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INVIVO INVESTIGATIONS OF ANTI-DIABETIC POTENTIAL OF TECOMA CAPENSIS ON STZ INDUCED DM IN ALBINO WISTAR RATS

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ABSTRACT

In the past decade, scientists have become increasingly interested in free radicals and antioxidant phytochemicals due to their wide spectrum of biological effects. Free radicals are linked to cancer, cardiovascular disease, rheumatoid arthritis, inflammation, cataracts, diabetes, Alzheimer's disease, and ageing. Reactive oxygen or nitrogen species induce many illnesses' pathogenic alterations. Tecoma capensis is used as a diuretic, tonic, anti-syphilitic, vermifuge, and for skin problems. Tecoma capensis methanol and aqueous extracts were tested for antidiabetic and antihyperlipidemic efficacy. Tecoma capensis Methanol extract was yellowish green, slimy, and yielded 27.15 percent w/w. Tecoma capensis aqueous extract was thick, sticky, and yielded 25.66% w/w. The study used the maximum standard dosage. Weight and conduct didn't alter. Normal eyes, skin, and tails were found. Animal was active and healthy till day 14. With extracts, rats had no death or morbidity. As 2000mg/kg of the extract proved safe, the effective dosage ED50 was identified as 250mg/kg, and 500mg/kg was investigated for anti-diabetic activity. STZ raised the rats' blood glucose levels, causing diabetes. This deteriorated until the experiment's 29th day. The glucose level soared. The inciting chemical produced substantial pancreatic injury. Extracts and the conventional medication lowered glucose levels. At 250mg/kg, water extracts raised glucose levels to 193mg/dL, which is not significant compared to normal rats. Same extract at 500mg/kg reduced glucose to 130mg/dL, which is not significant compared to normal rats. At 250 mg/kg, methanol extracts lowered glucose to 120mg/dL. Extract at 500mg/kg lowered glucose to normal levels. The usual synthetic medication produced the same effects. This group lowered blood glucose more than any extract. Extracts showed comparable action.

Key Words: Capensis, Anti-Diabetic, Extracts, Herbal..

INTRODUCTION

Diabetes Mellitus, also known as food-related diabetes, is a metabolic disorder. It indicates high blood sugar levels for an extended period of time. The diabetes epidemic is most visible and affects the world's ten million people. There are two types of DM. Type 1 diabetes and type 2 diabetes are the two types of diabetes. As a result of the loss of beta cells in type 1 diabetes, the pancreas produces less insulin, whereas type 2 diabetes impacts the generation of glucose in the circulation.

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The mechanism of action reveals that the pancreas produces less insulin and forms insulin receptors that are insensitive to control the improper process of blood glucose and reuptake by the muscles. Diabetes is also prevalent among the elderly and fat. Complications include cardiovascular disease, stroke, hyperglycemia, chronic kidney disease, neurological dysfunction, nerve and eye injury, and the development of foot ulcers [1].

Synthetic medications are used to treat diabetes, but they have complications and side effects as a result of their use. The majority of medicinal plants are used in diabetic treatment to produce medication protection and

efficacy in the body. Chemicals found in herbal medications are effective against diabetes mellitus [2].

Tecoma capensis is a medicinally valuable plant, according to research. There were numerous research reports on phytochemical screening of Tecoma capensis flowers and other parts, as well as reports on anti-diabetic, anthelmintic, anti-cancer, anti-oxidant, anti-nociceptive, anti-spasmodic, and anti-spasmodic activity. In light of the foregoing claims and facts, the current study was conducted to determine the chemical constituents of flower extract and to investigate the potential anti-diabetic properties of Tecoma capensis flower extracts [3,4]. Tecoma capensis is a traditional medicinal plant used for medical purposes all over the world. Studies have shown that it is useful as a diuretic, tonic, anti-syphilitic, and vermifuge, as well as for anthelmintics and skin problems. Methanol and aqueous extracts of Tecoma capensis were tested for their anti-diabetic properties.

