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Pre-Clinical Acute Toxicity And Hypoglycemic Study Of Phyllanthus Nirurion Mice Model

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Abstract

Phyllanthus niruri is herbal plants having potential medicinal remedial properties to cure many disease and metabolic disorders. Here, we study the in vivo acute toxicity and hypoglycemic effect of whole phytochemical extract of P niruri different varieties collected from Northern Uttar Pradesh and Uttarakhand region and their phytochemicals ethanolic extract collected for the in vivo study in mice model. Plant extracts enriched with different type biochemical, amino acids, saponins, tannins, flavonoids were administrated in mice model in different concentration to check the acute toxicity. Subsequently, hypoglycemic effect of the plant extract was studied in Streptozotocin (STZ) induced induce hyperglycemia or diabetic mice contrary to control and standard drug group i.e., glibenclamide. Results showed that doses upto 1000 mg/kg body weight has lethal effect on animals. However, the aqueous extract increases upto 400mg/kg body weight significantly reduces the blood glucose level in hyperglycemic mice and shown that each sample has positive hypoglycemic efficiency.

Keywords: Hypoglycemic, blood, phytochemical, extract, toxicity

1. Introduction

Unhealthy lifestyle and environmental pollution is the root causing factors that may lead severe health complications and diseases. Prolonged metabolic abnormality and medication results hormonal imbalance, metabolic diseases, acute-chronic inflammation and loss of organ functioning. Therefore proper diet, regular exercise and uptake of nutritional food sources are necessary to prevent all these problems. Herbal plants are the rich source of nutrients, minerals, vitamins and secondary metabolites that helps in metabolism, proper regulation of hormonal balance and cure health problems. In case of hypoglycemia an abnormal low plasma glucose concentration i.e., <70 mg/dL (<3.9 mmol/L) results potential harm to the individual of any age group. Hypoglycemia is one of the key limiting factors that controls the type 1 and type 2diabetes (Fidler et al., 2011).

Chatterjee and Sil, (2007) recognized that P. niruri extract shields the liver from liver damage induced by nimesulide which appeared by measuring the levels of glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and alkaline phosphatase in serum in vivo using murine model. Besides, that it is also associated with severe medical complications such as cognitive dysfunction, seizures, myocardial infractions, coma and death. Herbal medications results changes in the metabolic activity by enhancing insulin secretion, uptake of glucose molecule by the adipose and muscle tissues vice-versa obstructing the glucose absorption from intestine and secretion by the hepatocytes (Davidson et al., 2017).

Wide range of medicinal plants across the worldwide level used as antidiabetic remedy possessing hypoglycemic activity and to maintain glucose level in the blood system (Ali et al., 2012). Phyllanthus niruri Linn. (Euphorbiaceae), is one of the elementary herbal plant found annually in the rain forests, tropical and sub-tropicalareas and used as traditional remedy to cure many metabolic disorders (Mellinger et al., 2005; Sagar et a., 2022).

In the studies, it was found that P. niruri extract inhibited α-amylase (IC50: 2.15 ± 0.1mg/mL) and α-glucosidase (IC50: 0.2 ± 0.02mg/mL) activities and inhibits the glucose absorption in the vascularity system, suppress hemoglobin glycation and thereby increased body weights and glycogen storage in diabetic rats (Okoli et al., 2011).In several in vitro and in vivo pre-clinical studies reported that the whole plant extract of the P.niruri containing wide range of phytochemicals showed antidiabetic activity (Rani and Kumar, 2018).

Therefore, on the basis of prior studies and research showing the antidiabetic effect of P. niruri, we focused on the hypoglycemic effect of different varieties of P. niruri species collected from different climatic conditions. Subsequently standardization of oral dose for less acute toxicity and hypoglycemic efficiency in diabetic animal mice model.

2. Experimental

2.1 Materials: Sample plant Phyllanthus niruri collected from the different regions Uttarakhand and Uttar Pradesh. Collected samples were air-dried at room temperature and a fine powder in cryomill was prepared. Powdered samples were stored in air-tight container for further analysis.

2.2 Preparation of aqueous extracts: 200gm of each powdered sample dissolved in 1 L distilled water and kept on an orbital shaker at 24°C for 48h. After mixing, the plant extract solution filtered through Whatman No 1 filter paper and dried in rotary evaporator at 40ºC. Subsequently, dissolved 100gm of each dried sample in 70% ethanol and concentrated upto 9-10% to obtain greenish gummy extract at 650^0C for 4 hrs in a soxhlet assembly. The viscous plant extracts samples stored in air-tight bottle at 40^0C.

2.3 In vivo hypoglycemic efficiency: The pre-clinical study protocol was approved by the Institute Animal Ethics Committee, Department of Biotechnology (IRB Registration number-838/PO/Re/S/04/CPCSEA), Dr. KNMIPER, India. In vivo hypoglycemic activity and acute toxicity of aqueous extract of P. niruri checked by following the procedure as given in the literature (Hilaly et al., 2004). Two months old, 24 healthy **albino mice (both sex)** having weight 100-200g were procured from the Institute animal house. All the animals were kept under hygienic conditions, supplied with food (Grams grains, Soyabean grains, Wheat grains and breads) and ad lib water.

