IJPSR (2019), Volume 10, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 February 2019; received in revised form, 05 July 2019; accepted, 21 August 2019; published 01 November 2019

# ANTI-DIABETIC ACTIVITY OF HYDROALCOHOLIC EXTRACTS OF *ANACARDIUM OCCIDENTALE*, *AEGLE MARMELOS* AND *ACHYRANTHES ASPERA* IN ALLOXAN-INDUCED DIABETIC RATS

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**Keywords:**

Alloxan,
Hydroalcoholic extracts,
Anti-diabetic activity, *Anacardium*

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**ABSTRACT:** Diabetes mellitus is a major endocrine metabolic disorder of multiple etiologies characterized by chronic hyperglycemia; with disturbances of carbohydrate, protein, and fat metabolism. It is caused by the deficiency or ineffective production of insulin by pancreas, which results in increase or decrease in concentrations of glucose in the blood. There is number of chemical agents available to control and to treat diabetic patients, but total recovery from diabetes has not been reported till date. An alternative to these synthetic agents, many herbal plants with hypoglycaemic properties are known from across the world. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. The aim of the present study was to determine the anti-diabetic activity of the hydroalcoholic extracts of bark of *Anacardium occidentale*, whole aerial part of *Achyranthes aspera* and leaf of *Aegle marmelos*. Wistar rats were made diabetic by a single dose of alloxan monohydrate (120 mg/kg, i.p.). Hydroalcoholic extracts were screened for antidiabetic activity in alloxan-induced diabetic rats at a concentration of 100 mg/kg and 200 mg/kg of body weight in different groups of 6 diabetic rats each orally once a day for 15 days. Glibenclamide is also given to another group to support the result at a dose of 600 µg/kg of body weight orally once a day for 15 days. Blood glucose levels, body weights, lipid profile of rats were measured. Oral administration of the extracts for 15 days caused a significant ($p<0.05$) reduction in blood glucose levels and lipid profile in diabetic rats. The body weight of diabetic animals was also improved after the administration of extracts.

**INTRODUCTION:** Diabetes mellitus is a severe metabolic disorder which is indicated by hyperglycemia due to lack of insulin or the action of insulin on its target tissues or both.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(11).4932-36

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(11).4932-36

It is one of the major public health problems which is now becoming a global epidemic worldwide [1]. The rising glucose level in blood, in Type 2 diabetes, results due to combination of unhealthy diet, physical inactivity, defect in insulin secretion in response to food and reduced sensitivity of the target tissues to insulin action [2].

The chronic metabolic disorder, which affects about 150 million people in the world, is going to increase to 300 million by the year 2025. [3] India has today become the diabetic capital of the world with over 20 million diabetes and this number is

likely to increase to 57 million by 2025 [4]. This astronomic increase in the prevalence of diabetes has made diabetes a major public health challenge for India and is become important human ailment afflicting many from various walks of life in different countries and once again the whole world being looked upon Ayurvedic the oldest healing system of medicine for the treatment of diabetes [5]. The synthetic oral anti-diabetic drugs and insulin which are being currently used for the control of diabetic complications are effective in controlling the elevated blood glucose levels, but they have various side effects and do not control the complications related to diabetes [6].

Traditional medicinal plants are being used worldwide for many diabetic complications. Various herbal drugs and minerals have been described in olden traditional literature for the treatment of diabetes mellitus. Herbal drugs are considered to be safe and do not have much side effects compared to synthetic drugs [7]. Therefore, exploring the hypoglycemic potential of medicinal plants has become very important to provide mankind with the safer alternative of herbal drugs. The present study has been carried out to determine the hypoglycemic potential of bark of *Anacardium occidentale*, whole aerial part of *Achyranthes aspera* and leaf of *Aegle marmelos* in diabetic rats.

## MATERIALS AND METHODS:

**Plant Material:** *Anacardium occidentale*, *Achyranthes aspera*, and *Aegle marmelos* were collected in November 2016, from the Vindhya herbal garden, Bhopal, Madhya Pradesh. The plant material was authenticated by Dr. Sumantrivedi Professor & Head, Department of Botany, Govt. MLB Girls PG College Bhopal and Voucher specimen (Herb/2016/01) was deposited as Herbarium at the Department of Botany. The collected plant materials were air-dried in darkness at room temperature (20 °C). Dried plant parts were cut and stored in tight-seal dark containers until needed.

