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HYPOGLYCEMIC ACTIVITY OF ETHANOLIC EXTRACT OF LAWSONIA INERMIS LINN. (HENNA) IN ALLOXAN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Type 2 Diabetes mellitus (DM) is the most common form of disease globally with rapidly developing countries being at the forefront of this epidemic. The drugs used for treatment are associated with several adverse effects and are expensive. The current focus has been shifted to treat DM and its complications through plant derived drugs due to their high efficacy and safety. ***Aims:*** *In our study the main objective is to find out the hypoglycemic activity of ethanolic extract of leaves of Lawsonia inermis linn in alloxan induced diabetic rats and its comparision with the standard drug Glibenclamide.* ***Methodology:*** *Albino rats weighing 200-250gms were selected and diabetes was induced by injecting alloxan monohydrate (180mg/kg b.w) intra peritonially. Animals were divided into six groups, 1st group was kept as a normal, 2nd group as a diabetic control and these two groups were treated with 0.5 ml of normal saline orally. 3rd group was treated with standard drug glibenclamide 5mg/kg. Remaining three groups were treated with different doses of (150,200 and 400mg/kgb.w) of ethanolic extract of leaves of Lawsonia inermis for a period of 3weeks. Results were analyzed by estimating the fasting blood glucose levels using Glucometer. on days 3, 7, 14 and 21.* ***Results:*** *An ethanolic extract of Lawsonia inermis showed a significant fall in fasting blood glucose at all the doses. But ,at the dose of 400mg/kg showed highly significant (p value < 0.001) fall in bl.glucose level as compared to 150mg and 200mg/kg of extract.* ***Conclusion:*** *This study can be recommended for further evaluation.*

KEYWORDS

Diabetes mellitus, Hypoglycemia, Lawsonia inermis.

INTRODUCTION

Diabetes Mellitus (DM) is a global disease. It is a class of metabolic disorder characterized by hyperglycemia as a result of disturbances in carbohydrate, fat and protein metabolism due to defect in the insulin secretion, insulin action or both [1]. The global prevalence of DM in 2010 was 284 million people worldwide, which is all most around 6.4% of the world population which is higher than was projected in earlier studies.[2]. It has been rising steadily in the last decade in Asian countries; it's mainly due to urbanization, increased obesity and lack of physical activity [1]. India expected to become the Diabetic capital of the world in the year 2025, during which maximum numbers of patients are seen [3, 4].

Among adults, it is one of the leading causes of death also cases of legal blindness and lower extremities amputation [5][6]. The drugs associated with DM like hypoglycemic agents such as biguanides and sulphonylureas are associated with several side effects and sometimes they are found to be ineffective in chronic diabetic patients and are expensive.[7]. So currently the focus has been shifted to treat DM and its complications through plant derived

drugs due to their less side effects and high efficacy [8].

Plants have played a significant role in maintaining the health and improving the quality of life for thousands of years. Their important use being mainly in medicine, beverages, cosmetics and dyes. They contain natural substances that can promote health. Many herbs are used to treat metabolic disorders, cardiovascular problems, liver disorders, central nervous system and digestive system related disorders [9]. Due to their potential to produce significant therapeutic effect to produce their significant therapeutic effect, they can be useful as a drug or supplementary in the treatment of various diseases. In this one such plant, Lawsonia inermis linn.(Henna) has shown a various pharmacological activities like anti inflammatory, anti diabetic, anti oxidant, antibacterial, wound healing and anticancer[10,11] . Lawsonia inermis linn. is a much branched glabrous shrub or small tree (2-6m in height), cultivated for its leaves . Stem, bark, roots, flowers and seeds have also been used in traditional medicine. This plant is a worldwide known to be cosmetic agent used to stain hair, skin and nails. Henna invites more attention of the researchers because of its various potential medicinal therapeutic applications. This study evaluates the effect of extract of leaves of Lawsonia inermis on blood glucose levels in alloxan induced diabetic rats.

MATERIALS AND METHODS

Drugs: Glibenclamide, Normal saline, Alloxan monohydrate were used in this study.

Study animals

Wistar albino rats (200-250gms) of either sex selected from animal house, S. Nijalingappa medical college, Bagalkot, Karnataka, India were used. The animals were housed under standard laboratory conditions maintained at 25 ± 10°C and under 12/12 h light /dark cycle and fed with standard pellet diet and water ad libitum. The experimental protocol was approved by the institutional animal ethics committee and by the animal regulatory body of the Indian Government.

(Registration No: 829/AC/04/CPCSEA).

