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ORIGINAL ARTICLE **OPEN ACCESS**

Methanolic Extract of *Homonoia retusa* Aerial Parts shows Anti-Diabetic Effect against Streptozotocin Induced in Diabetes Rats

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ABSTRACT
To control diabetes mellitus, there must be an adequate supply of insulin. Many initiatives to limit or control have recently been launched. Growing numbers of people are turning to natural blood sugar-lowering remedies. Studies on herbs that can control diabetes have been published in a wide range of journals. Today, herbal medicine, also known as phytomedicine, is the oldest and most widely used type of healthcare practice. The use of these medicinal plants has been documented in writing more than 5000 years ago, in China, India, Greece, Rome and Syria. Homonoia retusa, belongs to family Euphorbiaceae of flower, leaves and stem were collected and dried, then they were grounded into a powder and passed through sieve 60. After being roughly crushed, the powder was extracted with methanol, then fractionated with non-polar to polar solvent and dried. The anti-diabetic activity of Homonoia retusa aerial parts of methanolic extract and its fraction is currently used to investigation. Present studies shown anti-diabetic activity effectiveness is the similar comparable to that of the widely used medication drug i.e. Glipizide, according to the results.
***Key words**: Diabetes, Homonoia retusa, Leaves, Arial parts.*

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INTRODUCTION

The most prevalent chronic illness and leading cause of death in contemporary society is diabetes mellitus. When there is insufficient insulin, a complex chain of events occurs that eventually lead to hyperglycemia, which is a clinical symptom of diabetes(1). It is common knowledge that glucose is a crucial source of energy for the tissues, and that its function is impacted if it is lost due to diabetes. Hyperglycemia decreased insulin production, and both are symptoms of the chronic disease diabetes. High blood sugar levels brought on by diabetes mellitus increase the generation of free radicals [1].Oxidative stress has a significant impact on the diabetes etiology and its complications. It is believed that the hyperglycemia generates due to Reactive Oxygen Species (ROS), which is in turn to lead to oxidative stress in a number of tissue [2]. However, when a person has diabetes, there is enough glucose present to allow it t reach to the tissues and different loop works for construct both sorbital & fructose. Because they have a poor ability to cross membranes, these cells accumulate them. A number of diabetic complications, including cataracts, retinopathy, neuropathy, and nephropathy, have been linked to these abnormal metabolic outcomes [3-6].

Herbal medicine is the oldest and most widely used medicine in the world. Herbal medicine is the fulcrum of complementary and alternative medicine, which is gaining popularity worldwide. Due to the side effects of synthetic products, medicinal herbs are gaining popularity globally. *Homonoia retusa* is a small shrub or tree that is typically found in the tropics. It has been long used in traditional medicines to treat a broad range of illnesses, i.e., Malaria, Blodder Stone, Urinary Discharge, Imflamation, Ulcer, Uterine Disorders and Blood Disordes [7-9]. Additionally, the plant's roots have emetic, anti-urolithiatic, diuretic, and laxative properties. The fruits, flowers and leaves are used to treat inflammation, cuts, antibacterial, antifungal, anticancer and dermatitis [10, 11].Therefore, the present study was crried out to determine whether the plant *Hormonoia retusa* aerial parts, Methonolic Extract and its different fraction (non-polar to polar) were have any anti- diabetic properties against in streptozotocin – indused diabetes rats.

MATERIAL AND METHODS

The present research work have been execute at Department of Pharmacology & Toxicology, UCPSc, Kakatiya University, Warangal. The collection and processing of aerial parts of Homonoia retusa plant

material was acquire & authenticated by a toxonomist and plant voucher specimen given number is 208 and preserved at departmental meusium. All the chemicals and reagent used for this consider were purched research–grade quality and procure in our laboratory according its preservative conditions. Estimation of glucose , triglycerdes, creatinine, cholesterol, Alnine Aminotransferase (ALT) and Aspartate Aminotransferase (AST). Alkaline phosphate (ALP) were studies by using standard Merck analytical kit methods. (Merck specialities private limited, Mumbai, India).

COLLECTION OF PLANT MATERIAL AND PREPARATION OF EXTRACTS:

Homonoia retusa plants aerial parts were collected in December 2019 in Gundlasingaram, Manuguru, Telangana. India. Dr. Md. Mustafa, a taxonomist and professor of botany at K.U. Warangal. *Homonoia retusa* aerial parts (Flower, Leaves, and Stem) were powdered, dried, and stored in an airtight container after passing through sieve number 60. 1 kg of powder was roughly crushed in separate round bottom flask and extracted with methanol (by maceration) for 7 days at room temperature. The content of the round bottom flask was frequently shaken to ensure efficient extraction. After seven days, the content of container was filtered, and the marcs required a second extraction with methanol for 5 days to fully extract the plant's chemical components. Rota evaporator was used to obtain their concentrated extract at a lower pressure. Then extracts were pooled together in china dish and dried. This methanolic extract was taken further for fraction with nonionicr to polar solvents i.e., n- Hexane, Chloroform, Ethyl acetate and n-Butanol. Thes extracct were dried and preserved in desicator.

