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Phytochemical investigation and evaluation of Antidiabetic and antioxidant potency of *Adansonii Monstera*

M Meenakumari [1], Divya Jella [2*]
[1-2] Department of Pharmacology, SSJ College of Pharmacy, Vattinagula Pally, Gandipet Main Road, Hyderabad, Telangana, India

* Corresponding Author: **Divya Jella**

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Abstract
Diabetes mellitus is currently considered a common lifestyle disease. The study aims to investigate phytochemicals and evaluate the antidiabetic and anti-oxidative potency of *Adansonii Monstera*. The *Adanson's monstera* flowers used in the study were collected from the herbal garden of our institution and authenticated by Sri Venkateshwara University. This study demonstrates that the ethanolic extract of *Adanson's monstera's* has promising antioxidant and antidiabetic activity to use for human health. The extract also has a very prominent antidiabetic effect to be used in many pharmacological as well as biological actions. Diabetic dyslipidemia is characterized by high TC, TG, low HDL, and high LDL. Consequently, the pharmacological and toxicity studies also proved the anti-diabetic potential of *Adanson's monstera's* could be a promising source of therapeutic demands.

Keywords: Diabetes Mellitus, antidiabetic activity, antioxidative property, *Adanson's Monstera*, Diabetic Dyslipidemia

Introduction

Diabetes Mellitus

Diabetes mellitus is a group of diseases characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both, altered metabolism of lipids, carbohydrates, and proteins, and an increased risk of complications of vascular diseases [1]. It is the most common endocrine disorder and can be associated with serious complications and premature death.

Classification of Diabetes mellitus

Diabetes mellitus is classified into two distinct forms

a. Type 1 or juvenile or insulin-dependent diabetes mellitus (IDDM).
b. Type 2, adult onset, or non-insulin-dependent diabetes mellitus (NIDDM).

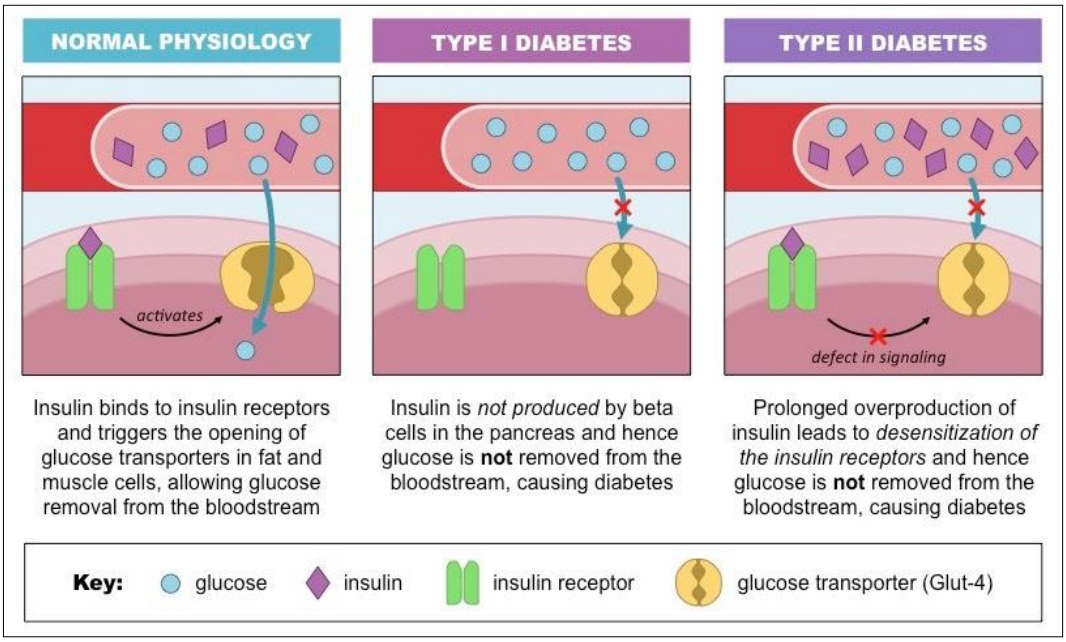

Fig 1: Types of diabetic

Materials and Methods

Collection of Plant Materials

The *Adanson's monstera* flowers used in the study were collected from the herbal garden of our institution.

