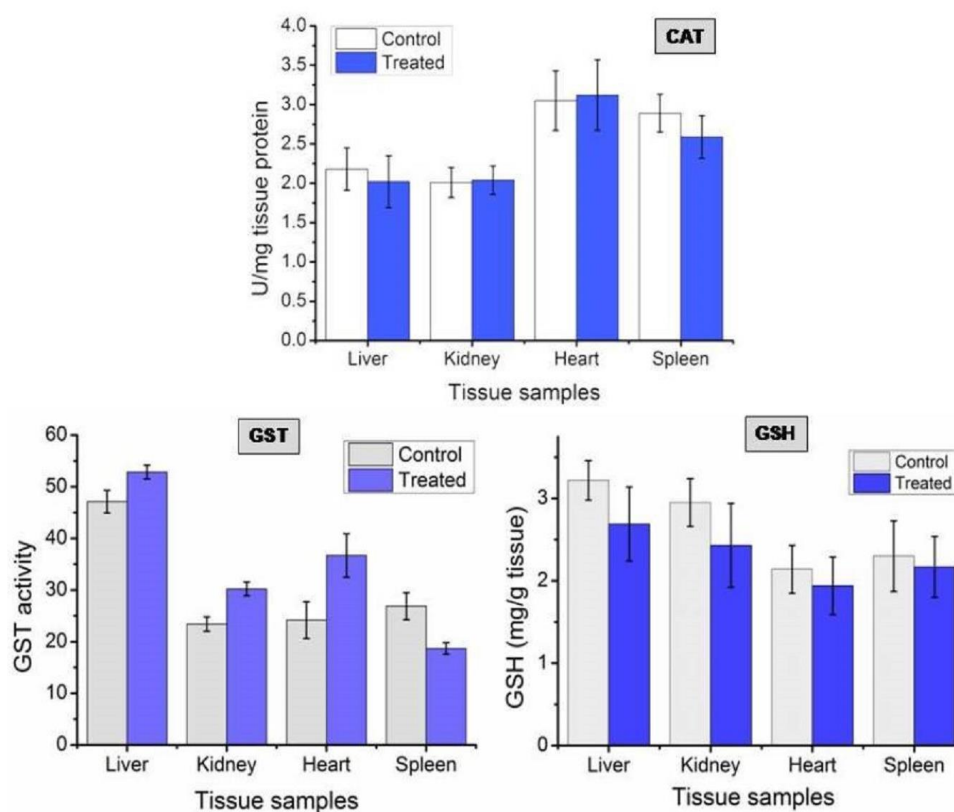


Fig. 3. Graphical representation of CAT, GST and GSH activities in control and *H. heteroclitra* treated mice. CAT = 1 U of enzyme activity has been defined as the micromole of product formed per minute per mg tissue protein.

Histological studies

Photographs of histological tissue sections of liver, kidney, heart, and spleen of control and MEHh-treated mice group is presented in the Fig. 4 and 5. Our study revealed no marked abnormalities in the tissue sections of liver, kidney, heart, and spleen section in plant treated group. However, slight alterations have been observed in liver tissue sections showing shrinkage and condensation of the hepatocytes. (Fig. 4a,b). Kidney tissue sections showed normal glomerulus and Bowman's capsules without any sign of inflammation or necrosis (Fig. 4c,d).

Histological observation in heart revealed normal heart muscle fiber with long nuclei in MEHh-treated mice (Fig. 5a,b) suggesting no toxicological effect during the period of the experiment. Spleen sections also revealed normal architecture of splenocytes with distinct nuclei. At the medulla region of the spleen, zones of slightly more coloured blue tint red pulps were prominent in both control and MEHh-treated mice suggesting no sign of toxicity in both the groups of mice. Histological observations basically supported the finding of biochemical studies from tissue homogenates.

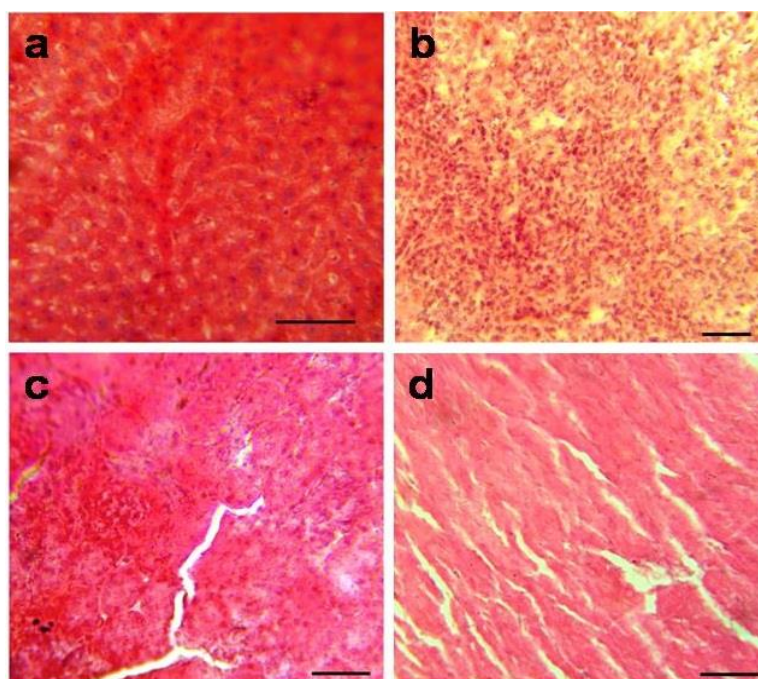


Fig. 4. Hematoxylin and eosin staining histological section of control and *H. heteroclita* treated liver and kidney. a,c = control of liver and kidney; b,d = *H. heteroclita* treated sections of liver and kidney. Scale bar = 100 μ m.