Plant material

The leaves of L.inermis were collected from the horticulture garden, Bagalkot, Karnataka, India, during the month of September 2011. The plant was identified by botanist Prof. Patil and voucher specimen was numbered and deposited in the Herbarium of Department of Pharmacology of S. Nijalingappa medical college, Bagalkot, Karnataka.

Preparation of extract

The leaves of the plants were collected, and then dried in a shade for seven days. The dried leaves were subjected to size reduction to a coarse powder by using dry grinder. The powdered dried leaves of one kg were extracted with 70% of aqueous ethyl alcohol by using the percolation process for 42 hrs. The concentrated extract 200gms was suspended in water. The concentrated extract was made to dry at 35-38 degree temperature. Solid material and was stored in an air and water proof container, kept in refrigerator at 4 degree temperature. The powdered dried leaves of one kg was yielded about 145gms of pure extract.

Phytochemical screening

The extract material was subjected for phytochemical investigation. The chemical constituents isolated from L. inermis are napthoquinone derivatives, lawsone, phenolic compounds, terpenoids, sterols, aliphatic derivatives, xanthones, coumarin, fatty acids, and amino acids.

Acute toxicity studies

The acute oral toxicity test of the extract was determined according to OECD (Organisation for Economic Co-operation and Development)

guidelines no 425. The female wistar rats weighing 150-180gms with the extract dose of 2000gms/kg orally were used for this study. The treated animals were monitored for 14 days, for mortality and general behaviour. No death was observed till the end of 14th day. The test material was found to be safe up to the dose of 2000mg /kg and from this three doses were chosen as 150mg, 200mg and 400mg/kg body wt. for further experimentation.

Diabetes induction:

The induction of diabetes was done by using Alloxan monohydrate in the dose of 160mg/kg bodywt. dissolved in chilled normal saline and given intraperitonially to a overnight fasted animals. The rats were kept for the next 24hrs on 10% of glucose solution in water bottles to prevent hypoglycemia and death. After 72 hrs of injection, fasting blood glucose levels were measured. The animals that did not show blood glucose levels more the 250mg/dl were rejected.

Grouping:

In experiment, the rats were divided into following groups with six animals in each group.

Group I: Normal -Normal controlled rats fed with 0.5 ml of normal saline.

Group II: Diabetic control rats; fed with 0.5ml of normal saline.

Group III: Diabetic rats treated with standard drug Glibenclamide 5mg/kg body wt.

Group IV: Diabetic rats; treated with ethanolic extract of L.inermis 150mg/kg.b.w.

Group V: Diabetic rats treated with ethanolic extract of L.inermis 200mg/kg.b.w

Group VI: Diabetic rats treated with ethanolic extract of L.inermis 400mg/kg.b.w

The treatment was given once a day for 21 days. The normal and control group received an equal volume of vehicle. All the procedures were performed under the guidelines of Institutional Animal Ethics Committee constituted as per directions of the committee for the purpose of Control and Supervision of Experiments on Animals(CPCSEA), under Ministry of Animal Welfare Division, Government of India, New Delhi, India.

Blood sampling and blood glucose estimation

Fasting glucose levels were estimated by using Glucometer on days 3, 7, 14 and 21. After matching the code number for glucometer with that of glucose strips provided, one glucose strip was introduced. Where A 26 gauze sized needle was used and it was pricked into one of the tail vein to obtain a drop of blood which was applied over the side of the strip to cover the target area. Within 30 seconds, the blood sugar levels were displayed in the Glucometer.

It requires very less amount of blood, simple and easy procedure. The strips can be stored at room temperature. The results were co-relatable with venous blood glucose level by laboratory methods with coefficient of variation ± 10%.

Throughout the experiment, animals were maintained without any cross infection and none of the animals showed any untoward side effects.

Statistical analysis

Data was presented in terms of Mean ± SD. Statistical differences between the means of the various groups were evaluated using one way analysis of variance followed by Post hoc Dunnett's multiple comparison test. Data were considered statistically significant at p value < 0.05 and highly significant at p value <0.0001.

RESULTS

With the help of this study we could evaluate the effect of ethanolic extract of leaves of lawsonia inermis in diabetic rats by using alloxan induced diabetes as an experimental model.

