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Phytochemical Evaluation and Pharmacological Screening of Ethanolic Leaf Extracts of Erythroxylum Monogynum and Pupalia Lappacea for Hepatoprotective, Nephroprotective, Antihyperlipidemic and Antihyperglycemic Activity in Alloxan-Induced Diabetic Albino Wistar Rats.

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Abstract: *Type 1diabetes is associated with damage to the liver, kidney and pancreas of patients. The damage varies in proportion and susceptibility among diabetic patients of type 1 class. This study assessed the hypoglycaemic, hepatoprotective, antihyperlipidemic and nephroprotective activities of whole methanolic leaf extract of erythroxylum monogynum and pupalia lappacea in alloxan-induced diabetic albino Wistar rats. Extraction of the ethanol extract of erythroxylum monogynum and pupalia lappacea was performed by maceration. Thirty six rats were divided into six groups. Group I consists of normal rats that were given only normal saline solution and served as a control group. Group II consists of normal rats that were given alloxan monohydrate (150mg/kg B.W). Group III consists of alloxan induced diabetic rats that were given daily sterile solution, drug extract A (200 mg/kg), Group IV consists of alloxan induced diabetic rats that were given daily sterile solution, drug extract B (200 mg/kg), Group V consists of alloxan induced diabetic rats that were given daily sterile solution, drug extract A (100 mg/kg) and drug extract B (100 mg/kg), Group VI consists of alloxan induced diabetic rats that were given daily sterile solution and glibenclamide(5mg/kg) respectively for 21 days by an instragastric tube with free access of food and water. Several biochemical parameters were assessed and histological studies were done. Oral administration of the extract resulted in significant reduction in mean values of blood glucose, cholesterol, triglycerides, LDL-C, VLDL, urea, uric acid, creatinine accompanied by an increase in the mean values of the total protein, albumin, HDL in diabetic rats. The effects produced by this extract were closely similar to a standard anti diabetic drug, glibenclamide. In conclusion, the present study indicates that the ethanolic extract of erythroxylum monogynum and pupalia lappacea to exhibit nephroprotective, hepatoprotective, antihyperlipidemic and antihyperglycemic activities in alloxan induced diabetic rats.*

Keywords : *Ethanol, Erythroxylum Monogynum, Pupalia Lappacea, Alloxan monohydrate 150 mg/kg b.w, Glibenclamide 5mg/kg b.w.*

I. Introduction

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period[1]. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers and damage.

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced[2].

As at 2013, 382 million people have diabetes worldwide. Type 2 makes up about 90% of the cases. This is equal to 8.3% of the adult population with equal rates in both women and men.

In 2014, the International Diabetes Federation (IDF) estimated that diabetes resulted in 4.9 million deaths. The World Health Organization (WHO) estimated that diabetes resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death. The discrepancy between the two estimates is due to the fact that cardiovascular diseases are often the cause of death for individuals with diabetes; the IDF uses modelling to estimate the amount of deaths that could be attributed to diabetes. More than 80% of diabetic deaths occur in low and middle-income countries[3].

Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in more developed countries. The greatest increase in rates was expected to occur in Asia and Africa, where most people with diabetes will probably live in 2030. The increase in rates in developing countries follows the trend of

urbanization and lifestyle changes, including a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present[4].

On the other hand, the kidneys are the vital organs that function to keep the blood clean and maintain chemical balance within[5]. They process blood to extract water. These by products become urine to be ultimately excreted from the body. The kidney serves many other important functions, including filtering out wastes to be excreted in the urine, regulating blood pressure via both urinary excretion of wastes and initiating the rennin angiotensen hormone regulatory system, regulating an acid-base balance via the bicarbonate system and stimulating red blood cell production via the release of the hormone erythropoietin.

The pancreas contains the cells that produce juices to break down fats and proteins and a hormone known as insulin to balance blood sugar content in the human body.

Any abnormality to these organs may lead to organ dysfunction and threat to human life. Diabetes is one of such causes of damage to these organs leading to organ dysfunction and endocrine related diseases which may be life threatening.

Several therapies are available for the treatment of diabetes-induced hepatotoxicity, oxidative stress and nephrotoxicity. These therapies include insulin and various oral antidiabetic agents such as sulfonylurea; biguanides, and α-glucosidase inhibitors, which are used as monotherapies or in combination to achieve blood sugar regulation. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge[6].

Several investigations have shown that herbs and plant materials rich in secondary compounds such as saponins, flavonoids, phenolic and polyphenolic compounds, arginine and glutamic acid possess hypoglycaemic, hepatoprotective and nephroprotective effects in animal models[7]. In the absence of reliable liver and kidney protective drugs in medical practice, herbs have become reliable substitutes and have so far played significant role in the ethanopharmacological management of various liver disorders and the accompanying oxidative stress[8].

Plants have long been known for their uses as food and medicine to humans. In some African cultures, vegetables like heinsiacrintia (atama in efik), vernoniaamygdalina (bitter leaf), ocimumgratissimum (ntong in efik), gongronemalatifolium (utazi in igbo) among others, are used in the treatment of many diseases including types 1 and 2 diabetic conditions.

The management of liver disease is still a challenge to modern medicine. The only drugs available for the treatment of liver disorders are corticosteroids and immunosuppressive agents. However, these suffer with several adverse effects. This has led to increased dependence on complementary and alternative medicine, especially herbal therapy. Plant drugs are known to play a vital role in the management of liver diseases. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but for minimal side effects and easy availability in nature

In this study, erythroxylum monogynum and pupalia lappacea were evaluated for its use in the management of diabetes.

Erythroxylum monogynum Roxb. (Erythroxylaceae) (E. monogynum) is a well known plant in traditional medicine which is found widely in southern parts of India[9]. Several parts of the plant are used in Indian traditional medicine for various therapeutic effects. In the southern parts of Andhra Pradesh, extract of the leaves mixed with yoghurt is administered to kill intestinal worms, and the leaf juice is used for the treatment of jaundice[10]. The infusion of bark and wood is used as stomachic, diaphoretic, stimulant, diuretic and also in mild cases of dyspepsia and continued fever. E. monogynum has been reported for its antibacterial activity[11][12].

Pupalia lappacea (L) Juss. (Amaranthaceae) is an erect or straggling under shrub found in the hedges of fields and waste places from Kashmir to Kanyakumari and commonly known as forest Burr or creeping cock's comb[13]. In folklore medicine, the leaf paste of Pupalia lappaceae with edible oil is used to treat bone fractures and inflammatory conditions. The fruit juice is applied locally for cuts, mixed with palm oil to treat boils and the fruit soup is used for cough and fever. In Africa, fruit is used as an ingredient in enema preparation; mixed with palm oil, it is applied as a dressing for boils and also applied to leprosy sores after making them bleed. Burnt plant is mixed with water to treat flatulence. Traditionally it is also used to treat jaundice, abdominal colics, cephalgias, diarrheas, paralysis, erectile dysfunction, vomiting and malaria. Chemical investigations of P. lappacea revealed that foliage of this plant consists of 8 compounds, namely 1-docosanol, stearic acid, stigmasterol, sitosterol, N-benzoyl-L-Phenyl alaninol acetate, setosterol-3-O-D-glucopyranoside, stigmasterol-3-O-D-glucopyranoside and 20- hydroxyl ecdysone. The seeds are reported to consist of glycosides, saponins, steroids and alkaloids. Aladedunye et al. reported the antioxidant activity of hexane and dichloromethane extract of P. lappacea foliage[14].

Literature review reports that no scientific validation has been done on the leaves of E. monogynum and pupalia lappaceae as a hepatoprotective agent, nephroprotective, and antihyperglycemic as well as antihyperlipidemic activities.

So the present work aims at evaluating these activities of leaves of E. monogynum and pupalia lappacea against alloxan- induced toxicity in the Wistar albino rats as study model.

II. Materials And Methods

2.1 Collection Of Drug:

Dried leaves of the plants Erythroxylum Monogynum, family Erythroxylaceae and Pupalia Lappaceae, family Amaranthaceae were collected. The plant was taxonomically identified and authenticated by DR.K. MADHAVA CHETTY Assistant professor of Botony, Department of Pharmacognosy, Sri Venkateshwara University, Tirupathi.

2.2 Chemicals:

The following chemicals were used during the experiment; to analyze and interpret the hepatoprotective, nephroprotective and anti hyperglycaemic and anti hyperlipidaemic activity of ethanol extracts of erythroxylum monogynum and pupalia lappaceae in alloxan induced diabetic rats.

Ethanol
Alloxan monohydrate 150 mg/kg b.w
Glibenclamide 5 mg/kg b.w

2.3 Preperation of plant extract:

The leaves of both plants were subjected to shade drying. On complete drying the leaves are powdered and stored in air tight containers at room temperatures. The powders of both the plant leaves was macerated with ethanol for 7 days and then filtered. The filtrate was evaporated to obtain dried extracts. The extracts thus obtained were subjected for evaluation of hepatoprotective, nephroprotective and antihyperlipidemic study. The plant extract was prepared by maceration process.