Authentication of plant materials

The collected plant materials were identified and authenticated based on macroscopic and microscopic characters at the Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh. The voucher specimens have been deposited at the Museum of the Department of Pharmacology of our institute for further reference.

Successive solvent extraction [2]

The collected flowers were washed properly and allowed to shade dry. After drying powder was made by grinder and used for extraction.

Petroleum Ether Extract: The coarsely powdered, dried plant parts (80 g) were extracted with petroleum ether by hot extraction process (soxhlet) for 4 hours. After completion of extraction, the solvent was removed by distillation and concentrated in a vacuum.

Chloroform Extract: The marc left after benzene extraction was dried and extracted with chloroform by hot extraction process (soxhlet) for 4 hours. After completion of extraction, the solvent was removed by distillation and concentrated in a vacuum.

Ethanol Extract: The marc left after the acetone extraction was dried and extracted with 95% ethanol by hot extraction process (soxhlet) for 4 hours. After completion of the extraction, the solvent was removed by distillation and concentrated in a vacuum.

Aqueous Extract: The marc left after the ethanolic extraction was dried and extracted with chloroform water by maceration process for one week. After the completion of the extraction, the solvent was removed by distillation and concentrated in vacuo. The above extracts were used for phytochemical studies. The extractive values for each extract were calculated and recorded.

Extraction Method

The experiments were carried out using air-dried plant materials which were reduced to moderately coarse powder using a mechanical grinder. The powder was passed through sieve no. # 40 and stored in air-tight containers for further use. The ethanolic and aqueous extracts of the dried powder drug were prepared as follows:

(a) Preparation of ethanolic extract

The air-dried coarse powder of the roots was well packed in a soxhlet apparatus and subjected to continuous hot extraction with 70% ethanol. The extract was filtered while hot and the filtrate was distilled under reduced pressure to remove solvent completely. The residue was dried and stored in the desiccator, used for subsequent experiments.

Preliminary phytochemical studies [3]

The aqueous and ethanolic extracts of the powdered drugs were subjected to various qualitative tests for the identification of plant constituents like Alkaloids, carbohydrates, flavonoids, saponins, Phenolic compounds, and Tannins**.**

Pharmacological Study

Selection of Animals

Healthy male adult Wistar albino rats (150-300 gm body weight) were selected for study. The animals aged between 2 - 3 months. They were housed in polypropylene cages, maintained under standard conditions (12 h light; 12 h dark cycle; 25±30 0C). They were fed with a standard rat pellet diet (Pranav Agro Ltd., Vadodra, India and water *ad libitum*. Animal ethical norms were strictly followed during all experimental procedures.

Acute Toxicity Study [4]

The acute toxicity study is used to establish the therapeutic index, i.e. the ratio between the pharmacologically effective dose and lethal dose on the same strain and species (LD50/ED50). The greater the index; the safer the compound and vice versa. The acute toxicity study was done according

to OECD (Organization of Economic Co-operation and Development) guidelines 425- Fixed Dose Procedure (FDP), as in annex 2D.

Procedure

The animals were divided into two groups and each group consisted of five mice. The defined or fixed dose level of aqueous and ethanolic extracts (2000 mg/kg) were given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioural, neurological and autonomic profiles. The toxicity signs were observed after 24 hours till fourteen days for any lethality or death.

Antidiabetic Study

The antidiabetic potential of aqueous and ethanolic extracts of the plant parts was evaluated by different parameters including antihyperglycemic study and biochemical analysis.

Induction of Type II Diabetes [5]

Model- STZ-induced Diabetic Model: A single dosage of 65 mg/kg streptozotocin produced in citrate buffer (pH 4.4, 0.1 M) will be given intraperitoneally to induce diabetes. The age-matched control rats received an identical volume of citrate buffer. Diabetes was proven after 48 h of streptozotocin injection, the blood samples are taken by tail vein and plasma glucose levels will be assessed by enzymatic GOD-PAP (glucose oxidase peroxidase) diagnostic kit method. The rats having fasting plasma glucose levels of more than 250 mg/dL were selected and employed for the present study. Bodyweight and plasma glucose levels were measured before and at the end of the experiment to see the influence of S-Equol on these parameters.

The diabetic rats after confirmation of stable hyperglycemia were divided into different groups of 6 rats each. That day was considered as the 0th day. Drug and doses were administered as mentioned. The animals taken for the study are 30 animals divided into 5 groups.