PRELIMINARY PHYTOCHEMICAL SCREENING:

The initial phytochemical analysis of the extracts were achieve by using standard described protocol (12, 13). The methanolic extract of aerial parts (Flower, Leaves, and Stem) of *Homonoia retusa* gave positive results for alkaloids, flavonoids, glycosides, saponins and tannins, phenols, steroids, and tri-terpinoids in test tube qualitative reactions.

Animal experiments have being performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines of the government of India. Wistar albino male rats weighing 200-250gm were purchased from Vyas labs in Hyderabad with earlier acceptance from our institutional animal social committee (IAEC /08/ UCPSc / KU/2020). The animals have been caged in a controlled environment 12 hour day light as well as12 hours dark cycle condition claimant at temperature 22±2^{o}C; 40±15% of RH) inside the UCPSc, Kakatiya university, Warangal. Animals ware maintained with standard pellet diet plan. The animals were free access to food & water.

ACUTE TOXICITY STUDY:

The acute oral toxicity study were carried out accordance with standard guidelines i.e., OECD 423. For this study methanolic extract of aerial parts of the plant *Homonoia retusa* (MEHR) **is** given to male wistar albino rats at doses up to 2000mg/kg. For 14 days, the animals were monitored for changes in autonomic and behavioral responses. The mortality observed in different dosing groups was recorded throughout the study.

EXPERIMENTAL DESIGN:

Table-1 Animals groups for the studies of anti-diabetes activity

Group (n=6)	Animals (Male Wister rats) with dose
Group-I	Normal control group with standard fed chow pallet diet and water.
Group-II	Standard drug Glipizize (10mg /kg/ day, orally) treated, STZ (45 mg/kg) induced diabetes rats with standard fed chow pallet diet and water
Group-III	200 mg/kg methanolic extract treated, STZ (45 mg/kg) induced diabetes rats with standard fed chow pallet diet and water
Group-IV	400 mg/kg methanolic extract treated, STZ (45 mg/kg) induced diabetes rats with standard fed chow pallet diet and water
Group-V	100 mg/kg ethyl acetate fraction treated, STZ (45 mg/kg) induced diabetes rats with standard fed chow pallet diet and water
Group-VI	200 mg/kg ethyl acetate fraction treated, STZ (45 mg/kg) induced diabetes rats with standard fed chow pallet diet and water

ASSEMENT OF HYPERGLYCEMIC ACTIVITY IN NORMAR RATS:

In order to evaluate the hyperglycemic action in healthy rats, the method outlined in literature is used (14, 15). Rats were classified into six identical groups (I-VI, n=6) after fastig for 18 hours. All of the animals were given fasting blood samples and different doses of aerial parts of *Hormonoia retusa* methanolic extract (200 & 400 mg/kg b.w) suspended in gum acacia is administered orally to standard of rats for 24 hours to test its impact on blood glucose levels.

INDUCTION OF DIABETES-II BY USING STREPTOZOTOCIN (STZ):

Streptozotocin (STZ) is used for diabetes induced into rats (II-VI, n=6) groups, immediately before use this, each rat received 45 mg/kg b.w intraperitoneally, STZ sample was break down in freshly made (0.1 M) citrate screen (PH 4.5). Then after 72 hours animal blood glucose levels were measured. Here, the diabetic rats blood glucose levels is more than 250 mg/ dL. The animal received the test component Methanolic Extract of *Hormonoia retusa* (MEHR), its fraction Ethyl acetate (EAHR) and reference drug is Glipizide given by orally once daily for up to 28 days. Animals were sacrificed at the conclusion of the end of the day study, and the pancreas was removed to examine the histological alterations.

EVALUATION OF ANTI-DIABETICS ACTIVITY IN RATS:

Results of anti- diabetics were judge by estimating in the blood plasma glucose levels every week. In this studies measurement of blood glucose levels with automated Glucometer by the collecting the drop of blood from the rat tails. The rats with blood glucose levels > 250 mg/dL were considered as diabetics and the calculated the % anti-diabetics activity (12).