2.4 Maceration: In maceration (for fluid extract), whole or coarsely powdered plant drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs. Using this process, 250gm powder was added in ethanol in the ratio 1:2 and vigorous shaking was carried out for 7 days continuously and was kept at room temperature. The filtrate thus obtained was ethanolic extract. The filtrate obtained was dissolved in 0.9% normal saline and used as vehicle in the experiment.

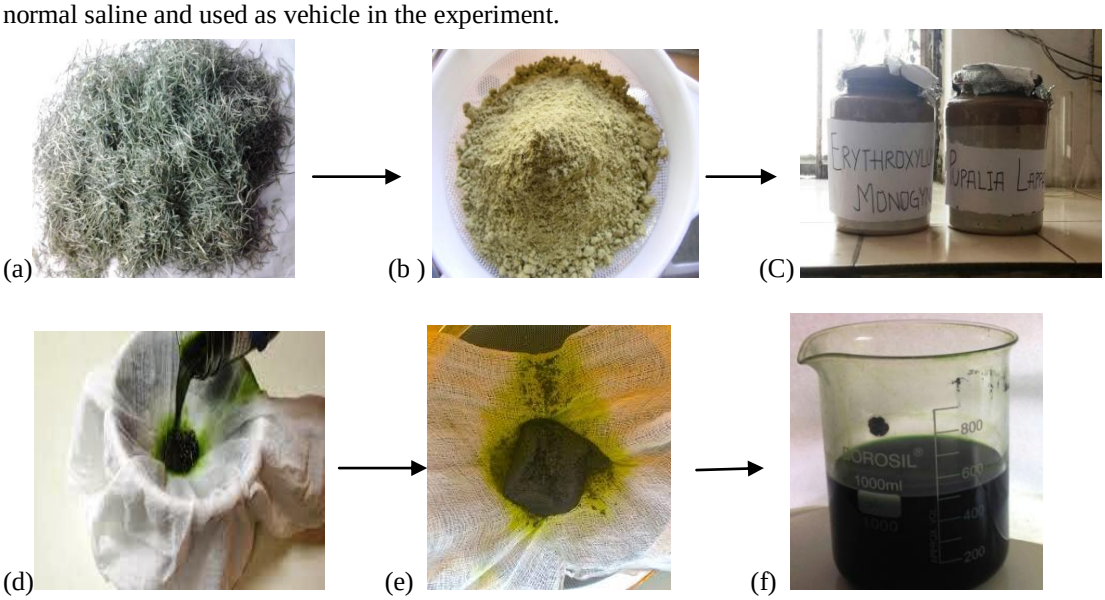


Figure No.1 : Maceration
(a) dried plant (b) coarsely powdered plant drug (c) drug is kept in contact with the solvent (d) filter the extract (e) filtering using muslin cloth (f) filtrate obtained was ethanolic extract.

III. Preliminary Phytochemical Screening:

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, glycosides and anthraquinones using standard procedures.

IV. Acute Toxicity Studies:

4.1 Acute toxicity test for erythroxylum monogynum:

The acute oral toxicity was carried out according to OECD-425 guidelines. Five male Wistar albino rats were selected and administered a dose of 3 g/kg. The respective dose was well tolerated by all the animals without showing any signs of toxicity and mortality. So we assumed that LD_{50} was beyond 3 g/kg. Three different graded doses of 100 mg/kg, 200 mg/kg and 400 mg/kg were selected to determine the activity.

4.2 Acute toxicity test for pupalia lappacea:

To determine the acute toxicity, a single oral administration of the ethanolic extract of P. lappacea in different doses (500, 1000 and 2000 mg/kg) were administered to different groups of mice (Ghosh, 1984). Control group received the vehicle (CMC). The animals were observed continuously for 72 h following drug administration for death and abnormality in behavioural changes.

Result:

In toxicity studies, ethanolic extracts of P. lappacea did not exhibit any mortality and abnormal behavioural changes up to the dose level of 2000 mg/kg b.w. in mice. Further the pharmacological studies were carried out at an oral dose of 200, 400 and 600 mg/kg.

V. Experimental Animals:

Wistar rats (180-250 Gms) of either sex housed in standard conditions of temperature (55±5%) or light (12 hrs light/dark cycles) were used. They were fed with standard pellet diet and water ad libitum. Animals were randomly selected for grouping. All experiments were performed according to the norms of the ethical committee (CPCSEA).

Figure no. 2: Experimental albino Wistar rats

5.1Experimental Design:

Evaluation Of Ethanolic Extract Of Erythroxylum Monogynum And Pupalia Lappacea For hepatoprotective, Nephroprotective, Antihyperlipidemic And Antihyperglycemic Activity In Alloxan- Induced Diabetic Albino Wistar rats.

GROUPS	EXPERIMENTAL DESIGN
Group I	Normal control rats received normal saline as a vehicle p.o
Group II	Diabetic control rats received alloxan i.p +normal saline p.o
Group III	Alloxan induced diabetic rats + ethanolic extract of leaf extract of erythroxylum monogynum 200mg/kg b.w in normal saline p.o
Group IV	Alloxan induced diabetic rats + ethanolic extract of leaf extract of pupalia lappacea 200mg/kg b.w in normal saline p.o
Group V	Alloxan induced diabetic rats + ethanolic extract of leaf extract of erythroxylum monogynum 100mg/kg b.w in normal saline p.o + ethanolic extract of leaf extract of pupalia lappacea 100mg/kg b.w in normal saline p.o
Group VI	Alloxan induced diabetic rats +glibenclamide 5mg/kg b.w in normal saline p.o

Table No.7 : Experimental design

VI. Preperation Of Drug Solution

Ethanolic extract of the leaves of erythroxylum monogynum and pupalia lappacea dissolved in normal saline to prepare dose level of 100, 200, 400 mg/kg body weight for administration into rats.

VII. Induction Of Diabetes Mellitus:

Figure no.3: Induction of alloxan (150mg/kg b.w) i.p.

Diabetes was induced in rats single intraperitoneal injection of alloxan (150mg/kg b.w) dissolved in freshly prepared NaCl saline, pH 5.5. Alloxan induces a triphasic blood glucose response when injected into an experimental animal. The first phase that comes into view within the first minutes after alloxan injection in transient hypoglycaemic phase that lasts maximally for 30 minutes. The 2nd phase appearing one hour after administration of alloxan leads to rise in blood glucose concentration. Moreover, the plasma insulin concentration has been noted to decrease at the same time. This is the first hyperglycaemic phase lasts for 2-4 hours which is accompanied by decreased plasma insulin concentrations. The 3rd phase is again a hypoglycaemic phase that is noted 4-8 hours after the alloxan injection, which lasts for several hours. It takes about 48 hours and after 48 hours of diabetes induction the treatment is given. The rats with effective and permanent elevated blood glucose levels (>/=220mg/dl) were selected and used for the study.

Grouping Treatment:
Groups II, III, IV, V and VI will be induced diabetes mellitus by i.p route using alloxan monohydrate (150mg/kg B.W). The diabetic state of animals is then checked and is conformed. Animals become diabetic if their blood glucose level is 217 ±17 mg/dl.

VIII. Biochemical Estimations

8.1 Collection Of Blood For Determination Of Serum Glucose:
Blood samples were collected by cutting the tail vein of rats and blood glucose levels are checked by glucometer.

Figure no.4 : Retro-orbital puncture.

The normal and diabetic control group rats were given 1 ml normal saline, p.o. Animals in the third, fourth and fifth groups were treated with ethanolic extract of the leaves of erythroxylum monogynum (200mg/kg), pupalia lappacea (200mg/kg) and combination of both (100mg/kg+a00mg/kg) the plants. Sixth group were treated with Glibenclamide at a dose of 5mg/kg b.w. for 21 days. Body weight and blood glucose were checked for every three day intervals during the duration of the experiment. The blood glucose levels were determined by tail tipping method using one touch select glucose monitor. On twenty first day blood from all the

groups were collected by retro-orbital puncture under mild anaesthesia, serum was separated quickly for estimating for estimating the following parameters to assess the hepatoprotective, nephroprotective and anti-hyperglycemic activity.

- Blood Glucose Levels,
- Total Cholesterol Levels (TC),
- Low Density Lipoprotein Cholesterol (LDLc) Levels,
- High Density Lipoprotein Cholesterol (HDLc) Levels,
- Very Low Density Lipoprotein Cholesterol (VLDLc) Levels,
- Triglyceride Levels,
- Cholesterol
- Total protein
- Albumin
- Urea, Uric acid
- Creatinine

One Touch Select Glucometer:

A **glucose meter** (or **glucometer**) is a medical device for determining the approximate concentration of glucose in the blood. It can also be a strip of glucose paper dipped into a substance and measured to the glucose chart. It is a key element of home blood glucose monitoring (HBGM) by people with diabetes mellitus or hypoglycaemia. A small drop of blood, obtained by pricking the skin with a lancet, is placed on a disposable test strip that the meter reads and uses to calculate the blood glucose level. The meter then displays the level in units of mg/dl or mmol/l.