Group I: Normal control rats administered saline (0.9% w/v);

Group II: Diabetic control rats administered saline (0.9% w/v);

Group III: Diabetic rats administered standard drug Glibenclamide (2.5 mg / kg) daily

Group IV: Diabetic rats administered ethanolic extract (250 mg/kg);

Group V: Diabetic rats administered ethanolic extract (500 mg/kg);

Blood samples were withdrawn from overnight fasted animals on day 5, 10 and day 15 following three hours after vehicle/extract/glibenclamide administration. Blood was withdrawn from the retro orbital plexus using heparinised haematocrits. The fasting blood sugar levels were determined by using glucose oxdiase peroxidase reactive strips.

Homogenate preparation and Biochemical profiling

The animals were sacrificed with excess pentobarbitone sodium, removing the liver from the animal and keeping it in the phosphate buffer of 0.2 M, pH 6.6. The liver was shattered and the homogeneous result was centrifuged and centrifuged for 10 min at 3000 rpm. The supernatant acquired was used for enzymatic and non-enzymatic testing. The determination of the decreased glutathione (GSH) non-enzymatic antioxidant estimate was evaluated. using the technique outlined. Determination of enzymatic anti-oxidant studies such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSSH), peroxides (Px) effect, glutathione peroxidase (GPx) effect was explored using standard conventional procedures. Estimation of lipid hydroperoxides and evaluation of malondialdehyde (MDA) lipid peroxidation evaluation as demonstrated by the creation of thiobarbituric acid explosive materials (TBARS) and hydroperoxides (HP) was assessed using methods.

Analysis of serum Lipid Profile and Total Protein

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein, cholesterol (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein cholesterol (VLDL) blood levels were evaluated according to conventional techniques. Total protein in the homogeneous tissue was measured by using bovine serum albumin as a standard. Similarly, blood urea assessment and serum creatinine analysis were done by standard technique and were reported.

***In vitro* antioxidant activity**

DPPH free radical scavenging activity Stock solution of leaf and stem extracts were prepared to the concentration of 1mg/ml. Different concentrations (50, 100 and 150 µl) of each extracts were added, at an equal volume, to ethanolic solution of DPPH (0.1%). The reaction mixture was incubated for 30min at room temperature; the absorbance was recorded at 517 nm. BHT was used as standard. The annihilation activity of free radicals was calculated in % inhibition according to the following formula.

Histopathology

At the end of the study, all the animals were sacrificed under light ether anesthesia, by Decapitation. The relevant organs liver and kidneys were dissected out and collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5 µ thickness were cut and stained haematoxylin and eosin (H&E) for histopathological examination.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's't'- test. P values <0.05 were considered significant.

Results and Discussion

The yield of the extracts is shown in Table 1 after the Adanson's monstera was pulverized and extracted with petroleum ether, Aqueous Extract, chloroform, and ethanol. In comparison to petroleum ether and chloroform, ethanol extracts had the greatest yield (3.84%, 3.52%, 3.63%, and 3.4%, respectively).

Table 1: Percentage yield of the extract of *Adanson's monster*

S. No	Solvents	% yield of extracts
1	Ethanol	3.84
2	Chloroform	3.63
3	Petroleum ether	3.4
4	Aqueous Extract	3.52

For *Adanson's monstera* extracts in ethanol, chloroform, and petroleum ether, the qualitative evaluation of phytoconstituents was done to check for the existence of medicinally active metabolites. Alkaloids, flavonoids,

carbohydrates, phenols, glycosides, tannins, amino acids, and protein were all present in the ethanol extract of both plants, but saponins, phlorotannins, thiols, anthraquinons, and resins were not (Tables 2 and 3).

In the chloroform extract of Adanson's monstera, phenols, thiols, polysaccharides, flavonoids, and saponins were all found. In the petroleum ether extract, saponins, carbohydrates, thiols, amino acids, and protein could be found.

Seven phytochemicals that were examined for were discovered in different solvent extracts. They include amino acids, proteins, alkaloids, flavonoids, carbohydrates, phenols, glycosides, tannins. The ethanol extracts from both plants had a higher concentration of phytochemicals.

