



Volume No: 1

Issue 2

June 2017

International Multidisciplinary Innovative Research Journal
-An International refereed e-journal - Science Issue

ISSN: 2456-4613

IMIRJ, 1(2), 2017 [1-8]

ANTIDIABETIC AND ANTIHYPERTENSIVE EFFECTS OF A METHANOLIC EXTRACT OF THE PLANT *TRIDAX PROCUMBENS* IN STREPTOZOTOCIN- INDUCED DIABETIC MICE

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ABSTRACT

Herbal drugs are frequently considered to be less toxic and also free from side effects. Hence, the present study was designed to evaluate antidiabetic principle of the Methanolic extract of *Tridax procumbens* for its antidiabetic and antihypertensive potential against streptozotocin-induced diabetic mice. The phytochemical characteristics of the methanolic extract of *Tridax procumbens* were analyzed. The present study revealed the presence of carbohydrates, phenols, tannins, steroids, alkaloids, flavonoids and saponins and absence of proteins, glycosides, terpenoids, and quinones in methanolic extract of *T.procumbens*. The biochemical parameters of glucose, triglycerides, LDL Cholesterol, HDL Cholesterol were measured in blood samples collected from both control and STZ induced diabetic mice. The diabetic control animals exhibited a significant decrease in body weight compared with control animals. A significant increase in serum glucose and triglycerides in STZ induced diabetic mice revealed the diabetogenic effect when compared to normal control group. The LDL Cholesterol was found to be increased and HDL Cholesterol was decreased in STZ induced diabetic mice compared to normal mice. The plant extract *Tridax procumbens* along with STZ treated mice revealed a value of decreased Serum triglycerides, LDL Cholesterol

and increased HDL Cholesterol. Hence our study suggested that the plant *Tridax procumbens* has significant therapeutic effect to control Diabetes and hypertension.

Keywords: *Tridax procumbens*, Streptozotocin, Antidiabetic, hypertension.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glucosuria and negative nitrogen balance occurs mainly due to lack of insulin secretion in beta cells of pancreas and desensitization of insulin receptors for insulin. The disease has reached epidemic proportions in the present century. It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people and is set to rise to 300 million by 2025 (Satyanarayana, 1969). Several drugs such as biguanides and sulfonylurea were presently available to reduce hyperglycemia. These drugs have side effects and thus searching for a new class of compounds is essential to overcome these problems (Kamaeswara Rao, 2001). Management of diabetes without any side effects is still a change to medical community. Traditional antidiabetic plants might provide new oral hypoglycemic compounds which can counter the high cost and poor availability of the current medicines / present day drugs for many rural populations in developing countries.

Tridax procumbens Linn (*Tridax*) family composite commonly known as 'Gnamra' and in English popularly called 'coat buttons' because of appearance of flowers has been extensively used in Ayurvedic system of medicine for various ailments and is dispensed "Bhringraj" by some of the practitioners of Ayurveda which is well known medicine for liver disorders (Bhagwat *et al.*, 2008). It is best known as a wide spread weed and pest plant. *Tridax procumbens* is a small perennial herb having short, hairy blade like leaves. Corolla is yellow in color. *Tridax procumbens* Linn (Asteraceae) is one of the common herbs present throughout India, and this plant extract was commonly used for treating the cut wound (anti-inflammatory) by rural Indians. Studies showed that *T.procumbens* has anti-inflammatory, hepatoprotective, wound healing, antimicrobial, antiseptic, hypertensive and immunomodulatory properties (Bhagwat and Pareek, 2009).

Hence, the study was carried out to investigate the antidiabetic and

antihypertensive activity of *Tridax procumbens* plant extract.

MATERIALS AND METHODS

Collection and Identification of Plants

Tridax procumbens plants were collected from a Garden in Virudhunagar. The collected plant parts (only leaf) were separated from undesirable materials or plant parts. They were dried for one week. The plant parts were ground into a suitable grinder. The powder was stored in an airtight container and kept in cool, dark and dry place for further analysis.

Preparation of Extract

The aerial parts of plant were collected and dried for three days. The dried powder of *Tridax procumbens* (180 gm.) was soaked in 500ml of 95% ethanol for 7 days in cold condition with occasional shaking and stirring. The whole mixture was success filtered through a piece of clean, white cotton material and whatmann NO.1 filter paper.