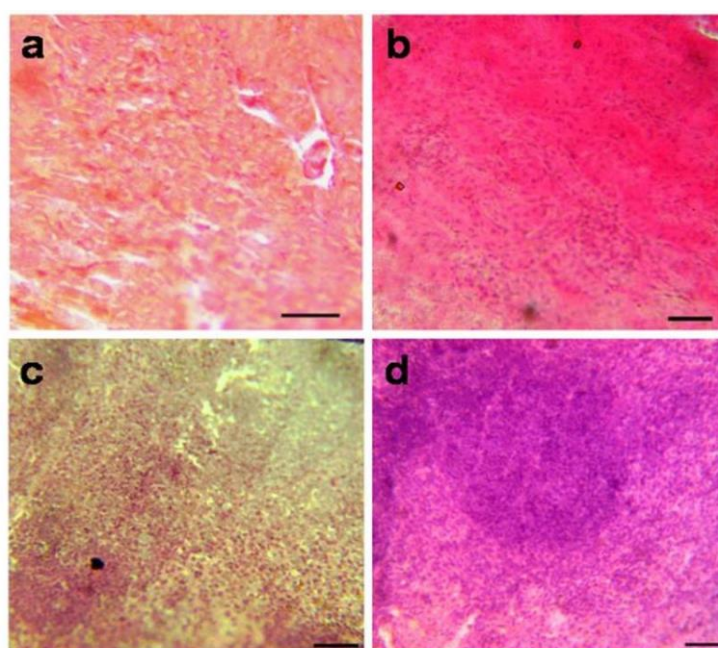


Fig. 5. Hematoxylin and eosin staining histological section of control and *H. heteroclita* treated heart and spleen. a,c = control of heart and spleen; b,d = *H. heteroclita* treated sections of heart and spleen. Scale bar = 100 μ m.

Discussion

Herbal drugs are always considered to be better and safer than the commercial drugs because of its less side-effects and eco-friendliness. Although beneficial, above certain concentration, all medicines or chemicals or plant extracts may become toxic to the

host body. Acute toxicity study is an important parameter of bioassay guided drug discovery pipeline. It enlighten the researcher about the threshold concentration of any drug or plant extract during any *in-vivo* study (Sur *et al.*, 2015; Roy *et al.*, 2016).

In view of the significance of ethnomedicines, we have tried to investigate the toxic effect of the fruit extract *H. heteroclita*, if any, in *in-vivo* system. Alterations of any biomolecules, enzymes, proteins or hormones etc. indicate the abnormal conditions of an organism. In the present study, no significant changes were observed in the concentrations of protein and carbohydrates between control and treated mice groups suggest that the plant extract at 100 mg/kg do not have any adverse effect on the mice group. The present study can be correlated to our earlier findings where we revealed very less concentration of toxic heavy metals in the methanolic crude extract of *H. heteroclita* (Swargiary and Brahma, 2017). Many toxicological studies have reported alterations in concentrations of protein and carbohydrate between control and treated animal models (Sareeratawong *et al.*, 2016; Madeleine *et al.*, 2017).

The introduction of any drug or chemical may be related to the development of oxidative stress in the body leading to toxicity in various tissues and organ systems. The reactive molecules produced during the metabolism of drugs or chemicals cause oxidative stress and can impair the function of drug metabolizing enzymes leading to toxicity. The presence of cellular antioxidant molecules (such as ascorbic acid, glutathione etc.) and enzymes (catalase, superoxide dismutase etc.) are disturbed (Deavall *et al.*, 2012; Banerjee and Ghosh, 2016; Pantelidou *et al.*, 2017). Increase in the activities of these enzymes suggests toxicity of drugs or chemicals on the host organism (Lim *et al.*, 2012). Our study revealed that the MEHh did not show any significant changes in the antioxidant and oxidative stress related enzymes and therefore no significant effect on the normal physiology of the treated mice. In accordance with our finding, many studies have reported similar nature of alteration in all these enzymes (Ingale *et al.*, 2013; Karale and Kamath, 2017; Lodhi *et al.*, 2009). Histological studies of all the tissues revealed no prominent changes in the architecture of cellular morphology, and thus supports the finding of biochemical analysis. Thus, the present study indicated that the MEHh do not have any

toxicological effect on enzymes and cellular morphology of the tissues. Therefore, it is concluded that *H. heteroclita* do not possess any toxic effect at a tested dose 100 mg/kg *b.w.* of albino mice.

Conclusion

Hodgsonia heteroclita is a very popular and commonly used medicinal plant in Kokrajhar district of Assam and used as an antihyperglycemic agent by many diabetic patients. However, our study is the first of its kind to reveal the pharmacological aspect of the plant. The present study confirms that the alcoholic fruit pulp extract of *Hodgsonia heteroclita* did not cause any adverse effect on mice. Earlier studies have also been reported to contain very less quantity of toxic elements in the plant. It can, therefore, be suggested that the plant could be a good source of medicine without any toxicological effects at a minimal dose and may be investigated further to explore the medicinal properties of the plant.

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