Advantages Of Glucometer:

- Simple and easy method
- Accurate readings in very less time
- Large volume of blood is not required
- Easy to operate

Disadvantages Of Glucometer:

- Mechanical errors are possible
- Cost of glucometer and strips
- Glucose values can be obtained only in a specific range of 10-600mg/dl.

8.2 Estimation Of Triglycerides:

Enzymatic (GPO/Tinder), End point Calorimetry, Single Reagent Chemistry with Lipid Clearing Factor (LCP).

Principle:

The triglycerides in the serum are hydrolysed enzymatically by the action of lipase to glycerol and fatty acids. The glycerol formed is converted to glycerol phosphate by glycerol kinase (GK). Glycerol phosphate is then oxidised to dihydroxyacetone phosphate by glycerol phosphate oxidase (GPO). The librated hydrogen peroxide is detected by a chromogenic acceptor, chlorophenol-4-aminoantipyrine, in the presence of peroxidase (POD). The red quinine formed is proportional to the amount of triglycerides present in the sample.

Triglycerides $\longrightarrow$ Lipase glycerol + fatty acids

Glycerol + ATP $\longrightarrow$ Glycerol kinase Glycerol-3-Phosphate-ADP

Glycerol-3-P $\longrightarrow$ glycerol Phosphate Oxidase Dihydroxyacetone –P $+H_2O_2$

2H2O+ Chlorophenol-4-aminoantipyrine $\longrightarrow$ Peroxidase Red quinine + $4H_2O$

Mode	End point
Wave length	505nm (490-550nmr)
Temperature	37°c
Optical path length	1cm
Blanking	Reagent blank
Sample volume	10µl
Working reagent volume	1000 µl
Incubation time	10min at 37°c
Concentration of standard	200mg/dl
Stability of colour	1 hour
Maximum absorbance limit	2000
Linearity	1000mg/dl
Units	mg/dl

Procedure:

Pipette in tubes marked	Blank	Standard	Test
Serum	-	-	10 µl
Standard	-	10 µl	-
Triglycerides reagent	1000 µl	1000 µl	1000 µl

Mixed well and incubated at 37°C for 10 min. Absorbance of standard and sample was measured against reagent blank at 505nm within 60 min.

Calculation:
Triglycerides concentration (mg/dl) = Absorbance of test / Absorbance of standard × 200
Triglycerides concentration (mmol/L) = Concentration (mg/dl) × 0.014

8.3 Estimation Of Total Cholesterol:
Method: Enzymatic (cholesterol oxidase-peroxidase), end point calorimetry, single reagent chemistry with lipid clearing factor (LCF).

Principle:
The estimation of cholesterol involves the following enzymatic reactions:
Cholesterol esters ⟶ cholesterol+ free fatty acids (in presence of cholesterol esterase)
H2O2 + Phenol +4- aminoantipyrine ⟶ quinoneimine dye + H_2O_2

Mode	End point
Wave length	505nm (490-550nmr)
Temperature	37°c
Optical path length	1cm
Blanking	Reagent blank
Sample volume	10µl
Working reagent volume	1000 µl
Incubation time	10min at 37°c
Concentration of standard	200mg/dl
Stability of colour	1 hour
Maximum absorbance limit	2000
Linearity	1000mg/dl
Units	mg/dl

Procedure:

Pipette in tubes marked	Blank	Standard	Test
Serum	-	-	10 µl
Standard	-	10 µl	-
Triglycerides reagent	1000 µl	1000 µl	1000 µl

Mixed well and incubated at 37°C for 10 min. Absorbance of standard and sample was measured against reagent blank at 505nm within 60 min.

Calculation:
Cholesterol concentration (mgldl) = absorbance of test /absorbance of standard ×200
Cholesterol Concentration (mmol/L) = Concentration (mg/dl) × 0.0259

8.4 Estimation Of HDL Cholesterol:
Method: Phosphotungstate
Principle: Chylomicrons, VLDL & LDL fractions in serum and plasma are separated from HDL by phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant, is assayed with enzymatic cholesterol method, using cholesterol esterase, cholesterol oxidase, peroxidise and the chromogen 4-aminoantipyrene/phenol.
Assay Parameter:

Mode	End point
Reaction slope	Increasing
Wave length	500nm(492-550nm)
Flow cell temperature	30°c
Incubation time	30°c
Sample volume (supernatant)	20µl
Standard concentration	100mg/dl
Blanking	Reagent blank
Working reagent volume	1.0ml
Units	mg/dl

Procedure:

Pipette into test tubes marked	**Blank**	**Standard**	**Test**
Reconstituted reagent	1ml	1ml	1ml
Standard	-	20 µl	-
Supernatant	-	-	20 µl

Mixed well and incubated at 37^0C for 5 min.

8.5 Estimation Of LDL Cholesterol:

The value of LDL cholesterol was calculated as follows: If the value of triglycerides was known LDL cholesterol can be calculated by friedwald's equation:

$$\text{LDL Cholesterol} = \frac{\text{total cholesterol} - \text{triglycerides} - (\text{HDL cholesterol})}{5}$$

8.6 Estimation of VLDL cholesterol:

$$\text{VLDL cholesterol} = \frac{\text{Triglycerides}}{5}$$

8.7 Estimation of cholesterol ratio:

$$\text{Cholesterol ratio} = \frac{\text{total cholesterol}}{\text{HDL cholesterol}}$$

Dose selection: different doses of the plant erythroxylum monogynum and pupalia lappacea selected on the basis of acute toxicity studies.

HDL<40 mg/dl, TC > 180mg/dl and LDL –C>100mg/dl and HbA1c> 7mmol/L indicates diabetes mellitus.

7.8 Estimation of total protein and albumin

Total protein is determined by (biuret method) and albumin is determined by (BCG method).

Assay method:

1. Albumin determination (BCG method)

   Albumin in the sample binds with bromocresol green (BCG) which produces a blue pigment. Quantitation of albumin in the sample can be made by measurement of the absorbance.
2. Total protein determination (biuret method)

   Protein in the sample forms a complex salt with copper ion, and which produces a blue-purple pigment. Quantation of protein in the sample can be made by measurement of the absorbance.

Preparation of reagents to be used:

- Albumin chromogen reagent

  Succinate buffer 75mmol/L, pH 4.2 (Bromocresol green 0.17 mmol/L)
- Total protein chromogen reagent

  Copper (II) sulphate pentahydrate 12mmol/L (potassium sodium tartrate sodium hydroxide)
- Albumin adjustment buffer

  (succinate buffer 75mmol/L, pH 4.2)

Procedure: Assay in a test tube.

Albumin

	TEST	STANDARD	BLANK
SAMPLE	Serum 20µL	Std. Soln. 20 µL	-
REAGENT	5.0mL	5.0mL	5.0mL

Mix well, and incubate at room temperature for 10 min. Measure the absorbance of wavelength 630nm of the test sample and standard solution with the blank solution as the control within an hour.

Total Protein:

	TEST	STANDARD	BLANK
SAMPLE	Serum 100 µL	Std. Soln 100 µL	Distilled water 100 µL
REAGENT	5.0mL	5.0mL	5.0mL

Mix well, and incubate at room temperature for 30 min. Measure the absorbance of wavelength 540nm of the test sample and standard solution with the blank solution as the control.

8.8 Estimation Of Urea:
Estimation of urea is done by DAM method.

Principle:
Urea in an acidic medium condenses with diacetylmonoxime at 100^0C to form a red coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample.

Procedure:
1. For calorimeter: mark the test tube with blank, test, and standard and add reagent as in tubes according to table given below.

	BLANK	TEST	STANDARD
SOLUTION 1	2.5ml	2.5ml	2.5ml
SAMPLE	-	0.01ml	-
REAGENT 3: WORKING UREA STANDARD, 30mg%	-	-	0.01ml
REAGENT 2 (DAM)	0.25ml	0.25ml	0.25ml

Mix well and keep the tubes in the boiling water exactly for 10 min. Cool immediately under running water for 5min, mix by inversion and measure the colour intensity within 10 minusing a green filter against blank.

2. For spectrophotometer: all the volume measured under colourimetric procedure can be adjusted proportionately depending on the flow cell, cuvette capacity. Rest of the procedure remains unchanged measure the OD at 525nm.

Calculations: urea in mg/100ml =OD test × 30/OD Standard

8.9 Estimation Of Uric Acid
Principle:
Uric acid is oxidised to allantion and carbon dioxide by a phosphotungstic acid reagent in alkaline solution. Phosphotungstic acid is reduced in this reaction in this reaction to tungsten blue which is measured at 710nm.Protein have been removed by precipitation with tungstic acid, TCA, phosphotungsticacid and heat coagulation. Other oxidizing agents have included arsenotungstic acid, arsenophosphotungstic acid, arsenomolybdic acid, and potassium ferricyanide. Urea –cyanide was latter used as the alkaline reagent, but this modification did not require the isolation of U.A. from the filtrate.
Chemical used- phosphotungsticacid method
Enzymatic - uricase method
Principle of determination of uric acid by uricase method:
Uric acid in the presence of uricase enzyme gets converted to allointoin and H_2O_2, and then 4-aminophenozone is converted to quinoid pigment in the presence of H_2O_2 and enzyme peroxidase.