Phytochemical Analysis

Preliminary phytochemical analysis was carried out to test the presence of tannins, flavonoids, terpenoids, alkaloids, reducing sugars, saponins, quinones and anthraquinones by

Ninhydrin test, iodine test, Shinoda test, Alkaline reagent test, Keller-Kiliani test, Libermann-Burchard reaction test respectively.

Selection, Maintenance and Mangement of Experimental Animals

Healthy young Swiss-albino mice aged 4 to 5 weeks, average weight of about 20 to 25 gm were selected for the present study. The mice were purchased from animal house Venture institute of Biotechnology and Bioinformatics Research center, Madurai (Subjective approval to ethical committee). They were housed individually in cages at an ambient temperature of $24 \pm 1^{\circ}\text{C}$, relative humidity of 55 to 65% with 12h light; 12h dark cycle and fed formulated mice food and water and libitum. Excreta were removed from the cages on every day. The animals were acclimatized to laboratory condition for one week prior to experimentation. All mice had free access of drinking water and food, *ad libitum*, during experimental period. All protocols followed for animal experiment were approved by the institutional animal ethical committee. (Zimmermann, 1983).

1. Induction of Diabetes by Administration of Streptozotocin

Preparation of Streptozotocin

STZ was purchased from sigma chemicals containing 100mg each vial and before used, it must be stored in -20°C. Streptozotocin was freshly dissolved in 1% tween 80 saline mixture in order to get concentration stock of 10mg/ml.

Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection with STZ (65mg/kg body weight) (Lenzen, 2008). Mice were fast overnight before injection with STZ.

Experimental Design

The animals were divided into three groups (each of 4 mice). All international and local rules and regulation for handling animals in experiments were followed. The experimental groups were illustrated as follows.

Group 1: Healthy mice fed on pellet diet served as normal controls.

Group 2: Diabetic mice fed on pellet diet.

Group 3: Diabetic mice fed on pellet diet plus *Tridax procumbens* extract (0.05 ml/day)

Symptoms accompanying *Diabetes mellitus* such as polydipsia, polyuria, and

loss of body weight were observed every day. STZ induced mice was considered as *Diabetes mellitus* when fasting body glucose level above 11.1 mol/l. Diabetes severity grade is classified into three types, severe, moderate and mild diabetes, based on fasting blood glucose level.

At the end of the experiment, the animals were anesthetized with chloroform after 12 hours fasting and whole blood samples were taken from hepatic portal vein. The blood samples left for 15 minutes at 37 °C for serum separation, then centrifuged at 3000 rpm for 10 minutes, then sera were separated and kept in plastic vials at 4°C until analyses.

Assay of Biochemical Parameters

Serum glucose was determined by enzymatic colorimetric method (*Trinder*, 1969). Total cholesterol (TC) was determined in serum using enzymatic method (*Richmond*, 1973). Triglyceride was measured in an aqueous extract (*Bucolo and David*, 1973). Finally results were compared with the respective controls.

RESULTS

The phytochemical characteristics of the methanolic extract of *Tridax procumbens* were analysed. The present study revealed the presence of carbohydrates, phenols, tannins, steroids, alkaloids, flavonoids and

saponins and absence of proteins, glycosides, terpenoids, and quinones in methanolic extract of *T.procumbens* (Table.1)

Table :1 Photochemical analysis of the extract of *Tridax procumbens*

S.No	Test	Result
1	Proteins (Ninhydrin Test)	Negative
2	Carbohydrates (Iodine Test)	Positive
3	Test for phenols and tannins	Positive
4	Flavonoids (Shinoda Test)	Positive
5	Saponins	Positive
6	Glycosides (Salkowski's Test)	Negative
7	Steroids	Negative
8	Terpenoids	Positive
9	Alkaloids	Positive
10	Quinones	Negative

Figure.1 -Effect of an ethanolic extract of *T. procumbens* on blood glucose levels in streptozotocin-induced diabetic mice