**Preparation of Plant Extracts:** Plant extracts were prepared according to a standard protocol. Prepared plant material (10 gm) was transferred to dark-colored flasks and mixed with 200 ml of solvents with different polarities of petroleum extract, hydroalcoholic extract, and aqueous extracts were stored at room temperature respectively. After 24 h, infusions were filtered through Whatman no. 1 filter paper and residue was re-extracted with an equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 °C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 °C [8].

**Chemicals and Reagents:** Alloxan monohydrate (Thomas Baker Pvt. Ltd., India), Glibenclamide tablets (Daonil; Aventis Pharma. Ltd., India). Kits for glucose estimation were procured from Labcare Diagnostic, India, Pvt. Ltd. The other chemicals and reagents were procured from Himedia Lab Pvt., Limited.

**Animals:** Wistar rats (150-200 g) were grouped (n=6) and housed under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25 ± 2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum* [9]. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC No. is TIT/IAEC/831/P'col/ 2015/61), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India [10].

**Acute Toxicity:** Acute toxicity studies were carried out in accordance with OECD guidelines, acute oral toxicity study of bark of *Anacardium occidentale*, leaves of *Aegle marmelos* and whole aerial part of *Achyranthes aspera*. The *Anacardium occidentale*, *Aegle marmelos* and *Achyranthes aspera* (50, 100, 150, 200, 300 mg/kg/day) were administered orally for 4 days of six groups of rats (n=6) and the animals were kept under examination for mortality as well as any other behavioral changes [11].

**Evaluation of Anti-diabetic Activity:**

**Induction of Experimental Diabetes in Rats:** After fasting, diabetes was induced by a single intraperitoneal injection of 120 mg/kg body weight of alloxan monohydrate' in distilled water.

The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. These animals were tested for diabetes after 15 days, and animals with blood glucose (fasting) were selected for experimentation [12].

**Experimental Protocol:** Animals were divided into nine groups of 6 rats each.

**Group I:** Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat).

**Group II:** Rats served as diabetic-control and received the vehicle (0.5 ml distilled water/day/rat).

**Group III:** Rats (diabetic) were administered Glibenclamide (600 µg/kg.) for 15 days.

**Group IV:** Rats (diabetic) were administered hydroalcoholic extract of *Anacardium occidental* (100 mg/kg) for 15 days.

**Group V:** Rats (diabetic) were administered hydroalcoholic extract of *Anacardium occidental* (200 mg/kg) for 15 days.

**Group VI:** Rats (diabetic) were administered hydroalcoholic extract of *Aegle marmelos* (100 mg/kg) for 15 days.

**Group VII:** Rats (diabetic) were administered hydroalcoholic extract of *Aegle marmelos* (200 mg/kg) for 15 days.

**Group VIII:** Rats (diabetic) were administered Hydroalcoholic extract of *Achyranthes aspera* (100 mg/kg) for 15 days.

**Group IX:** Rats (diabetic) were administered hydroalcoholic extract of *Achyranthes aspera* (200 mg/kg) for 15 days.

**Statistical Analysis:** All analysis was performed using Graph pad prism for Windows. All statistical analysis is expressed as the mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test [13].

**RESULTS AND DISCUSSION:** Preliminary Phytochemical analysis of bark of *Anacardium occidentale*, whole aerial part of *Achyranthus aspera* and leaf of *Aegle marmelos* showed the presence of alkaloids, flavonoids, phenols, tannins, proteins and steroids. Flavonoids are also known to regenerate the damaged β-cells in diabetic mice. The effect of hydroalcoholic extracts on blood glucose levels, body weight, triglyceride, and protein level in normal and diabetic rats after treatment of 15 days are shown in **Fig. 1-5**.