Procedure:

Pipette in cuvette	Blank	Standard	Sample
Reagent	1ml	1ml	1ml
Std	-	0.02ml	-
Sample	-	-	0.02ml

Mix and incubate for 10min and measure abs at 546nm.

CalculatioN:
Uric acid conc. = abs sample × 6/abs sample =_mg/dl
8.10 Colorimeteric Estimation Of Serum Creatinine:
Using the alkaline picrate method (jaffe's method)

Principle:
Creatinine + picric acid ⟶ creatinine picrate (ALKALINE MEDIUM)
The orange colour can be measured colorimetrically, where the intensity of the obtained color is directly proportional to the concentration of creatinine in the sample.

Procedure:

In a clean dry test tube add 0.5 ml distilled water (blank) or serum (test), then add 0.5 ml NaOH and 0.5 ml picric acid.

	Blank	Test
Distilled water	0.5 ml	-
Serum	-	0.5 ml
NaOH	0.5ml	0.5 ml
Picric acid	0.5ml	0.5 ml

Mix well the contents of each tube.

- Allow to stand for 15 minutes.
- Measure the absorbance value at λmax 500nm.
- If a standard creatinine solution (0.55mg/dl) has an absorbance value of 0.30, calculate the concentration of creatinine in the provided serum sample using the following equation:
  $\mathbf{C_{test} = C_{std} \times A_{test} / A_{std}}$

8.11 Histopathological Studies:

The animals were euthanized using anesthetic ether and their pancreas, livers and kidneys were dissected out. The isolated organ was sliced into 5mm pieces and fixed in neutral formalin (10% solution) for at least 3 days. Then the pancreas pieces were washed in running water for about 12 hours. This was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each and final dehydration was done using absolute alcohol with about three changes for 12 hours each. Clearing was done by using chloroform with two changes for 15 to 20 minutes each. After clearing the organ pieces were subjected to paraffin infiltration in automatic tissue processing unit.

Embedding in paraffin by vaccum:

Hand paraffin was melted and the hot paraffin was poured into L-shaped blocks. The organs pieces were then dropped into molten paraffin quickly and allowed to cool.

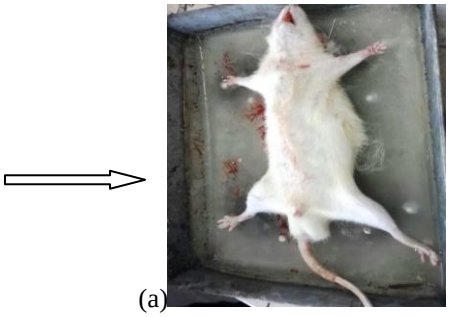
Sectioning:

The blocks were cut using microtone to get sections of thickness of 5µ. The sections were taken on a micro slide on which egg albumin (sticking substance) was applied. The sections were then allowed to remain in an oven at 600C for 1 hour. Paraffin melts and egg albumin denatures, thereby fixing tissues to the slide.

Staining**:** Eosinand Hematoxylin stain

Eosin is an acid stain. Hence it stains all the cell constituents which are basic innature to pink colour. Eg. (RNA,cytoplasm).

Haemotoxylin is a basic stain which stainsall the acidic cell components blue eg., DNA in the nucleus.

(a)   (b)

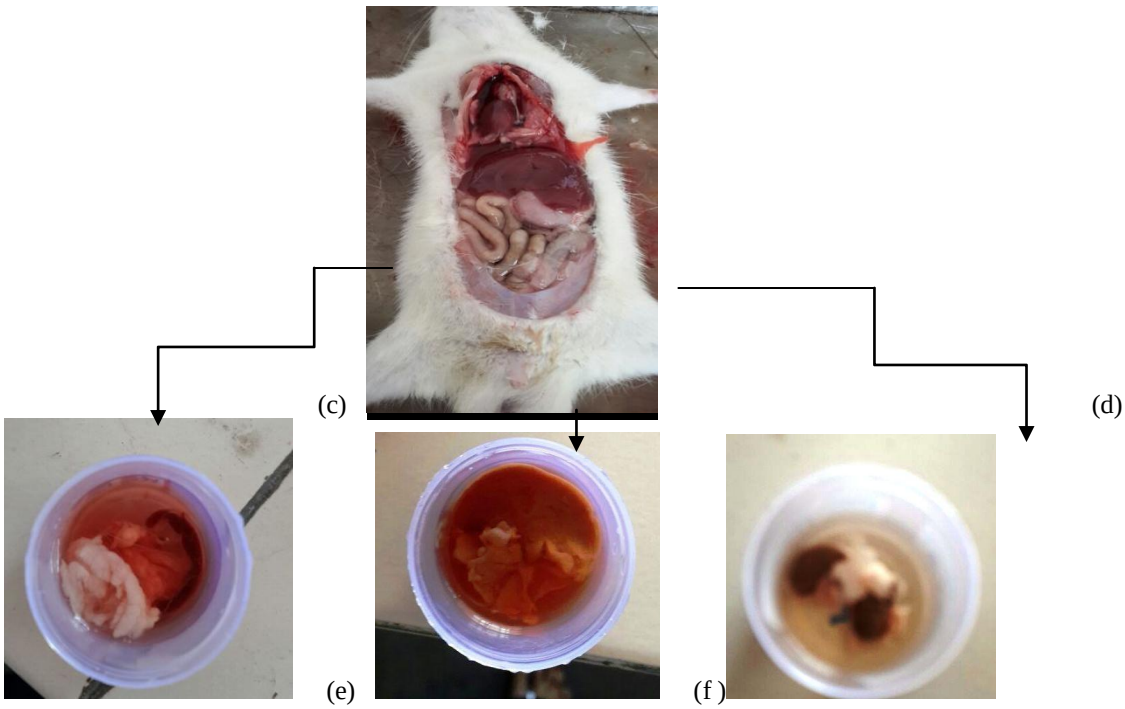


Figure no.5: Dissection of rat

(a) euthanized rat using ether and kept upside down on wax tray (b) removing the skin of rat (c) dissected rat (d) isolated pancreas from rat (e) isolated liver from rat(f) isolated kidneys from rat.

Procedure:

- The sections were deparaffinised by washing with xylene for about 15 minutes.
- The sections were dehydrated by washing in alcohol of decreasing strengths (100%, 90%, 80% and 70%).
- The sections were finally washed with water,
- The sections were stained with hydroxylin for 15minutes.
- It was rinsed in tap water.
- Acid alcohol was differentiated by 3 to 10 quick dips. The differentiation with a microscope showed that the nuclei were distinct and the background was very light or colourless.
- The slide was washed in tap water very briefly.
- The section was dipped in ammonia water, lithium carbonate until sections become bright blue by 3 to 5 dips.
- The slide section was washed in running tap water for 10 to 20 minutes. If washing is inadequate, eosin will not stain evenly.
- The slide was then stained with eosin for 15seconds to 2min, depending on the age of eosin and the depth of the counter stain desired. For even staining results, slides were dipped several times before allowing them to set in the eosin for the desired time.
- Slides were rehydrated in 95% alcohol and absolute alcohol until excess eosin was removed. Two changes of 2min. Interval were performed of different treatments (95% alcohol, absolute alcohol and xylene).
- The section was then mounted in DPX (diphenyl xylene) mountant.
- Staining results showed blue colour nucleus and cytoplasm with various shades of pink with change in different tissue components.

IX. Results

Table No.9:Phytochemical Analysis Of Ethanolic Extract Of Erythroxylum Monogynum

PHYTOCONSTITUENT	AMOUNT
Steroids	+++
Glycosides	+++
Saponins	++
Alkaloids	+++
Sugar	++
Phenol	+
Tannins	+

Keys: +++=appreciable amount, ++=moderate amount, +=trace amount, −=completely absent

TABLE NO. 10: Phytochemical Analysis Of Ethanolic Extract Of Pupalia Lappacea

PHYTOCONSTITUENT	AMOUNT
Steroids	+++
Glycosides	+++
Saponins	++
Alkaloids	+++
Sugar	++
Phenol	+
Tannins	-

Keys: +++=appreciable amount, ++=moderate amount, +=trace amount, −=completely absent