MEASUREMENT OF PANCREATIC WEIGHTS:

The result of pancreatic activity is determine after four weeks period of the post- STZ experimental (initially 2 week period is considered as pre experimental), on 28th day (after 4 week), finally animals were sacrificed with a higher quantity of general anesthesia and each rats pancreas were collected correctly and weight of pancreas were recorded (17).

ESTIMATION OF BIO-CHEMICAL PARAMETERS:

The Bio-chemistry parameters were estimated from the plasma profiles of metabolic parameters like triglycerides and total cholesterol in the plasma was estimated in all the animal experimental groups, these studies were estimated done by using commercially available Bio-chemical kits procured from Accurex, Bio-medical (Mumbai, India) (18,19).

HISTOPATHOLOGICAL EVALUATION:

In neutral buffered formalin for 48 hours, the pancreas that was removed was fixed. An apparatus for processing tissues was used to process the pancreas. The tissues underwent processing using embedded in paraffin blocks and sections were made about 5µm cute out from paraffin blocks (20).

RESULTS AND DISCUSSION

The present studies were initially carried out in the animal for acute toxicity of aerial parts of *Homonoia retusa* methanolic extract up to dose is 2000 mg/kg showed that this extract doesn't exhibit any signs of toxicity or mortality. However, only the quantity at 2000 mg/kg is reduces in physical activity noted. In order to conducted main objective of research work anti-diabetic activity, the current dose regime *Homonoia retusa* methanolic extract (MEHR) 200 and 400 mg/kg and its fraction ethyl acetate *Homonoia retusa* (EAHR) 100 and 200 mg/kg was chosen, to tests on rats and used standard drug is Glipizide (10 mg/kg) to determine the anti-diabetic activity. Additionally, the effects of the test drug-treated group of rats differentiate to the untreated (Normal control group rats) and Streptozotocin (45 mg/kg b.w.) induced diabetic-II, treated (standard control group rats) groups of rats were displayed the significance reduction of activity. Treatment with standard drug is Glipizide (10 mg/kg), crude methanol extract is MEHR (200, and 400 mg/kg) and its fraction is EAHR (100 and 200 mg/kg) was significantly reduced the fasting blood glucose levels these results were shown Table-2. These was studied compare with steptozotocin (STZ) induced diabetes rats Table-3. Than we observed bio-chemical parameters levels of insulin, serum cholesterol, triglycerides in STZ induced diabetes-II rats, the result were observed significantly increases with EAHR and MEHR Tablr-4 and redusece the levels of Alanine Amino Transferees (ALT), Aspartate Amono Transferase (AST) and Alkaline Phosphate (ALP) result shown in Table-3. Among the all extract *Homonoia retusa* methanolic extract of fraction is ethyl acetate *Homonoia retusa* (EAHR) significantly increased body weights shown Table-5.

The research work mainly shown the result is in Streptozotocin (STZ)-induced diabetic-II rats, at 400 mg/kg MEHR and 200mg/kg EAHR of *Homonoia retusa* aerial parts resulted in a significant decrease in the elevated serum biochemical parameter blood glucose and EAHR 200 mg/kg elevating serum biochemical variables like insulin, serum creatinine, and Serum triglycerides Table-4, and ALT, AST and ALP in STZ reduced diabetic rat's levels. Streptozotocin orally administration significantly reduced body weight. These effects were observed to be on par with standard drug, the result shown not greater than, those of standard drug Glipizide (10 mg/kg). In this study disease and standard control group, histological changes in the pancreas tissue were seen. These histological alterations had been significantly enhanced by the 400 mg / kg MEHR and 200mg / kg EAHR dose of *Homonoia retusa* extract Fig.1.

Table-2 EFFECT OF ETRACTS OF *Homonoia retusa* ON FASTING BLOOD GLUCOSE LEVELS IN NORMAL RATS

Group and Treatment (n=6)	Blood glucose levels (mg/kg) at different time point in hours							
	0 hr	1hr	2 hr	4 hr	6 hr	8 hr	16 hr	24 hr
Group-I Normal Control	88.5±4.9	88.8±6.08	87.2±6.05	**82.5±6.2**	84.7±7.3	82.3±6.3	83.01±4.6	83.4±5.3
Group-II STZ +Standard drug (Glipizide10mg/kg)	88.6±5.3	81. 0±3.9	71.32±2.3*	**60.15±3.4**	72.77±6.0	77.4±4.5	81.72±4.4	82.9±3.7
Group-III –STZ +MEHR (200mg/kg)	89.8±3.4	87.2±5.7	82.4±4.9	**77.1±4.6**	76.1±5.0	79.3±5.7	80.7±4.1	83.07±4.6
Group-IV – STZ+ MEHR (400mg/kg)	89.5±4.2	88.5±6.3	82.2±5.4	**71.8±3.2***	70.3±3.2*	76.7±4.0	82.1 ±4.3	82.4±2.9
Group-V – STZ + EAHR 100mg/kg	91.4±3.7	89.0±3.8	84.7±3.4	**75.1±2.6**	76.7±3.9	80.6±3.4	83.6±2.6	85.7±2.5
Group-VI- STZ + EAHR 200mg/kg	90.3±4.5	86.6±5.9	78.6±4.9	**65.9±3.8**	72.8±6.3*	80.0±6.11	82.8±2.8	86.1±3.1