MATERIALS AND METHODS

Collection of Plant Material

Flowers of Tecoma capensis were collected in February from the Pudiparthi area of Nellore. Botanist M. Johan Paul, Botanist, PRR & VS Government Degree College Vidavalur (V) SPSR Nellore identified and validated plant components (Dt). Tecoma capensis flowers were shade dried at room temperature for one week before being manually pulverised. The finely powdered flowers were kept separate until ready to use in an airtight container. About 50 gms of finely powdered flowers were extracted using Methanol and solvents in a Soxhlet apparatus, while the same quantity of drug powder was macerated for 24 hours under sonication, and the solvents were evaporated and concentrated using a rotary evaporator. These floral extracts were utilised to test for phytochemicals and anti-diabetic effects.

Animal Acclimitization

Wistar Albino Rats were used as the research animals for testing acute toxicity and diabetes screening. They were purchased from a Bengaluru-based vendor, and all of the creatures are roughly 3 months old. Their weights varied between 186 and 220 grammes. They were kept in cages under conventional laboratory settings and given time to acclimate to the environment. The animals were fed a regular pellet meal and were provided free access to fresh water. The rats were appropriately cared for, and the tests were carried out in accordance with the Institutional Animal Ethical Committee's recommendations, which were received prior to the research. They are kept in plastic cages in an air-conditioned chamber, where the humidity is controlled and the day and light cycles are repeated every 12 hours.

Acute Oral Toxicity

The extracts were given the names AETS and METS for aqueous extracts and Methanol extracts, respectively, and were examined for acute toxicity in albino rats of the Wistar strain. According to OECD rules, the animals were given a normal dose of medication of 2000mg per kg body weight of the rat. Prior to the toxicity investigation, three rats were chosen and their body weights were recorded. These were subsequently combined with the extract and given at the above-mentioned dose. After that, the rats were placed in a separate cage for around 2 hours of observation. Animals were spotted behaving abnormally, such as licking their feet and tails, itching, rubbing, and running. Tremors, convulsions, abnormal sleep, coma respiratory issues, and death were among the bodily ailments recorded. The rats were watched for two weeks under the above settings, with the results presented in the next section. Wister rats weighing 160-200gm were employed in the experiment. Because most extracts had LD50 values more than 200 mg/kg p. o., the starting dosage level for AETS and METS was 2000 mg/kg body weight p. o. The dose volume was given ad libitum to overnight starved rats. Following the delivery of AETS and METS, food was withheld for another 3- 4 hours while indicators of toxicity were examined.

Anti-Diabetic Investiagtion

Out of the various methods that are available to test the diabetic activity of any drug, Streptazotocin induction was the promising and most applied method to test the effect of the drug on the pancreas. The experimental section is as follows.

Grouping and allocation of rats

For the evaluation of anti-diabetic activity, rats that had been acclimated to laboratory settings were used. The animals were not fed during the night and were given unlimited access to water. These rats were separated into seven groups, each with six rats. The animals were then given medicines and extracts after being separated into groups. Individual cages were created from the split batches, and treatment and dosage guidelines were followed. Sodium chloride was used to make a standard saline solution. 9g of freshly weighed NaCl was combined with 1L of distilled water and completely dissolved. After that, the solution was filtered to eliminate any remaining undissolved materials. In the studies, this normal saline solution (NSS) was utilised directly.

Batch-I Animals were administered with NSS at a dose of 1.5ml/kg body weight of the rat.

The induction agent STZ was dissolved in normal saline at a dosage of 10 mg/ml, and the rats were administered this solution to induce hyperglycemia. Pioglitazone, the standard medicine, was dissolved in 100mL of saline, resulting in a concentration of 4 mg/mL.