TABLE NO.:11

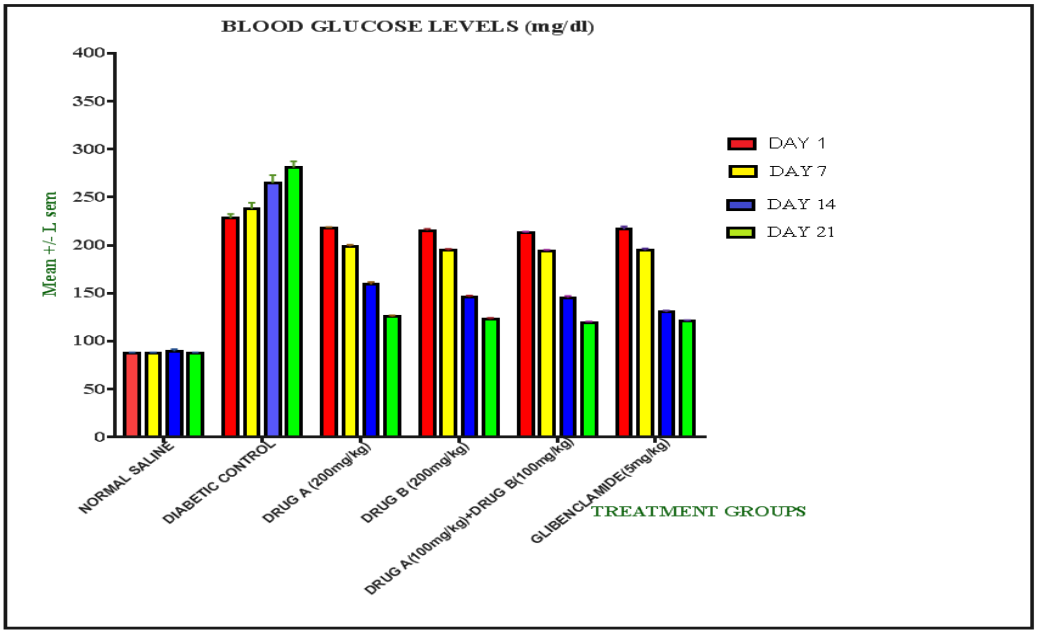
Effect of ethanolic extracts of the leaves of medicinal plants on blood glucose levels (mg/dl) in alloxan induced diabetic rats on 1st, 7th ,14th and 21st day of the treatment:

Treatment group	1st day	7th day	14th day	21st day
NORMAL CONTROL(NORMAL SALINE)	87.50±0.763	87.30±0.91	89.50±2.172	87.50±0.76
DIABETIC CONTROL ALLOXAN(150mg/kg)	228.3±4.4	238.2±6.28	265.8±8.312	281.0±6.512
ERYTHROXYLUM MONOGYNUM (200 mg/kg)	218.3±0.66	198.8±1.68	159.4±2.24*	125.4±1.47**
PUPALIA LAPPACEA (200 mg/kg)	215.3±1.68	194.5±1.68	146.1±1.44*	122.7±1.72**
EM(100mg/kg)+PL(100 mg/kg)	212.8±1.40	194.0±1.238	145.3±1.667*	119.5±0.99**
GLIBENCLAMIDE (5 mg/kg)	216.7±3.007	194.6±2.201	130.3±1.585**	120.7±1.17**

Values are given as ± SEM for group of six animals in each group.
Diabetic control rats were compared with normal rats.
Diabetic + erythroxylum monogynum (200mg/kg) b.w, diabetic + pupalia lappacea (200mg/kg) b.w , Diabetic + erythroxylum monogynum (100mg/kg) b.w + pupalia lappacea (100mg/kg) and Diabetic +glibenclamide (5mg/kg) were compared with diabetic control rats.
**P<0.05, **P<0.01, **P< 0.001 was considered significant comparing to diabetic control group.

GRAPH 1:

Effect of ethanolic extracts of the leaves of medicinal plants on blood glucose levels (mg/dl) in alloxan induced diabetic rats on 1st, 7th ,14th and 21st day of the treatment.



ANOVA followed by Dunnett's t-test
P<0.05, **P<0.01,P< 0.001 was considered significant comparing to diabetic control group.

Here the administration of ethanolic extracts of plant leaves for 21 days decreased the elevated blood glucose levels. The above result suggests that the combination of drug extracts (A+B) was more effective than single drug extracts and was almost comparable with the effect produced by standard drug glibenclamide treated group. The results are represented by means of graph 1 by using ANOVA followed by Dunnett's t –test.

TABLE NO.: 12

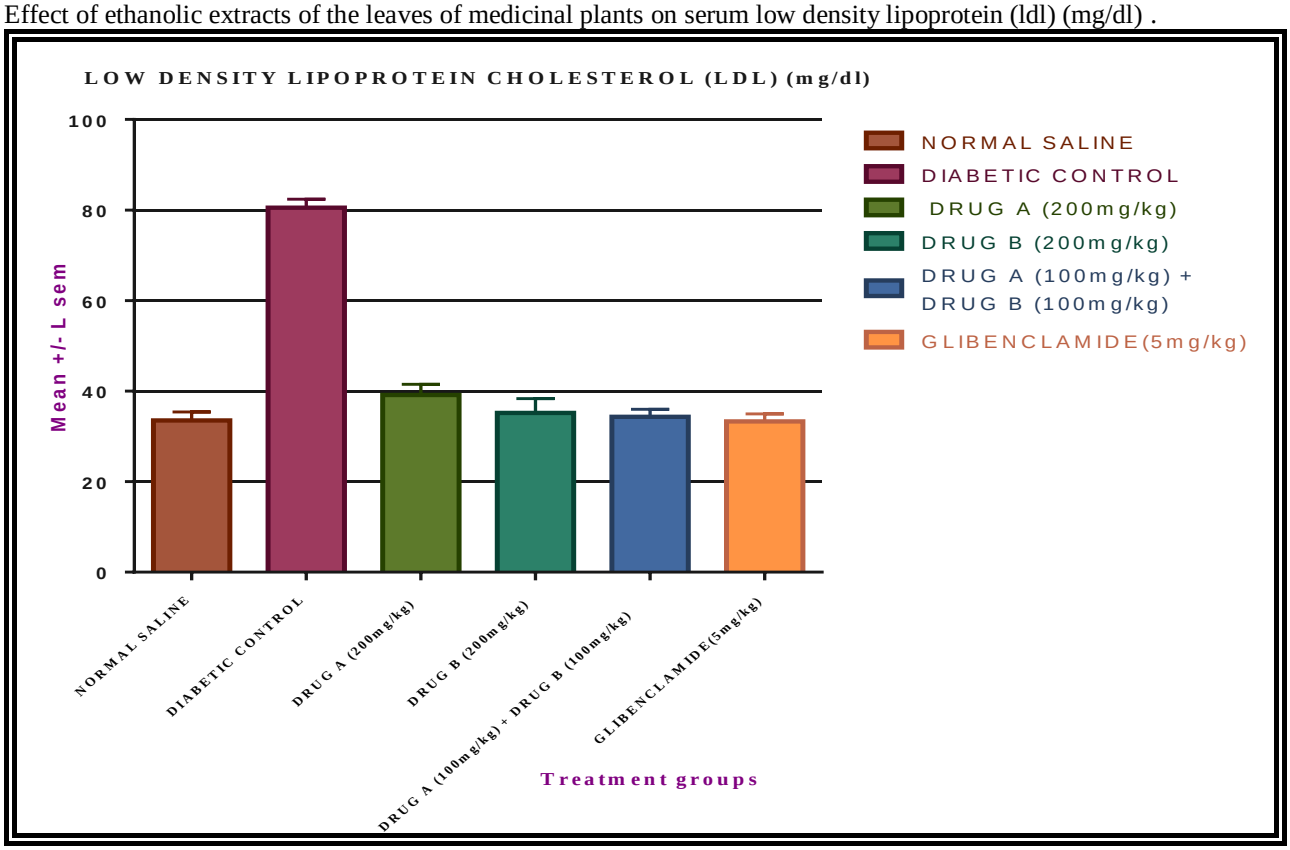
Effect of ethanolic extracts of the leaves of medicinal plants on serum lipid profile in alloxan induced diabetic rats after 21 days of treatment.

Treatment group	SERUM LOW DENSITY LIPOPROTEIN (LDL) (mg/dl)	SERUM VERY LOW DENSITY LIPOPROTEIN (VLDL) (mg/dl)	SERUM HIGH DENSITY LIPOPROTEIN (HDL)(mg/dl)
NORMAL CONTROL(NORMAL SALINE)	33.50±0.763***	16.80±0.477***	32.83±0.945***
DIABETIC CONTROL ALLOXAN(150mg/kg)	80.50±0.76	27.34±0.881	18.67±0.881
ERYTHROXYLUM MONOGYNUM (200 mg/kg)	39.17±0.94***	21.5±0.763**	30.50±0.768**
PUPALIA LAPPACEA (200 mg/kg)	35.17±1.302***	20.33±0.666***	31.50±0.763***
EM(100mg/kg)+PL(100 mg/kg)	34.33±0.66***	17.50±0.7638***	32.00±0.6831***
GLIBENCLAMIDE (5 mg/kg)	33.43±0.66***	16.50±0.7638***	32.67±0.667***

Values are given as ± SEM for group of six animals in each group.
Diabetic control rats were compared with normal rats.
Diabetic + erythroxylum monogynum (200mg/kg) b.w, diabetic + pupalia lappacea (200mg/kg) b.w , Diabetic + erythroxylum monogynum (100mg/kg) b.w + pupalia lappacea (100mg/kg) and Diabetic +glibenclamide (5mg/kg) were compared with diabetic control rats.
**P<0.05, **P<0.01, **P< 0.001 was considered significant comparing to diabetic control group.