Table 2: Phytochemical analysis of various extracts of Adanson's monster

S. No	Phytoconstituents	Ethanol	Chloroform	Petroleum ether	Aqueous Extract
1	Alkaloids	+	-	-	-
4	Carbohydrates	+	+	+	+
2	Flavonoids	+	+	-	+
3	Saponins	-	+	+	+
5	Phenols	+	+	-	-
6	Glycoside	+	-	-	-
7	Tannins	+	-	-	-

+ (Positive) = Presence of the compound;
- (Negative) =Absence of the compound

Pharmacological studies

Acute Toxicity study

For the acute toxicity study, the ethanolic extract of Adanson's monstera was administered orally and animals were observed for any change in body weight, gross pathology and behavior pattern. The body weight of the rats before and after administrations were noted and was found that there was a slight increase in the body weight. An acute toxicity study of a test drug in BALB/c mice indicated that the dose of 2.0 g/kg b.w do not produce significant dose-related changes of the histopathology of internal organs. The acute toxicity study in mice showed that at 2000 mg/kg dose, the plant is safer for consumption and for medicinal uses.

Table 3: Acute toxicity of ethanol extracts of Adanson's monstera

Mice Number	Sex	Initial Bwt (gm)	Dose gm/kg	7 Day Bwt (gm)	14 Day Bwt (gm)	Fate
1	F	24	2.0	23	25	Survived
2	F	23	2.0	30	37	Survived
3	F	22	2.0	26	30	Survived
4	F	25	2.0	27	31	Survived
5	F	24	2.0	26	31	Survived
6	F	23	2.0	26	33	Survived

Values are in mean ± SEM (n=5), *P<0.05, **P<0.01, ***P<0.001 Vs Diabetic Control

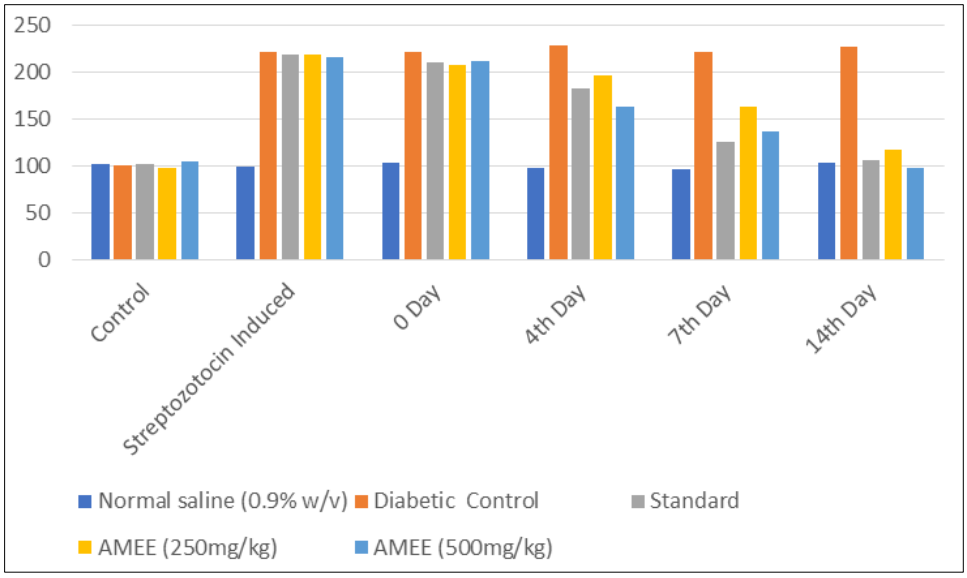

Fig 1: Effect of AMEE on Blood glucose Levels

Effect of AMEE on lipid levels of Streptozotocin induced diabetic in rats

The effect of ethanolic extracts of Adanson's monstera flower on serum sample were studied to lipid analysis and biochemical parameters like Total cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) were analyzed and the results are summarized and biochemical parameters analysis is tabulated in Figure 2.