This was immediately applied to the rats. 1 gramme of extract was weighed and dissolved in 10 millilitres of normal saline. The extract was ultrasonicated to ensure proper mixing, and the resulting solution was filtered through a Whatmann filter paper. The solution is transparent, and the extract was appropriately combined, resulting in a concentration of 100 mg/ml, which was consistent with AETS and METS doses of 500 mg/kg for batches IV and VI. For the lower dose 250mg/kg batches, Batches V and VII, 50ml of the aforesaid 100mg/ml solution was produced up to 100ml in a volumetric flask using normal saline. This was then utilised for the extract batches' lower doses.

Except for Batch-I, all of the batches received 60mg/kg body weight of streptazotocin (STZ) via the intraperitoneal route47. After 30 minutes of relaxation, the animals were given the medication. Dextrose solution was given to the rats at a dosage of 1.5ml/kg in a single dose before the extract was given to prevent hypoglycemia shock induced by the immediate action of streptazotocin on the insulin receptors and pancreas.

Batch-II animals were induced with STZ as per above procedure and then the rats were allowed to rest for 30mins. After making sure the rats were not unconscious, they were allowed back in their cages and allowed to have food and water normally.

Batch-III animals were induced with STZ and the same above procedure similar to Batch-II was followed and after 30mins of the administration of the STZ, a standard drug, Pioglitazone was administered at a dose of 2mg/kg via oral route through an injection syringe.

Batch-IV animals were induced with STZ and 30mins after induction they were administered with the solution of AETS at a dose of 250mg/kg via oral route using syringe.

Batch-V animals were induced with STZ and 30mins after induction they were administered with the solution of AETS at a dose of 500mg/kg via oral route using syringe.

Batch-VI Animals were induced with STZ and 30mins after induction they were administered with the solution of METS at a dose of 250mg/kg via oral route using syringe.

Batch-VII animals were induced with STZ and 30mins after induction they were administered with the solution of METS at a dose of 500mg/kg via oral route using syringe.

After that, all of the animals were permitted to rest for the day, and medications were administered in the morning at a certain dose, once a week. The rats were given four dosages on the first day of the week, and the experiment was continued for roughly 28 days. The animals' blood glucose levels were determined using a digital glucometer and Accu sugar check chipped strips, which assess the glucose level using the glucose oxidase technique. On the tip of the rats' tails, a tiny incision was made, and one drop of blood was sampled on the strip. The readings were taken down and written down. The tails were taped and the animals were returned to their cages. Blood samples were taken on the first day after the rats were given STZ, the eighth day, the fifteenth day, the twenty-second day, and the thirty-ninth day. During the trial, the conduct of the rats was observed, and any rat exhibiting anomalous behaviour was eliminated from the research [5,6].

Statistical Significance analysis

The evaluation and comparison of the data was done using the ANOVA with the values in the format of Means and their standard errors for the rats in each group. The unpaired student T-test was applied for comparison between the groups (all groups with normal, all groups with DM induced group). The values that are achieved were considered as significant if the P value is less than 0.01.

Table 1: Grouping and Treatment pattern for testing Anti-diabetic activity of AETS and METS

Sl. No.	Grouping name	Group Label	Treatment
1	Batch-I	Normal control	Normal Saline
2	Batch-II	Negative Control	Normal Saline+STZ
3	Batch-III	Positive Control	Pioglitazone+STZ
4	Batch-IV	Test 1	AETS+STZ
5	Batch-V	Test 2	AETS+STZ
6	Batch-VI	Test 3	METS+STZ
7	Batch-VII	Test 4	METS+STZ

STZ-Streptazotocin; Test1-4-extract testing groups.