GRAPH 2:

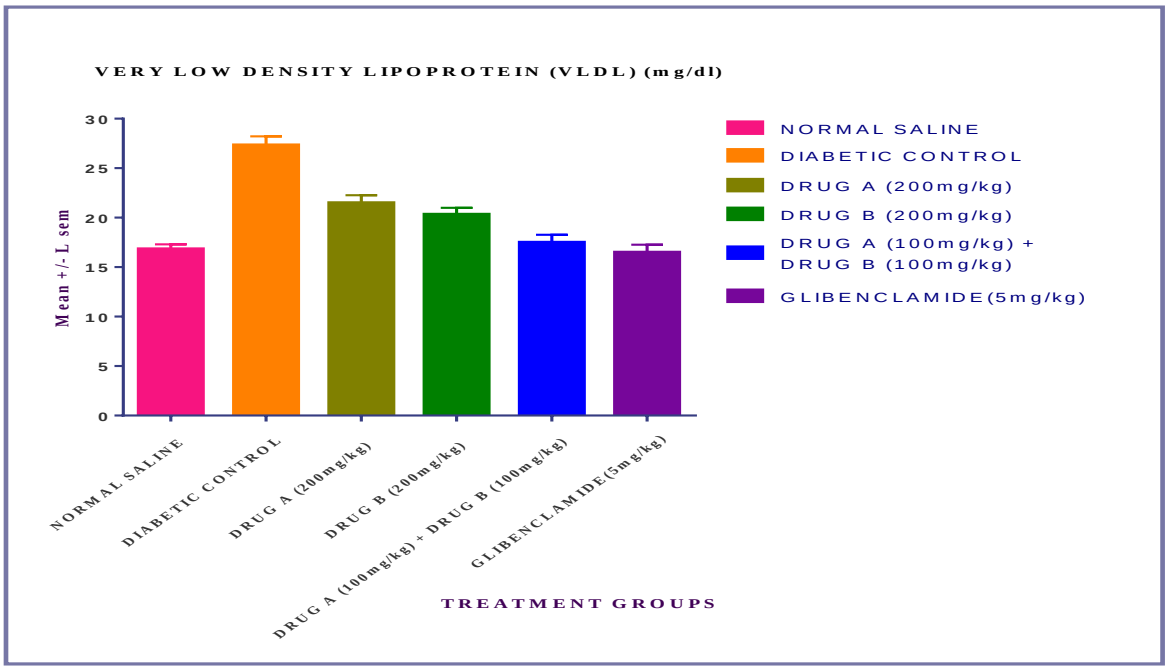
Effect of ethanolic extracts of the leaves of medicinal plants on serum low density lipoprotein (ldl) (mg/dl) .



ANOVA followed by Dunnett's t-test
P<0.05, **P<0.01,P< 0.001 was considered significant comparing to diabetic control group.

GRAPH 3:

Effect of ethanolic extracts of the leaves of medicinal plants on serum very low density lipoprotein(Vldl)(mg/dl)



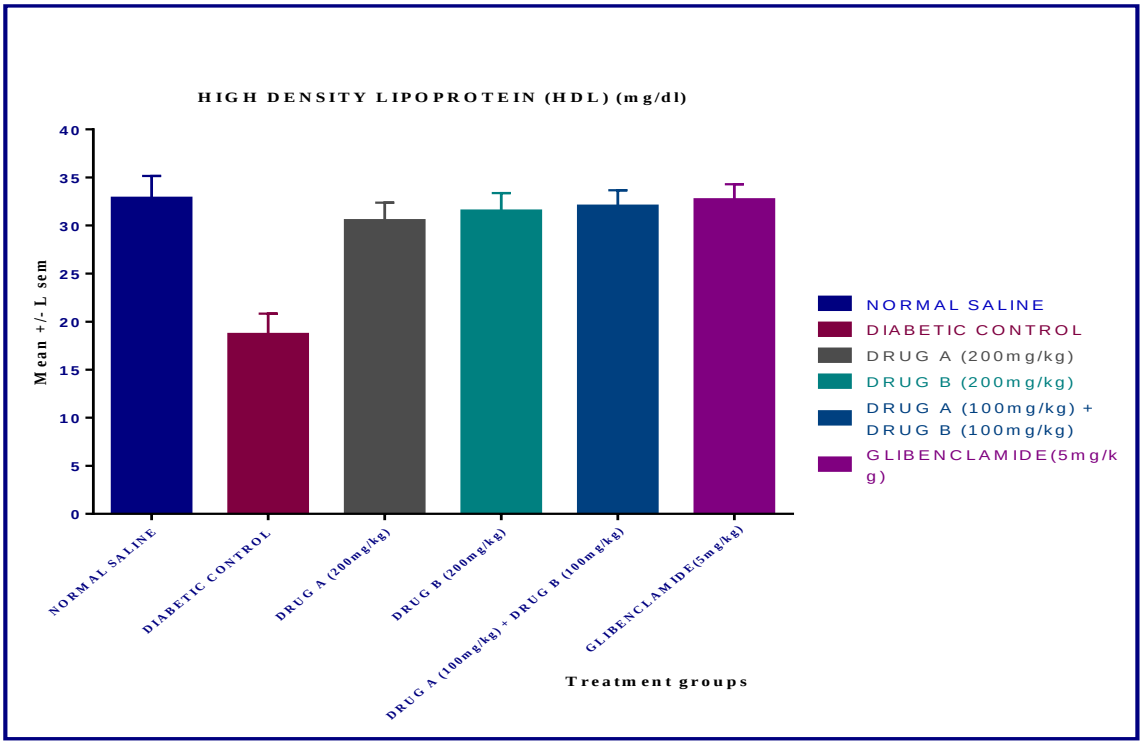
.

ANOVA followed by Dunnett's t-test

P<0.05, **P<0.01,P< 0.001 was considered significant comparing to diabetic control group.

GRAPH 4:

Effect of ethanolic extracts of the leaves of medicinal plants on serum high density lipoprotein (hdl) (mg/dl)



.

ANOVA followed by Dunnett's t-test

P<0.05, **P<0.01,P< 0.001 was considered significant comparing to diabetic control group.

TABLE NO.: 13

Effect of ethanolic extracts of the leaves of medicinal plants on serum lipid profile in alloxan (150mg/kg b.w) induced diabetic rats after 21 days of treatment.

Treatment group	TOTAL CHOLESTEROL (mg/dl)	TOTAL TRIGLYCERIDES (mg/dl)	CHOLESTEROL RATIO (TC/HDL)
NORMAL CONTROL(NORMAL SALINE)	81.84±0.6009***	83.50±0.763***	2.35±0.076
DIABETIC CONTROL ALLOXAN(150mg/kg)	126.83±0.600	132.33±1.054	6.44±0.066
ERYTHROXYLUM MONOGYNUM (200 mg/kg)	94.5±0.7632**	110.00±1.238*	3.15±0.084**
PUPALIA LAPPACEA (200 mg/kg)	85.5±0.7638**	97.50±0.562*	2.85±0.076**
EM(100mg/kg)+PL(100 mg/kg)	84.40±0.666***	92.50±0.7638***	2.61±0.042***
GLIBENCLAMIDE (5 mg/kg)	82.00±0.5774***	85.50±0.763***	2.5±0.0763***

Values are given as ± SEM for group of six animals in each group.

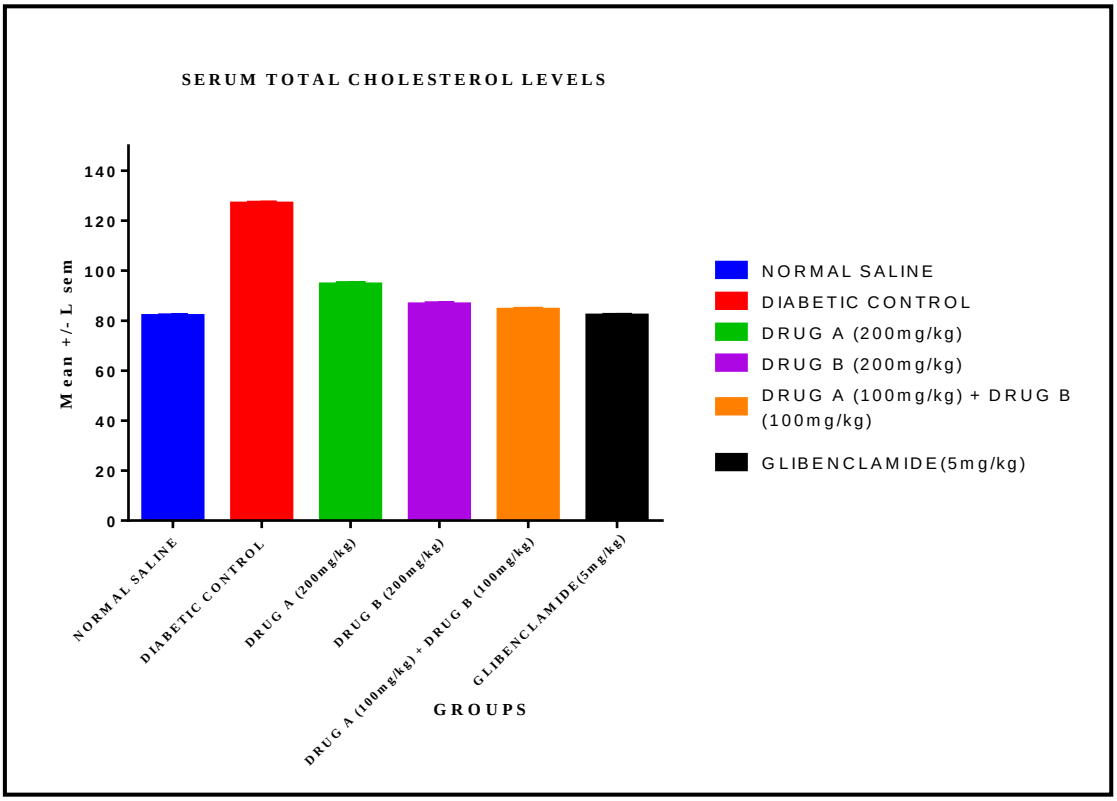
Diabetic control rats were compared with normal rats.