Acute toxicity test: For the experimental work mice were fasted for 16 h and randomly divided into 5 groups of six mice per group. Plant extract graded doses (dose/body weight) of different concentration were administrated to mice i.e., 200, 400, 800 and 1000 mg/kg.

Hypoglycemic activity: However, in control group STZ (Streptozotocin) was used to induce hyperglycemia and glibenclamide used as standard drug. After drug and test sample administration, provide adequate supply of food and water to animals, and observed the signs acute toxicity over a period of 48 h. Hyperglycemia was induced by multiple intra-peritoneal injection of freshly prepared STZ solution in 0.05 M sodium citrate (pH 4.5) at the dose of 35 mg/kg body weight followed by an hour of fasting. Mice with fasting blood glucose level of 200 mg/dl or higher were considered to be diabetic for the study. A parallel set of control mice (non-diabetic) were injected with citrate buffer only.

The hyperglycemic animals were randomly divided into five groups (n=5) and received oral administration of aqueous extract (200 and 400 mg/kg), Distilled water (5ml/kg) and Glibenclamide (0.2 mg/kg) respectively. The blood glucose was then measured by glucometer before (i.e. 0 h) and at 0.5, 1, 2 and 4 h after treatment.

2.4 Statistical analysis:

The experimental data analysed quantitatively as mean ± standard deviation and by the analysis of variance (ANOVA) and p-value (< 0.05)

3. Results and discussion

3.1 Sample preparation:

Pure aqueous extract and ethanolic extract were successful prepared for the analysis and in vivo hypoglycemic activity.

3.2 Acute toxicity

In vivo acute toxicity of the aqueous plant extracts at concentration of 200, 400, 800 and 1000mg/ kg was measured by counting the number of deaths within this period of time. After 48 hrs administration of sample, it was reported that the death occurred only in the 1000 mg/kg body weight dose group. Hence it has been considered as toxic. The aqueous extract of P. niruri at low concentration has significant anti-oxidant property to reduce oxidative stress and cell death (Narendra et al., 2012; Agarwal et al., 2015).

In other study, it was reported that P. niruri extract have tissue restoration activity and completely healed liver cirrhosis in rat model (Amin et al., 2013). Muthulakshmi and co-worker, reported that the P. niruri aqueous leaf extract have immune stimulating properties (Muthulakshmi et al., 2016).

The acute oral toxicity (LD50) and elevated activation of neutrophil and antibody response was shown in Albino mice kidney due to the toxic effect at higher dose (Singh et al., 2016).

3.3 Hypoglycemic effect

The Plant extracts has potential hypoglycemic effect to reduce the blood sugar level at different concentration. Here, it has been shown that aqueous extract of Phyllanthus niruri has significant potential to suppress the blood sugar level and positive Hypoglycemic efficiency against Hyperglycemic animals as compare to the standard drug sample (Table 3). The blood sugar level

decreases as the increased phytochemical dose concentration upto 400mg/kg body weight (Fig. 5,6). Thus, it was concludes that the efficiency of the plant extract is good and non-toxic upto higher concentration of half of their body weight.

Moreover, the entire plant of Phyllanthus niruri employed to have significant
reduction in biochemical parameters against oxidative stress induced by Staphylococcus aureus in the rat (Ramamurthy & Abarna, 2014).

Streptozotocin causes hyperglycaemia and glucose intolerance or syndromes similar to either type 1 or type 2diabetes. Phyllanthusniruri methanol extracts was affirmed to have a hypoglycemic strength in bringing down lowering blood glucose on streptozotocin induced mice diabetic models. The research also suggested that the extract might be considered as a safe valuable treatment for long term and effective management of diabetic patients (Paithankar et al., 2015)

