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RESEARCH ARTICLE

Neuropharmacological activity of *Azadirachta indica* Leaves on Diabetes Rodunts

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ABSTRACT

Azadirachta indica have been known for their anti diabetic activity since long time. Many attempts for observe antidiabetic potency of *Azadirachta indica* have been made utilizing foe anti diabetic activity tools. In an attempt according to the obtained results in the present study, ethyl acetate extract of *Azadirachta indica* leaves (50, 100 & 200 mg/kg/day, p.o.) can exert positive effects within two weeks in the treatment and decreasing the physiological symptoms of diabetic neuropathy in rats. In the present study, response time to immobility in tail suspension and force swim test showed significant decrease in comparison to the diabetic group.

Keywords: hyperglycaemia, endocrine disorder, insulin, neuropathy

Introduction

Diabetes is a group of metabolic disorder characterized by high blood glucose levels resulting from defects in insulin secretion, insulin action or both. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism. Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke (Genuth et al. 2003, Edelman et al. 2004). As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy (Sato et al. 2009), neuropathy (Shimazaki et al. 2007), nephropathy (Droumaguet et al. 2006) cardiovascular complications (Metzger et al. 2008) and ulceration (Landon et al. 2009) Thus, diabetes covers a wide range of heterogeneous diseases. Diabetes is the most common endocrine disorder and by the year 2010, it is estimated that more than 200 million people worldwide will have DM and 300 million will subsequently have the disease of 2025.

Material and Methods

Extraction of plant material

The leaves of *Azadirachta indica* were dried under shade in laboratory. It was pulverized to coarse powder. The coarse powder of leaves was passed through sieve No.18 to maintain uniformity and stored in cool and dry place. Briefly, 140 g of the coarsely powdered dry leaves were extracted using petroleum ether for 72 h at 40-50°C. The extract was collected and dried at 40-50°C in hot air oven and subsequently extracted with ethyl acetate at 77-80°C. The extract was collected and dried at room temperature for 4-5 days.

Phytochemical Screening of the extract

The obtained ethylacetate fraction of the extract was screened as per the reported procedure for loss on drying, total ash value, acid insoluble ash value, water soluble ash and the presence of alkaloids (Neelam and Madhulika, 2017).

Neuropharamacological Screening

Tail Suspension Test

Rats both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period.

Rats were considered immobile only when they hung passively and were motionless. The total duration of immobility induced by tail suspension were measured.

Force Swim Test

Rats of either sex were individually forced to swim in an open cylindrical container (diameter

10 cm, height 25 cm), containing 19 cm of water at 25±1 °C. The total duration of immobility were recorded during the last 6 min of the 10-min period. Each Rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect (Vogel G. 2002).

Elevated plus maze test (EPM)

The plus-maze consists of two open arms, 16×5 cm, and two enclosed arms, 16×5×12 cm, with an open roof, arranged so that the two open arms are opposite to each other. The maze is elevated to a height of 25 cm.

Each Rat individually placed in the center of the maze, facing one of the open arms. During test period, the transfer latency was recorded. The procedure was conducted preferably in a sound attenuated room (Kulkarni, S.K. & Vogel G. 2002).

Evaluation of transfer latency

Transfer latency is the time in which animal moves from the open arm to the enclosed arms putting all his 4 legs completely inside the enclosed arm.

Animal

The animals used for experiment were procured from the authorized animal house of Sapience Bioanalytical Research Lab, Bhopal (M.P.). All Wistar albino rats were healthy and 120 g to 140 g of body weight. The animals were kept in air conditioning environment with temperature range of 25±20C with conventional laboratory food and fresh drinking water. The bedding of animals was changed every 3rd day.

Acute toxicity study

Acute toxicity studies were performed according to the OECD guidelines (425).Three rats were administered single dose of extract (2000 mg/kg p.o.). The animals were observed for 4 hours after dose administration for any alteration in their behavior and also after 24 h. Extract did not show any signs of toxicity up to the dose of 2000 mg/kg p.o.

Experimental design

In the experiment, a total of 24 rats were used. The rats were divided into 6 groups comprising of 4 animals in each group as follows:

Group I: Normal control group

Group II: Negative control rats received Alloxan 120mg/kg, i.p. for induction of diabetes

Group III: rats received Glibenclamide (5mg/kg, p.o.) for 14 days and Alloxan 120mg/kg, i.p. on 1st day

Group IV rats received ethyl acetate extract of Azadirachta indica, (50mg/kg p.o.) once daily for 14 days and Alloxan 120mg/kg, i.p. on 1st day

Group V rats received ethyl acetate extract of Azadirachta indica of Tamarindus indica,

(100mg/kg p.o.) once daily for 14 days and Alloxan 120mg/kg, i.p. on 1st day

Group VI rats received ethyl acetate extract of Azadirachta indica, (200mg/kg p.o.) once daily for 14 days and Alloxan 120mg/kg, i.p. on 1st day

Sample collection

Blood samples were collected by tail vein and blood glucose levels were estimated using an electronic glucometer (Gluco chek).