Diabetic + erythroxylum monogynum (200mg/kg) b.w, diabetic + pupalia lappacea (200mg/kg) b.w , Diabetic + erythroxylum monogynum (100mg/kg) b.w + pupalia lappacea (100mg/kg) and Diabetic +glibenclamide (5mg/kg) were compared with diabetic control rats.

**P<0.05, **P<0.01, **P< 0.001 was considered significant comparing to diabetic control group.

GRAPH 5:

Effect of ethanolic extracts of the leaves of medicinal plants on total cholesterol (mg/dl).

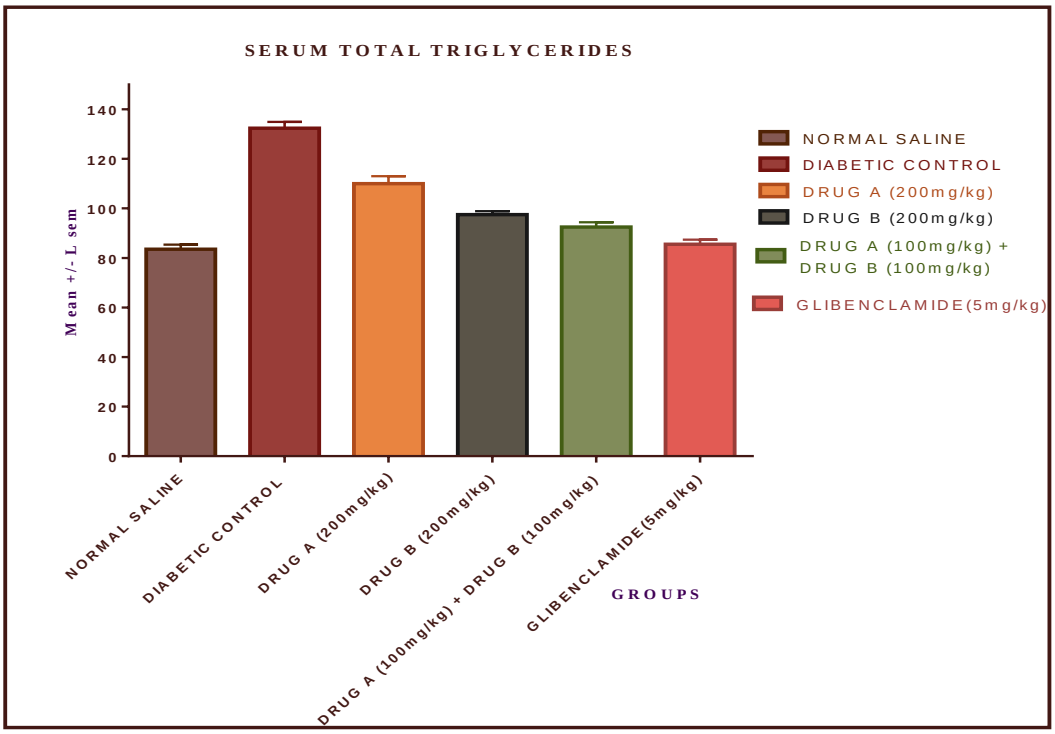


ANOVA followed by Dunnett's t-test

P<0.05, **P<0.01,P< 0.001 was considered significant comparing to diabetic control group.

GRAPH 6:

Effect of ethanolic extracts of the leaves of medicinal plants on serum total triglycerides (mg/dl)

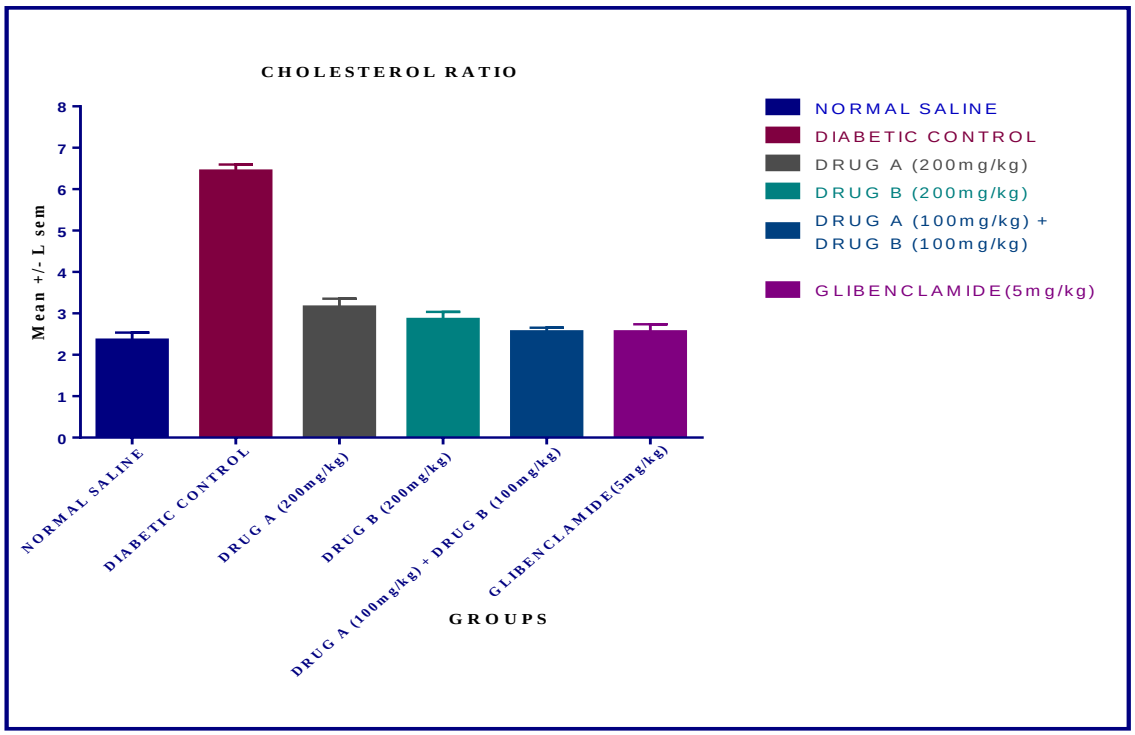


ANOVA followed by Dunnett's t-test

P<0.05, **P<0.01,P< 0.001 was considered significant comparing to diabetic control group.

GRAPH 7

:Effect of ethanolic extracts of the leaves of medicinal plants on cholesterol ratio (tc/hdl).



ANOVA followed by Dunnett's t-test

P<0.05, **P<0.01,P< 0.001 was considered significant comparing to diabetic control group.