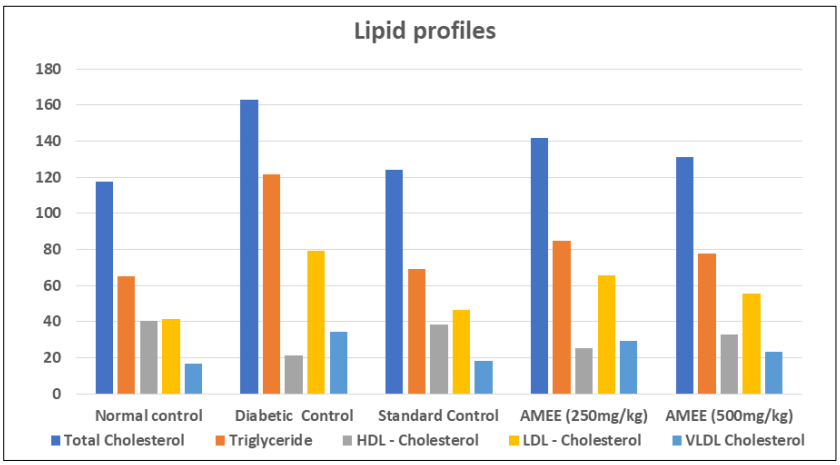

Fig 2: Effect of AMEE on Lipid Levels

3.3.4. Effect of AMEE on kidney functions of streptozotocin-induced diabetic in rats

The effects of ethanolic extracts of Adanson's monstera flower on serum blood urea nitrogen (BUN) and creatinine in Streptozotocin-induced diabetes in rats, and the results were tabulated in Figure 3.

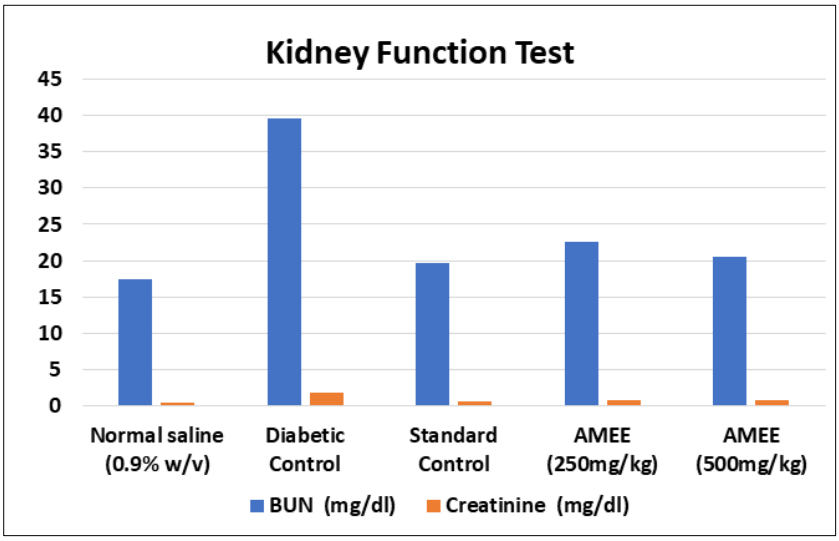

Fig 3: Effect of AMEE on Kidney functions

3.3.5. Effect of AMEE on liver MDA and LH

The effects of ethanolic extracts of *Adanson's monstera* flower on MDA and LH in Streptozotocin induced diabetes in rats, and the results were tabulated in Figure 4.

Values are mean ± SEM; n=6 in each group; [a]P <0.01 when compared to normal control; [b]P<0.01, [c]P<0.05, when compared to diabetic control (one-way ANOVA followed by Dunnett's 't' test).

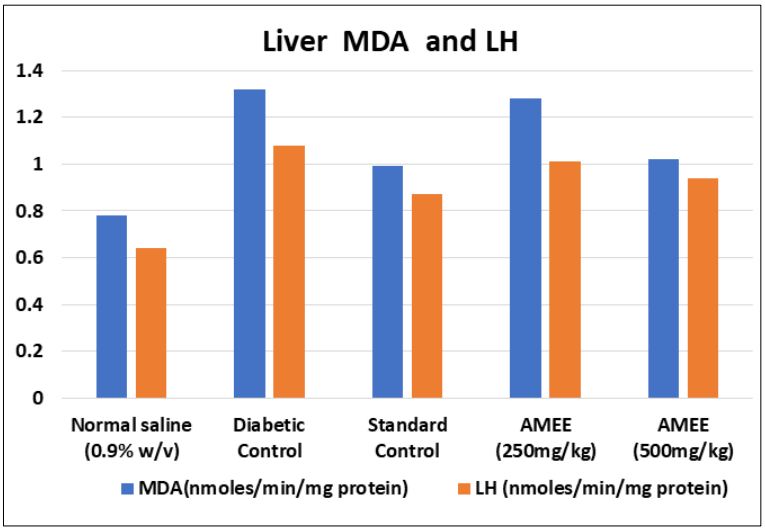

Fig 4: Effect of AMEE on liver MDA and LH

3.3.6. Effect of AMEE on liver enzymatic antioxidants

The effects of ethanolic extracts of Adanson's monstera flower on liver enzymatic antioxidants in Streptozotocin induced diabetes in rats, and the results were tabulated in Figure 5.