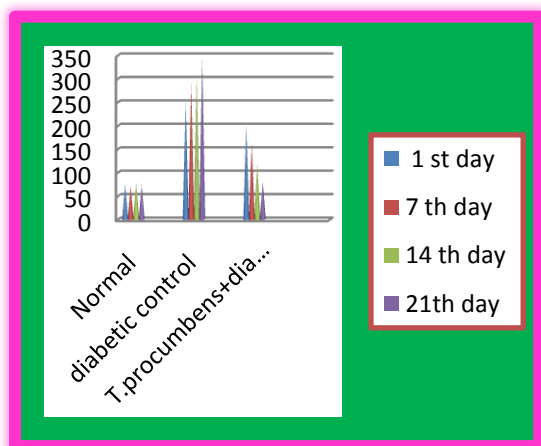
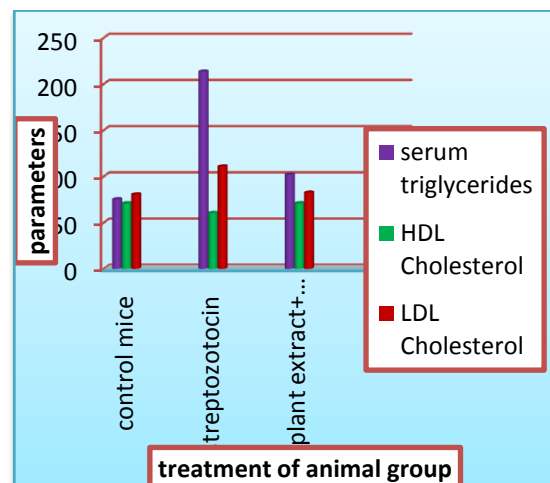


FIG.2. Effect of plant extract of *Tridax procumbens* on Serum Triglycerides, HDL Cholesterol and LDL Cholesterol in Streptozotocin induced diabetic mice (mg/dl)



In our study, blood glucose level was found to be increased in STZ induced diabetic mice when compared to control mice. Oral administration of plant extract *Tridax procumbens* along with STZ in mice revealed a decrease in blood glucose (Fig.1) The increase in serum triglycerides in STZ induced diabetic mice revealed a significant increase when compared to normal control group mice. Oral administration of plant extract *Tridax procumbens* along with STZ in mice has shown a value of decreased serum triglycerides and LDL Cholesterol. The level of HDL Cholesterol was found to be high in the plant extract *Tridax procumbens* along with STZ treated mice (Fig.2)

DISCUSSION

Tridax procumbens is a weed found throughout India. The phytochemical screening revealed the presence of alkaloids, carotenoids, flavonoids, saponins and tannins. It has known for its number of pharmacological activities like immunomodulatory, hepatoprotective and antidiabetic activity.

Natural antioxidant mainly arises from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc, (Ali, 2008). Tannins bind to proline rich protein and interfered with protein synthesis.

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation (Just, 1998). Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo, 2000). Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). Alkaloids have been associated with medicinal uses for centuries and one of their common

biological properties is their antibacterial property (Okwu, 2004).

The presence of oleanolic acid in *Tridax* was found to be a potential antidiabetic agent. The flavonoids present in a plant regenerate the damaged beta cells of pancreas. Simultaneously, the polyphenolic compounds and saponins present in *Tridax* inhibit glucose transport by inhibiting sodium glucose transporter in intestine.

In the present study STZ induced diabetes provides a relevant example of endogenous chronic oxidative stress results in hyperglycemic condition was reported (Low *et al.*, 1997). Our study has been demonstrated to induce diabetic condition by administration of STZ in mice model. This led to complete destruction of β -cells and also damaged the islets of Langerhans, thereby triggering an inflammatory process leading to damage of macrophage and subsequent lymphocyte infiltration followed by the onset of insulin deficiency. Antidiabetic condition was provided by the extract of *T.procumbens* because the plant is endowed with carotenoids, oleanolic acid and various ions. A decrease in serum triglycerides was also observed in plant extract treated mice.

Antidiabetic study was carried out in mice model using *T.procumbens* on blood glucose and lipid profile in STZ induced mice. The study clearly showed STZ induced diabetes which causes oxidative stress resulting in hyperglycemic condition and hypertension were found to be alleviated by *T.procumbens* plant extract.

CONCLUSION

The methonolic extract of the plant of *T.procumbens* revealed an antidiabetic activity against STZ- induced diabetes mellitus in mice. It is also found to be effective in managing the complications associated with diabetes mellitus leading to defects in lipid metabolism. Hence it is concluded that plant extract of *T.procumbens* possess some active principles responsible for its antidiabetic and antihypertensive effects.

ACKNOWLEDGEMENT

This work was supported by Venture Institute of Biotechnology Research, Madurai.

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