Table 2: Effect of AETS and METS on the lowering of blood sugar levels

Group	Blood Glucose Level (mg/dL)				
	1st day	**8th day**	**17th day**	**22nd day**	**29th day**
Normal	97.32±0.442	98.671±0.352	97.915±0.036	95.429±0.323	95.460±0.13
DM induced	97.208±0.033	194.32±1.157	220.09±2.127	237.67±1.354	248.12±0.521
Standard	96.698±0.111	98.702±0.556[a]	97.439±0.623[a]	93.692±0.073[a]	92.026±0.169[a]
AETS-250	98.956±0.136	128.06±0.766[b]	121.95±1.616[a b]	109.68±0.487[a b]	105.80±0.057[a b]

AETS-500	95.985±0.060	120.11±0.778 [b]	103.08±1.230 [a b]	98.732±0.419 [a]	96.032±0.077 [a]
METS-250	95.201±0.257	116.92±0.464 [a b]	106.80±1.581 [a b]	100.80±0.264	97.989±0.171 [a]
METS-500	97.186±0.421	97.55±0.148 [a]	94.062±0.182 [a]	91.287±0.294 [a]	89.057±0.198 [a]

AETS-Aqueous Extract of TS; METS-Methanol Extract of TS; DM-Diabetes Mellitus; Standard-Pioglitazone; a-Significant compared to the induced group where p<0.001; b-Significant compared to the normal group where p<0.001

Physical characteristics of extracts

The Methanol extract of Tecoma capensis.was thick Yellowish Green color, slimy in nature and the percentage yield of the extract were found to be 27.15% w/w. The Aqueous extract of Tecoma capensis.was thick Browish green color, sticky in nature and the percentage yield of the extract were found to be 25.66% w/w.

Acute Toxicity Studies

The maximum standard dosage was used in the acute toxicity investigation. Weight and animal behaviour remained same. The eyes, skin, and tails are all normal, and there are no aberrant indicators. Until the 14th day, the animal was healthy and active. The therapy with extracts had no effect on the rats' mortality or morbidity. Because the extract was proven to be safe at 2000mg/kg, the effective dosage ED50 was determined to be 250mg/kg, and the higher dose 500mg/kg was also investigated for anti-diabetic action. There were no changes in the rats' skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, motor activity, and behaviour pattern before and after administration, and there were no signs of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, or coma. The beginning of toxicity, as well as symptoms of toxicity, were not observed. Further research found no harm or fatality at this concentrations.

Anti-Diabetic Activity

The usage of STZ in rats resulted in the development of diabetes, as evidenced by the rodents' elevated blood glucose levels. This continued to degrade until the experiment's last day, which was the 29th day. The glucose level had risen dramatically. Due to the inducing agent's substantial damage to the pancreas, this was the situation. The normal glucose level in the rats' blood sample varied from 94 to 98 mg/dL. This was increased to 261mg/dL, approximately three times the usual range, indicating a severe induction and an emergency condition in which the body was collapsing.

The extracts and the usual medicine brought the high glucose levels back to normal. When compared to the normal group of rats, the water extracts at a dosage of 250mg/kg revealed a glucose level of 193mg/dL, which is not that significant. When the same extract was given at a greater dose of 500mg/kg, the glucose level dropped to 130mg/dL, which is not noteworthy when compared to normal rats. Methanol extracts, on the other hand, at a dosage of 250 mg/kg, significantly reduced glucose levels to 120 mg/dL. On the other hand, the extract at 500mg/kg resulted in a considerable reduction in glucose to normal levels. The same outcomes were observed in the groups given the conventional synthetic medication. This group outperformed any extract in terms of decreasing blood glucose levels. The extracts, on the other hand, were competent enough to demonstrate comparable action.

CONCLUSION

The present work revealed that the extracts of flowers of *Tecoma capensis* by phytochemical screening of ethanol extracts contains flavonoids and phenols and steroids & triterpenoids and it may be possess significant antioxidant activity. Methanol and Aqueous extracts exhibit Antidiabetic activity at the dose of 500mg/kg when compared to standard which may be due to the presence of high phenolic content and high flavonoid content and other constituents. Present study also indicates that the possible antioxidant mechanism of the extract can be due to hydrogen or electron donating and direct free radical scavenging activity of the extracts, but exact antidiabetic mechanisms should be further studied.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

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