*Stastically significant $p < 0.05$, ** $p < 0.01$, compared with normal control and standard drug Glipizide

Table-3 EFFECT OF ETRACTS OF *Homonoia retusa* ON FASTING BLOOD GLUCOSE LEVELS IN STZ INDUCED DIABETES-II RATS

Group and Treatment (n=6)	Blood glucose levels (mg/kg) at different time point in hours							
	0 hr	1hr	2 hr	4 hr	6 hr	8 hr	16 hr	24 hr
Group-I Normal Control	88.5±4.9	88.8±6.08	87.2±6.05	**82.5±6.2**	84.7±7.3	82.3±6.3	83.01±4.6	83.4±5.3
Group-II STZ + Standard drug (Glipizide10mg/kg)	88.6±5.3	81. 0±3.9	71.32±2.3*	**60.15±3.4**	72.77±6.0	77.4±4.5	81.72±4.4	82.9±3.7
Group-III –STZ + MEHR (200mg/kg)	89.8±3.4	87.2±5.7	82.4±4.9	**77.1±4.6**	76.1±5.0	79.3±5.7	80.7±4.1	83.07±4.6
Group-IV – STZ+ MEHR (400mg/kg)	89.5±4.2	88.5±6.3	82.2±5.4	**71.8±3.2***	70.3±3.2*	76.7±4.0	82.1 ±4.3	82.4±2.9
Group-V – STZ + EAHR 100mg/kg	91.4±3.7	89.0±3.8	84.7±3.4	**75.1±2.6**	76.7±3.9	80.6±3.4	83.6±2.6	85.7±2.5
Group-VI- STZ + EAHR 200mg/kg	90.3±4.5	86.6±5.9	78.6±4.9	**65.9±3.8**	72.8±6.3*	80.0±6.11	82.8±2.8	86.1±3.1

*Statically significant $p < 0.05$, ** $p < 0.01$, compared with normal control and standard drug Glipizide

Table-2 EFFECT OF FRACTIN EAHR OF *Homonoia retusa* ON INSULIN, SERUM CHOLESTEROL AND TRIGLYCERIDES IN STZ INDUCED DIABETES-II RATS

Group and Treatment (n=6)	ALT (IU/L)		AST (UI/L)		ALP (UI/L)	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
Group-I Normal Control	186.4 ±4.5	192±3.6	119.6±3.2	128.3±3.6	186.4 ±4.5	192.8±3.6
Group-II STZ + Standard drug (Glipizide10mg/kg)	175.3±3.9	84.9±3.9** (51.56%)	128.3±2.6	72.46±2.8** (43.52%)	175.3±3.9	84.9±3.9** (51.56%)
Group-III- STZ + EAHR 200mg/kg	172.2±3.7	92.2±2.7** (46.45%)	125.4±1.7	84.16±1.7** (37.28%)	172.2±3.7	92.2±2.7** (46.45%)

*Statically significant $p < 0.05$, ** $p < 0.01$, compared with normal control and standard drug Glipizide

Table-2 EFFECT OF FRACTIN EAHR OF *Homonoia retusa* ON BODY WEIGHTS AND BLOOD GLUCOSE LEVES IN STZ INDUCED DIABETES-II RATS

Group and Treatment (n=6)	Body Weight (gm)		Blood glucose level (mg/dl)	
	Day 1	Day 28	Day 1	Day 28
Group-I Normal Control	213.3±10.4	178.2±6.7 (-16.4%)	252.1±7.6	291.1±6.6 (15.47%)
Group-II STZ + Standard drug (Glipizide10mg/kg)	202.5±8.2	230±8.4 (13.8%)	281.6±4.6	140.4±5.8** (50.14%)
Group-III- STZ + EAHR 200mg/kg	196.9±8.6	223.7±7.1 (10.3%)	278.3±4.8	156.7 ±7.4** (43.69%)

*Statically significant $p < 0.05$, ** $p < 0.01$, compared with normal control and standard drug Glipizide