Induction of diabetes

Diabetes mellitus was induced in rats by a single i.p. injection of alloxan monohydrate (120 mg/kg body weight) in normal saline (0.9% NaCl). Hyperglycemia was confirmed by fasting blood glucose level measurement by glucometer on the 3rd day after the alloxan injection. rats with consistent hyperglycemia on 3rd day (fasting blood glucose levels > 140 mg/dl) was considered diabetic and was used for further studies (Jyoti M, et al, 2002).

Result

In acute toxicity study, there were no behavioral changes seen up to 4hrs and no mortality was observed up to the end of 24 hrs even at the maximum tested dose level of 2000mg/kg per oral, it was considered maximum safe dose. The coarse powder of the shed dried parts of the plant was subjected to extraction by using soxhlet apparatus using ethyl acetate as solvent. In the extract yield was obtained in alcoholic extract that was 8.14%. After the extraction identification of various phytoconstituents was done. The extract contains carbohydrates, tannins, flavonoids, glycosides, alkaloids etc. In the process of diabetic neuropathy, nerve cells and vessels' membranes are not dependent to insulin for transferring glucose and in diabetes disorder great amount of glucose enter cells. In nerve cells, glucose changes to sorbitol by aldose reductase enzyme and sorbitol accumulation increases free radicals such as hydroxyl-super oxide and hydrogen peroxide and eventually causes cell damage. Based on this mechanism of injury, different prevention and treatment approaches are under investigation (Gonzalez ME, 1998 & Brownlee M, 2001). As it was mentioned in the introduction, anti-oxidant property of cerebrolysin is 300 times less compared to vitamin E (Babenkova, et al, 1999).

Discussion

In acute toxicity study, there were no behavioral changes seen up to 4hrs and no mortality was observed up to the end of 24 hrs even at the maximum tested dose level of 2000mg/kg per oral, it was considered maximum safe dose. The coarse powder of the shed dried parts of the plant was subjected to extraction by using soxhlet apparatus using ethyl acetate as solvent. In the extract yield was obtained in alcoholic extract that was 8.14%.After the extraction, pharmacognostical evaluation was done including

determination of Ash value and moisture content was determined. Extract was subjected to various chemical tests for preliminary identification of various phytoconstituents. The extract contains carbohydrates, tannins, flavonoids, glycosides, alkaloids etc.

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Neuropharmacological study was evaluated in diabetic rats through neuropharmacological screening tests (Tail suspension test, Force swim test & Elevated plus maze test).

Results of Tail suspension test and Force swim test revealed that the ethanol extract exhibited significant decrease in Immobility period as compared to diabetic rats. Results shown in table: -7.8, 7.9

Results of Elevated plus maze test revealed that the ethanol extract exhibited significant decrease in transfer latency as compared to diabetic rats. Results shown in table: -7.10.

Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas (Lenzen S *et al* 1988, Oberley LW, 1988). *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis (Jorns A *et al* 1997, Ledoux SP, *et al* 1986). The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells (Szkudelski T, 2001).

Treatment with extract at different dose 50, 100 and 200mg/kg was elicited significant inhibition of blood glucose level. The Results were compared with glibenclamide was more effective in reducing blood glucose level in normal as well as diabetic control group.

Conclusion

According to the obtained results in the present study, ethyl acetate extract of *Azadirachta indica* leaves (50, 100 & 200 mg/kg/day, p.o.) can exert positive effects within two weeks in the treatment and decreasing the physiological symptoms of diabetic neuropathy in rats. In the present study, response time to immobility in tail suspension and force swim test showed significant decrease in comparison to the diabetic group.

References

American Diabetes Association. Gestational diabetes mellitus. Diabetes Care 2001; 24 (suppl 1): S77-S79.

Babenkova IV, Teselkin IuO, Makashova NV, Guseva MR (1999) Antioxidative activity of histochrome and some other drugs used in ophthalmolog Vestn Oftalmol 115: 22-24.

Biswas, K., Chattopadhyay, I., Banerjee, R.K., et al., (2002) Biological activities and medicinal properties of Azadirachta indica (Azadirachta indica). Curr Sci, 82: 1336- 1345.

Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414: 813-820.

Neelam Singh and Madhulika Singh (2017) An Evaluation of Phytochemicals in Maceration and Infusion Extracts of *Cyperus rotundus* Linn Leaves in Two Different Solvents. Journal of Pharmacology and Biomedicine 1 (2): 77-84