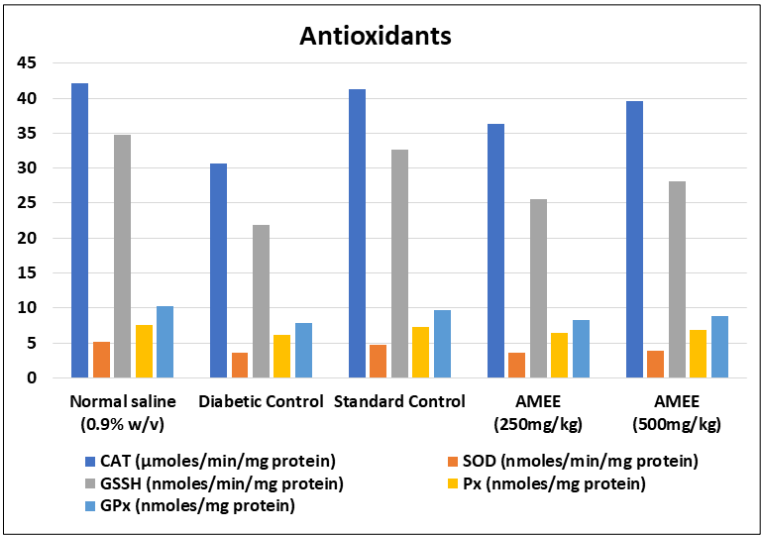

Fig 5: Effect of AMEE on liver enzymatic antioxidants

DPPH scavenging activity of ethanolic extract of *Adanson's monstera*

Antioxidants protect against oxidative damage brought on by free radicals and may be utilised to treat inflammatory and cardiovascular conditions. It is generally known that plants act as natural antioxidants. The goal of the current research was to determine the plant *Adanson's monstera's* antioxidant potential.

The stronger inhibitory effects on alpha amylase are shown by the ethanol extract of *Adanson's monstera's in vitro* _ amylase inhibition activity. As a result, *Adanson's monstera* ethanol extract was chosen for its *in vitro* antioxidant activities.

In vitro antioxidant activity of ethanolic extract with DPPH free radical scavenging activity, ferric reducing antioxidant power test, superoxide anion scavenging activity, and nitric oxide scavenging activity was carried out as part of my research study on *Adanson's monstera*.

Table 4: Histological investigation of Liver, Kidney and Pancreas

Extract/standard	Concentration of extracts (µg/ml)	% of inhibition
Ethanol extract of Adanson's monstera	50	86.84±0.007
	100	93.59±0.014
	150	97.10±0.007

3.4.1. Histological investigation of Liver

The histopathological study of the liver of Streptozotocin induced Wistar albino rats with the ethanolic extracts of Adanson's monstera flower was revealed in Figure 7 to 9.

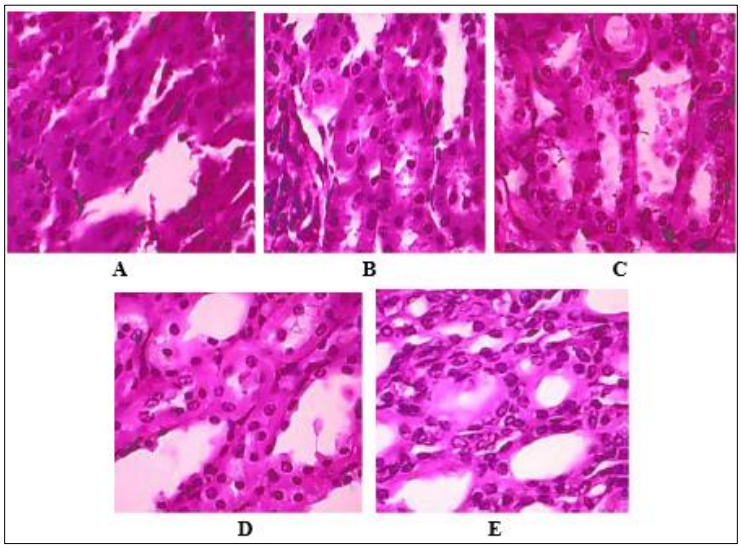

Fig 7: *In-vivo* study of Liver. A: Control (0.1% CMC), B: Diabetic Control, C: Standard Control (Glibenclamide), D: AMEE (250 mg/kg), E: AMEE (500 mg/kg)

3.4.2. Histological investigation of Kidney

The histopathological study of the kidney of streptozotocin-induced Wistar albino rats with different ethanolic extracts of Adanson's monstera flower was revealed in Figure 8.