HISTO-PATHOLOGICAL STUDIES OF THE PANCREAS:

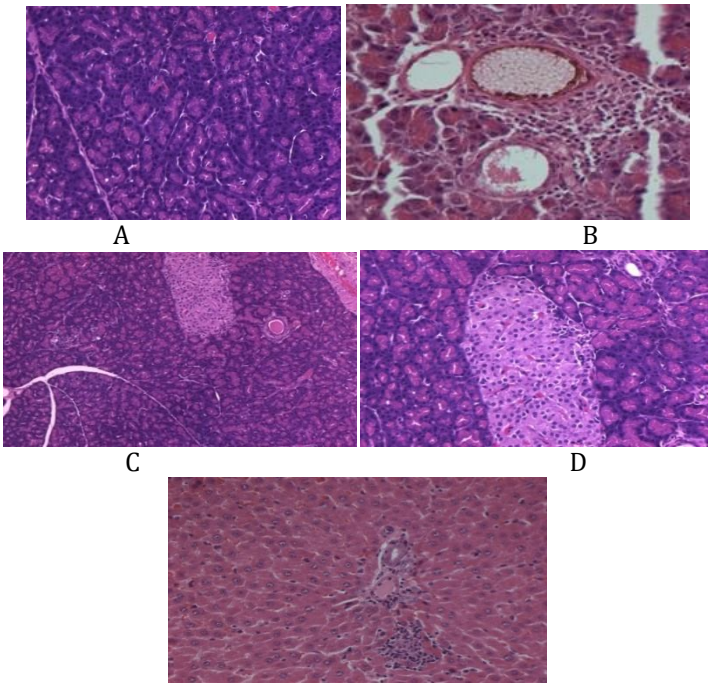

Fig. 1. Histo-pathological change was observed in the pancreas under 100X using Digital Moti Microscope and Hematoxylin-eosin stain. A. Normal control group Rats. B. STZ induced Diabetics Rats. C. STZ induced Diabetes Rats treated with standard drug Glipizide (10 mg/ kg b.w). D. STZ induced Diabetic rats treated with MEHR (400 mg/ kg). E. STZ induced Diabetic Rats treated with EAHR (200 mg/ kg).

CONCLUSION

The conclusion of present research work main objective is analyzing the anti-diabetic activity for *Homonoia retusa*. First we collected aerial (Flower, Leaves and Stem) this plant and authenticated then according to standard procedure prepared methonolic extract and it fraction non polar solvents to polar solvents. Here, the finding LD50 of MEHR extract up to 2000 mg/kg resulted in observed that the no animal mortality indicating results were nontoxic and safe. For the estimating Anti-diabetes activities we used standard drug is Glipizide (10 mg/kg), and extract is MEHR (200 and 400 mg/kg), and its fraction EAHR (100 and 200 mg/kg), significantly reduced bio chemical parameters serum blood glucose levels, increased insulin, serum creatinine, and Serum triglycerides and also increased body of STZ idused in diabetic-II rats results werw shown Table- 2,3,4. The positive results were observeddue to the plant material contain secondary metabolite i.e., flavonoids, triterpinoids, steroids glycoside and saponins. The results of selected methanolic extract and its fraction ethyl acetate of *Homonoia retusa* plants of aerial parts contain more number of secondary metabolites. The significant results of extract if methanoli extract is 400 mg/kg and its fraction ethyl acetate is 200 mg/kg shown better results when compared with standard drug is Glipizide is (10 mg/ kg).

The positive results were due to consists of flavonoids, steroids, triterpenes, glycosides, and saponins were present in MEHR extract and its fractions. Present studies were shows that the methanolic extract of *Homonoia retusa* aerial parts (Flower, Leaves, and Stem) i.e MEHR (400 mg/ kg) and its fraction EAHR

(200 mg/ kg) has anti-hyperglycemic activity, which could contribute to its ethno-medical use of *Homonoia retusa* plants. More research is needed to determine the active constituents and mechanism of action.

In this study we also collected pancreas at end of the day of the study and observed its weights. Then go for Histo-pathological duties change was observed in the pancreas under 100X using Digital Moti Microscope and Hematoxylin-eosin stain, the results of these studies is pacreas more heal and more improvement of β-cells in STZ induced diabetes-II rats with MEHR (400 mg/ kg) and its fraction EAHR (200 mg/ kg) when compared with the standard drug is Glipizide (10 mg/ kg). This is the results we drown from present research work. Finally concluded that the plant *Homonoia retusa* have ethono-medical uses, and it requires more research is needed to evaluating and active compounds constituents and also require mechanism of action.

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