Wistar albino rats of either sex were selected for the study and were fasted overnight prior to the administration of alloxan. Initially the basal fasting blood glucose level of each animal was

noted by tail vein puncture which revealed no significant difference in the blood glucose levels. Later, alloxan monohydrate was given in the dose of 160mg/kg body wt. by intra-peritoneal route. Significant rise in fasting blood glucose occurred after three days of administration. Alloxan is a chemical used conventionally to produce diabetes and hyperglycaemia in experimental animals by selectively destroying β-cells.[12]. Thirty six rats were taken and marked separately, viz. Normal, Diabetic control, Standard, Lawsonia inermis in the dose of 150, 200 and 400mg/kg body wt. the study carried out for three weeks. At the end of three weeks of oral treatment with an ethanolic extract of leaves of L. Inermis has produced significant fall in blood glucose levels at all the doses in study rats. But comparison of blood glucose levels on day 3 and day 21 reveals that the extract of leaves of Lawsonia inermis at the dose 400mg/kg has produced a highly significant decrease (p value< 0.001) in blood glucose levels as compared to L.inermis 150mg/kg and 200mg/kg and control group as shown in **Table 1 and Table 2.** Even standard drug Glibenclamide has shown fall in fasting blood glucose level significantly in diabetes induced rats.

Table 1: Blood glucose levels by tail vein method on days 3 and 21 in terms of Mean and SD.

Sr.No.	Group	Days	N	Mean bl.glu levels(mg/dl)	Standard Deviation
1	Normal	3 21	6	91.33 111.33	17.143 10.463
2	Control	3 21	6	313 309	37.320 76.801
3	Std. drug Glibbenclamide	3 21	6	302 161	37.197 21.194
4	L.inermis (150mg/kg)	3 21	6	190 66.83	28.332 7.333
5	L.inermis (200mg/kg)	3 21	6	362 64.83	61.605 4.070

N= Number of animals in each group

Table 2: Effect of 21 days administration of glibenclamide and ethanolic extract of L.inermis in alloxan induced diabetic rats

	Group	F	T	P value
1	Normal group	3.205	2.439	0.040*
2	Control	4.272	0.115	0.912**
3	Glibenclamide Std. drug	2.149	8.067	0.000***
4	L.inermis 150mg/kg	2.846	10.351	0.000
5	L.inermis 200mg/kg	16.610	11.790	0.000
6	L.inermis 400mg/kg	17.885	13.854	0.000

***, **, *** represents the significance rise at p<0.05, p<0.01 and0.001 respectively.**

DISCUSSION

Diabetes mellitus is the name given to a group of disorders characterized by chronic hyperglycemia, polyuria, polydipsia, polyphagia and weakness due to disturbance in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and its action. These metabolic dysregulations leads to secondary patho physiological changes in multiple organ system including both micro & macro vascular dysfunctions [13]. The treatment strategies mainly include nutritional therapy, oral hypoglycemic agents, insulin preparations and or combination of any of these strategies [14]. But currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects. Thus, managing diabetes without any side effects is still a challenge. Inspite of the presence of known antidiabetic medicine available in the pharmaceutical market, remedies derived from medicinal plants are successfully used in the treatment of diabetes. Complementary and alternative systems of medicine have been suggested as traditional treatments for treatment of DM. In this study evaluated the effect of L.inermis leaves extract on fasting blood glucose estimation done in diabetic induced albino wistar rats. Diabetic induction was done by administrating alloxan monohydrate in the dose of 160mg/kg bodywt by intra-peritonial route. This chemical induces necrosis to islets β-cells through free radical mediated damage, thus producing partial destruction of pancreatic beta cells, so insulin deficiency will lead to marked increase in blood glucose level producing type 2 DM [15, 16]. It has induced diabetes up to 380mg/dl. After stabilization of hyperglycemia the study has been conducted for 21 days. Oral treatment with an ethanolic extract of leaves of L. Inermis has produced significant fall in fasting blood glucose levels.The extract of leaves of Lawsonia inermis at the dose 400mg/kg has produced a highly significant decrease (p value< 0.001) in blood glucose levels as compared to the doses of L.inermis 150mg/kg and 200mg/kg body wt. As per Arayne MS, 95% of methanolic extract of leaves of L.inermis has shown a potent antihyperglycemic effect [11]. Many traditional plant treatments for diabetes mellitus are using throughout the world. Few of the traditional plants treatments for diabetes have received scientific scrutiny as in the World Health Organisation has recommended.[17] like Trigonella foenumgraecum, Momordica charantia, Tinospora cordifolia, Enicostema littorae, Gymnema sylvestre, Azadirachta indica, Syzi-gium cumini are some of the most effective and the most commonly studied Indian plants in relation to diabetes.[18, 19]. In this study the possible mechanism by which L.inermis leaves bring about its hypoglycemic action may be by potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to peripheral tissue and also presence of alkaloids, tannins, flavonoids, napthaquinones, lawsone.

CONCLUSION

In conclusion, L. inermis leaves extract at dose 400mg/kgBW and glibenclamide showed significant hypoglycaemic activity in diabeticrats after oral administration. Thus, the present efforts are directed to isolate the active constituents from extracts of L. inermis leaves for the elucidation of mechanism of action.

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