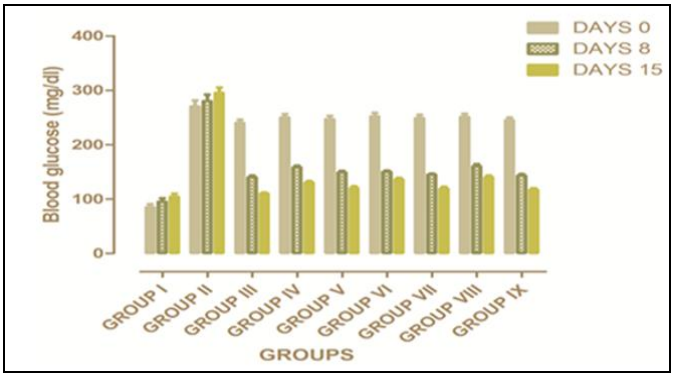


**FIG. 1: ANTI-DIABETIC EFFECT OF HYDROALCOHOLIC EXTRACTS OF *ANACARDIUM OCCIDENTALE, AEGLE MARMELOS* AND *ACHYRANTHES ASPERA* TREATMENT BLOOD GLUCOSE (MG/DL)IN NORMAL AND DIABETIC RATS.** Values are expressed as mean ±S.E.M (n = 6).Values are statistically significant at # P <0.001 vs. normal group; *P < 0.05 vs. diabetic control group (followed by Dunnett's test -tests).

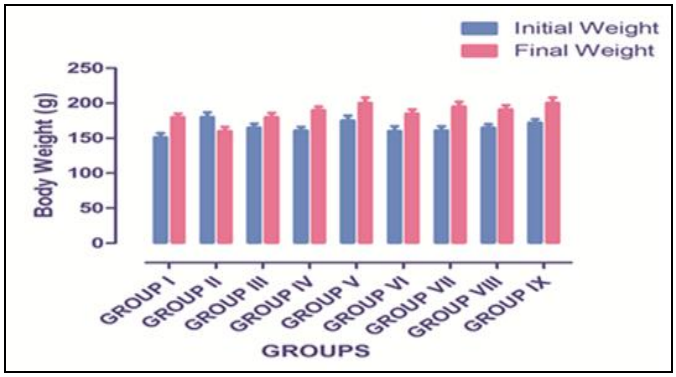


**FIG. 2: ANTIDIABETIC EFFECT OF HYDROALCOHOLIC EXTRACTS OF *ANACARDIUM OCIDENTALE, AEGLE MARMELOS* AND *ACHYRANTHES ASPERA* TREATMENT ON BODY WEIGHT.**

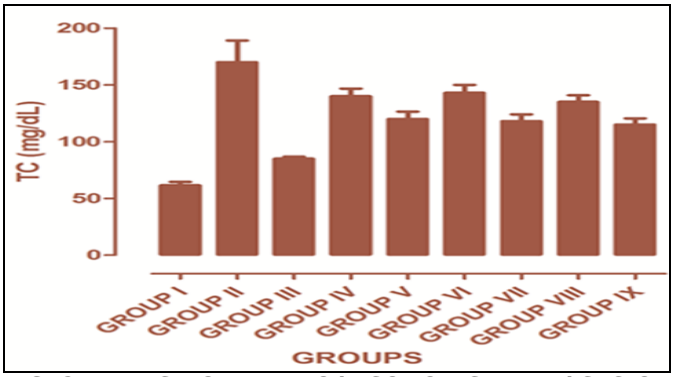


**FIG. 3: EFFECT OF HYDROALCOHOLIC EXTRACTS OF *ANACARDIUM OCIDENTALE, AEGLE MARMELOS* AND *A. ASPERA* TREATMENT ON TOTAL CHOLESTEROL LEVEL IN NORMAL AND DIABETIC RATS**

Values are expressed as mean ±S.E.M (n = 6).Values are statistically significant at # P <0.001 *vs.* normal group; *P < 0.05 *vs.* diabetic control group (followed by Dunnett's test -tests).

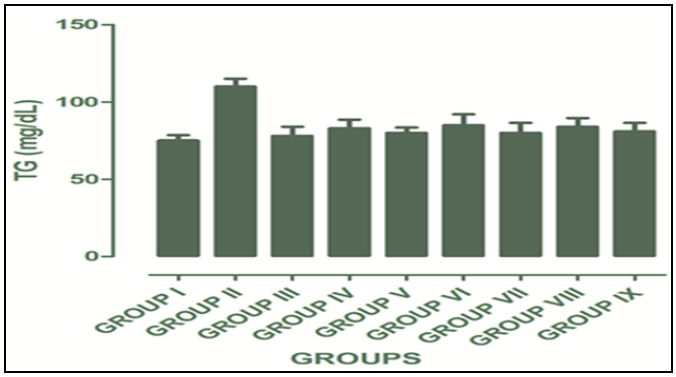


**FIG. 4: EFFECT OF HYDROALCOHOLIC EXTRACTS OF *ANACARDIUM OCIDENTALE, AEGLE MARMELOS* AND *A. ASPERA* TREATMENT ON TRIGLYCERIDE LEVEL IN NORMAL AND DIABETIC RATS**