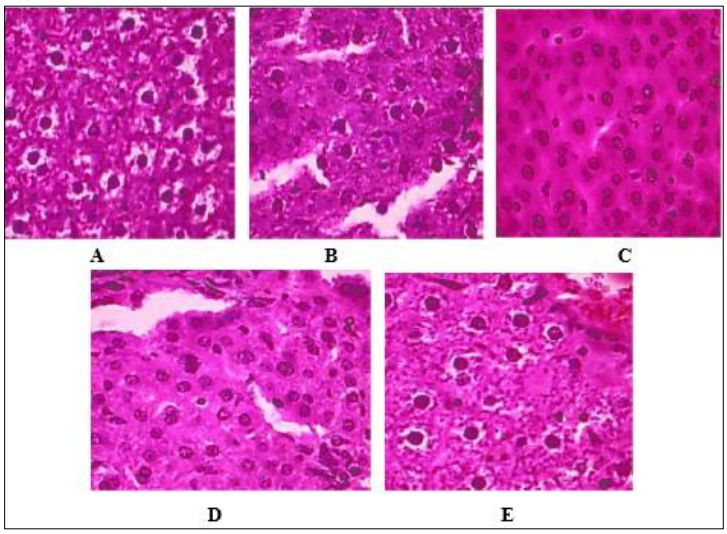

Fig 8: *In-vivo* study of kidney. A: Control (0.1% CMC), B: Diabetic Control, C: Standard Control (Glibenclamide), D: AMEE (250mg/kg), E: AMEE (500mg/kg)

3.4.3. Histological investigation of Pancreas

The histopathological study of Pancreas of streptozotocin induced Wistar albino rats with different ethanolic extracts of Adanson's monstera flower was revealed in Figure 9.

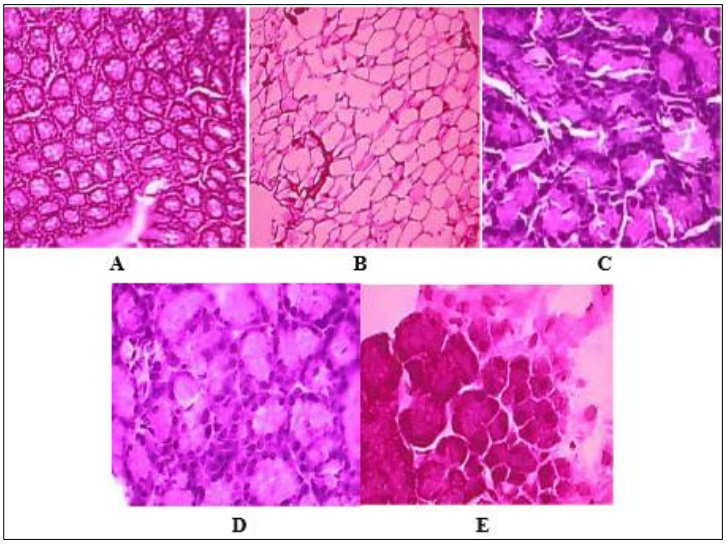

Fig 9: *In-vivo* study of Pancreas. A: Control (0.1% CMC), B: Diabetic Control, C: Standard Control (Glibenclamide), D: AMEE (250mg/kg), E: AMEE (500mg/kg)

Conclusion

In conclusion, this study demonstrates that the ethanolic extract of *Adanson's monstera's* has promising antioxidant and antidiabetic activity to use for human health. The extract also has very prominent antidiabetic effect to be used in many pharmacological as well as biological actions. Diabetic dyslipidemia is characterized by high TC, TG, low HDL, and high LDL. Consequently, the pharmacological and toxicity studies also proved the anti-diabetic potential of *Adanson's monstera's* could be a promising source of therapeutic demands.

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