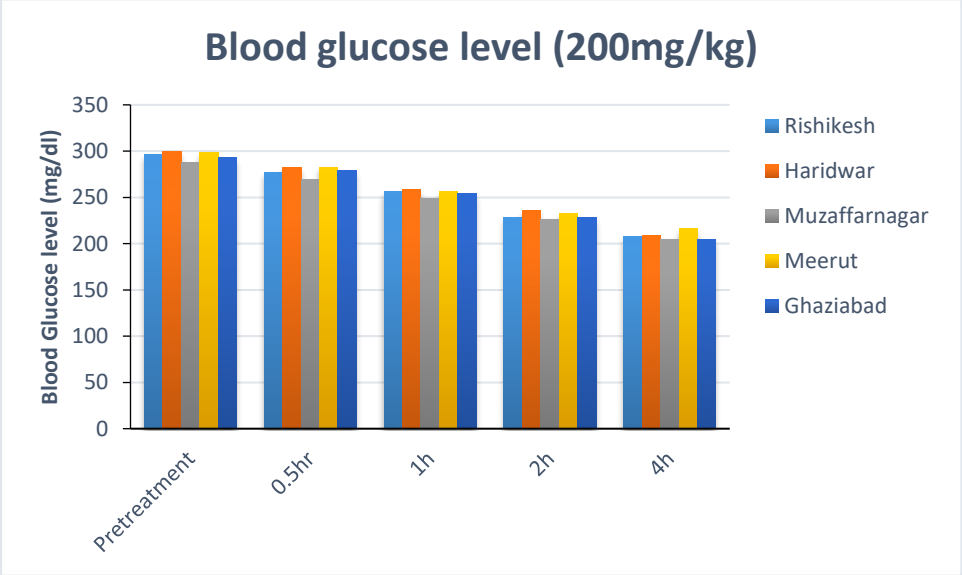

Figure 1: Hypoglycaemic effect of plant extracts at dose of 200mg/kg body weight.

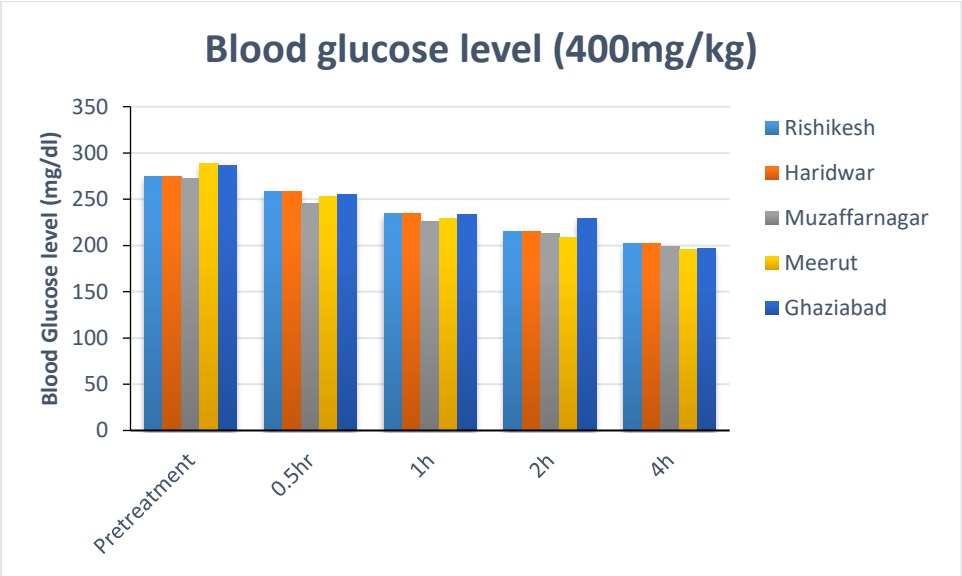

Figure 2: Hypoglycaemic effect of plant extracts at dose of 400mg/kg body weight.

Table 1: Comparative effect of plant extracts and standard drug on blood sugar level at regular time interval.

Site	Aqueous Extract Dose(mg/kg)	Blood Glucose Level (mg/dl)				
Rishikesh		**Pretreatment**	**0.5hr**	**1h**	**2h**	**4h**
	200	**296.5**	**276.8**	**256.4**	**228.5**	**208.0**
	400	**274.0**	**258.8**	**234.5**	**215.5**	**202.0**
Haridwar	**200**	**299.5**	**282.6**	**258.5**	**235.8**	**208.9**
	400	**268.5**	**252.8**	**228.6**	**218.5**	**200.5**
Muzaffarnagar	**200**	**288.0**	**269.5**	**248.5**	**226.6**	**204.8**
	400	**272.5**	**245.8**	**226.0**	**212.5**	**198.6**
Meerut	**200**	**298.5**	**282.8**	**256.5**	**232.8**	**216.5**
	400	**288.5**	**252.6**	**228.6**	**208.8**	**195.5**
Ghaziabad	**200**	**292.8**	**278.6**	**254.5**	**228.6**	**204.8**
	400	**286.6**	**254.8**	**233.6**	**212.8**	**196.5**
Glibenclamide	**0.2**	**302.6**	**268.8**	**216.5**	**198.6.0**	**288.4.0**
Control	**2ml/kg**	**318.5**	**365.8**	**352.5.0**	**354.5**	**352.5**

4. Conclusion:

The result of the above study has confirmed that the plant Phyllanthus nirui has potent hypoglycemic efficiency as well as acute toxicity effect at concentration of 400mg/ml.

This may be due to the high phytochemical content in the plant sample having anti-microbial, ani-oxidative and anti-inflammatory property. The sample of Haridwar and Muzaffarnagar region showed constant hypoglycemic effect as with the progression of time the blood glucose level reduces. Thereby we can estimate the effective lethal dose for oral administration in the animal models. Conclusively this study provides an insight for the future pharmaceutical application of the P. niruri varieties of different regions.

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