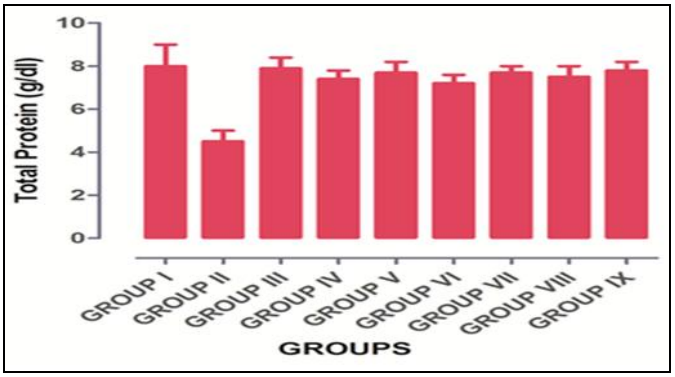


**FIG. 5: EFFECT OF HYDROALCOHOLIC EXTRACTS OF *ANACARDIUM OCIDENTALE, AEGLE MARMELOS* AND *A. ASPERA* TREATMENT ON TOTAL PROTEIN LEVEL IN NORMAL AND DIABETIC RATS**

Values are expressed as mean ± S.E.M (n = 6).Values are statistically significant at # P <0.001 *vs.* normal group; *P < 0.05 *vs*. diabetic control group (followed by Dunnett's test -tests).

It was observed that standard drug Glibenclamide lowered the blood glucose levels, significantly bringing it back to normal, which is stimulate the insulin secretion from β-cells. The extracts showed significant reduction of blood glucose level and lipid profile in diabetic rats. A significant reduction in average weight was observed in alloxan-induced diabetic rats. The decrease in weight in diabetes was due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis. Hydroalcoholic extracts showed the increases in body weight might be due to improving the glycemic control mechanisms and insulin secretions from remnant pancreatic cells in diabetic animals. The extracts also showed significant increase in protein level in diabetic rats[14].

**CONCLUSION:** In the present study, the bark of *Anacardium occidentale*, whole aerial part of *Achyranthes aspera* and leaf of *Aegle marmelos* were selected for anti-diabetic studies owing to its traditional uses. Therefore, the study was undertaken to justify its claimed uses. Wistar rats were selected as experimental animals for the anti-diabetic activity. The extract was screened for alloxan-induced anti-diabetic activity. The hydroalcoholic extracts of plant showed significant anti-diabetic activity at doses, 200 mg/kg of body weight. However, this is preliminary work, and more work is needed to determine the active chemical nature in the extract which may help in improving management of diabetes. The study reveals that the hydroalcoholic extract of bark of *Anacardium occidentale*, whole aerial part of *Achyranthes aspera* and leaf of *Aegle marmelos* could be added in the list of herbal preparation, beneficial in diabetes mellitus. Further studies can be undertaken at the cellular and molecular levels, which may further elucidate its mechanism in detail. The present investigation has also opened avenue for further research especially with reference to the development of potent polyherbal formulation for diabetes mellitus [15].

**ACKNOWLEDGEMENT:** The authors are thankful to the Head of Scan Research Laboratory, Bhopal (MP), for providing data related to blood glucose level, bodyweight loss, and lipid profile.

**CONFLICT OF INTEREST:** The author declares no conflict of interest.

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**How to cite this article:**
Brijyog, Singh LP, Kumar S and Verma S: Antidiabetic activity of hydroalcoholic extracts of *Anacardium occidentale*, *Aegle marmelos* and *Achyranthes aspera* in alloxan-induced diabetic rats. Int J Pharm Sci & Res 2019; 10(11): 4932-36. doi: 10.13040/IJPSR.0975-8232.10(11).4932-36.