TABLE NO.: 14

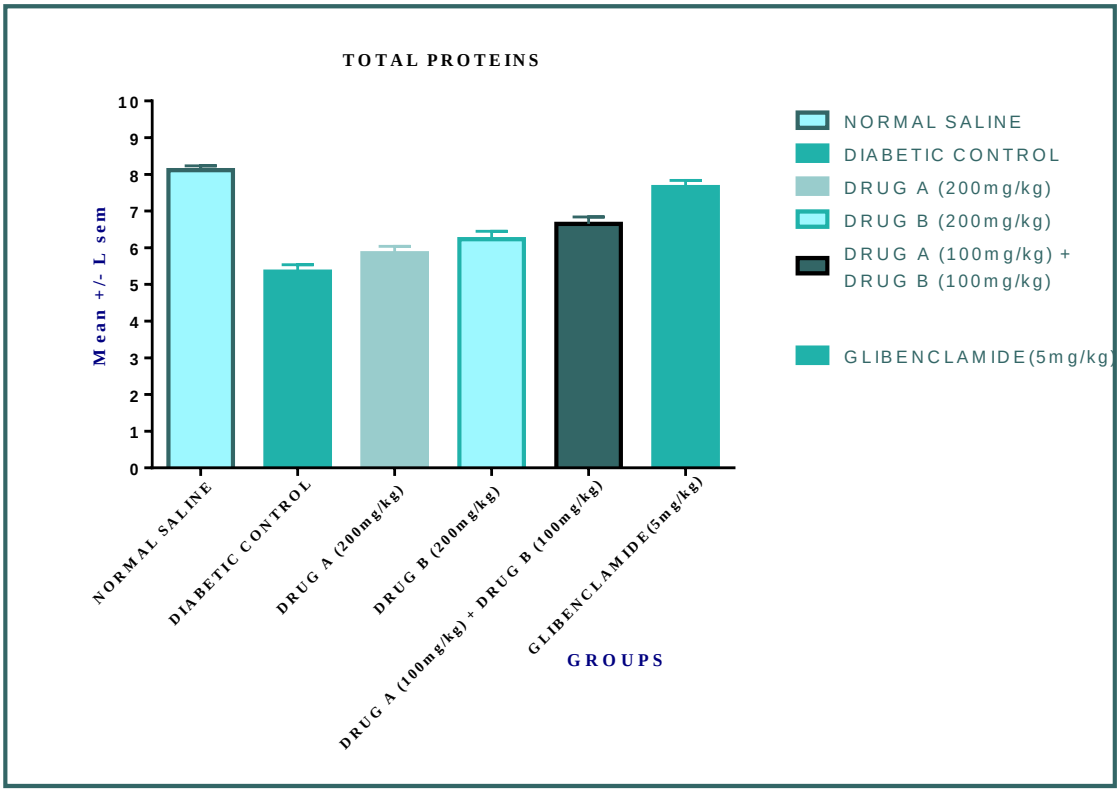
Effect of ethanolic extracts of the leaves of medicinal plants on total protein, albumin, urea, uric acid and creatinine levels in alloxan (150mg/kg b.w) induced diabetic rats after 21 days of treatment:

TREATMENT GROUPS	TOTAL PROTEIN	ALBUMIN	UREA	URIC ACID	CREATININE
NORMAL CONTROL(NORMAL SALINE)	8.117±0.04	3.39±0.006	26.7±066	1.65±0.076	1.05±0.763
DIABETIC CONTROL ALLOXAN(150mg/kg)	5.35±0.07*	1.85±0.07*	37.5±0.07*	2.65±0.26*	2.75±0.76*
ERYTHROXYLUM MONOGYNUM (200 mg/kg)	5.85±0.0763*	2.45±0.07**	34.5±0.076**	2.15±0.076**	1.95±0.76**
PUPALIA LAPPACEA (200 mg/kg)	6.23±0.088**	2.56±0.66**	33.5±0.76**	1.85±0.076**	1.85±0.75**
EM(100mg/kg)+PL(100 mg/kg)	6.65±0.076**	2.95±0.07**	30.50±0.76**	1.75±0.076**	1.65±0.763**
GLIBENCLAMIDE (5 mg/kg)	7.55±0.07**	3.05±0.07**	29.50±0.7**	1.65±0.07**	1.35±0.74**

Values are given as ± SEM for group of six animals in each group.
*:Statistically significant when compared to control group (I) at P< 0.05;
**: statistically significant when compared to untreated diabetic group (II) at P<0.005.

GRAPH 8:

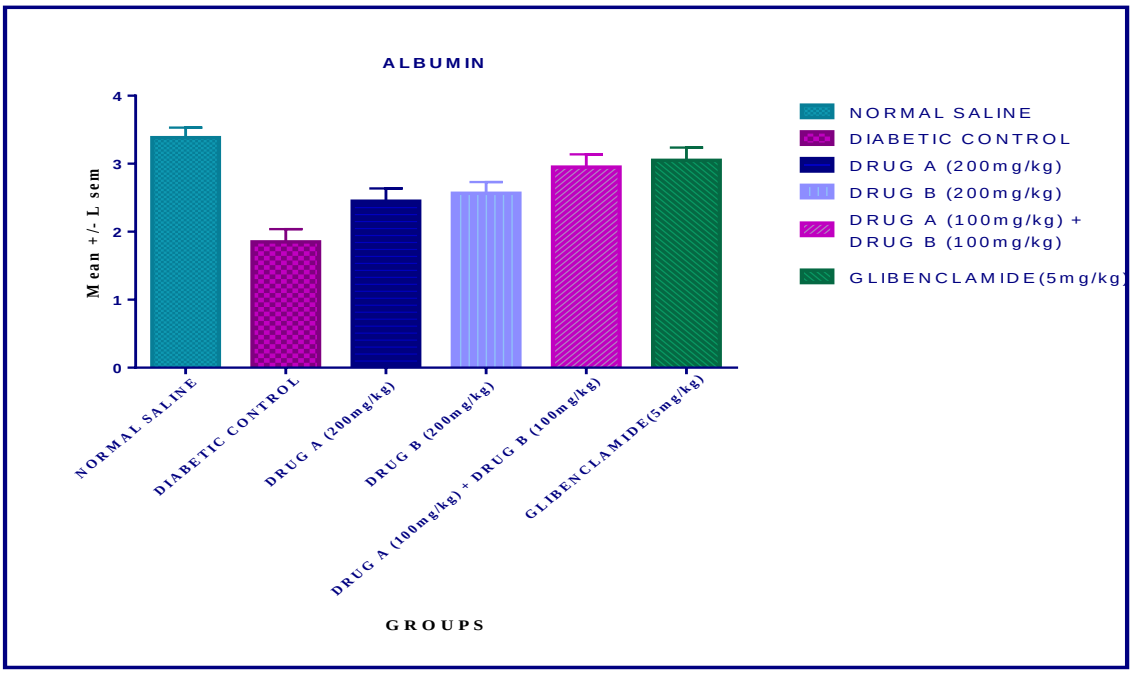
Effect of ethanolic extracts of the leaves of medicinal plants on total protein.



ANOVA followed by Dunnett's t-test
**P<0.05, was considered significant comparing to diabetic control group.

GRAPH 9:

Effect of ethanolic extracts of the leaves of medicinal plants on total albumin.

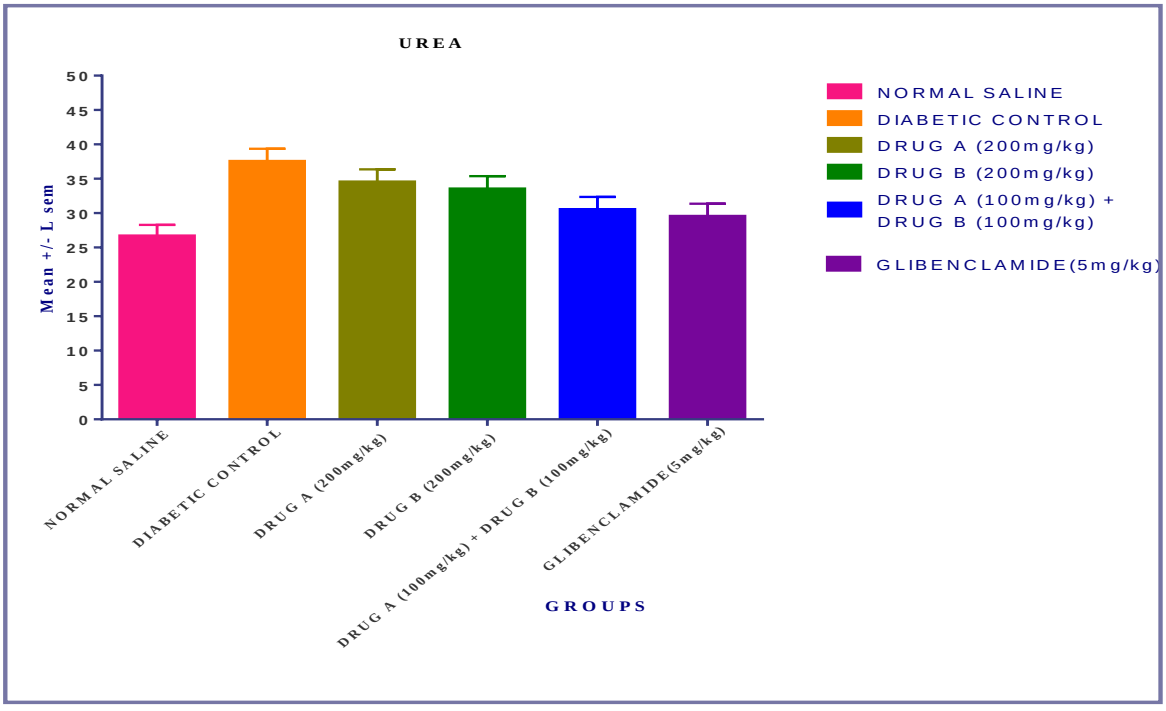


ANOVA followed by Dunnett's t-test

**P<0.05, was considered significant comparing to diabetic control group.

GRAPH 10:

Effect of ethanolic extracts of the leaves of medicinal plants on total urea.

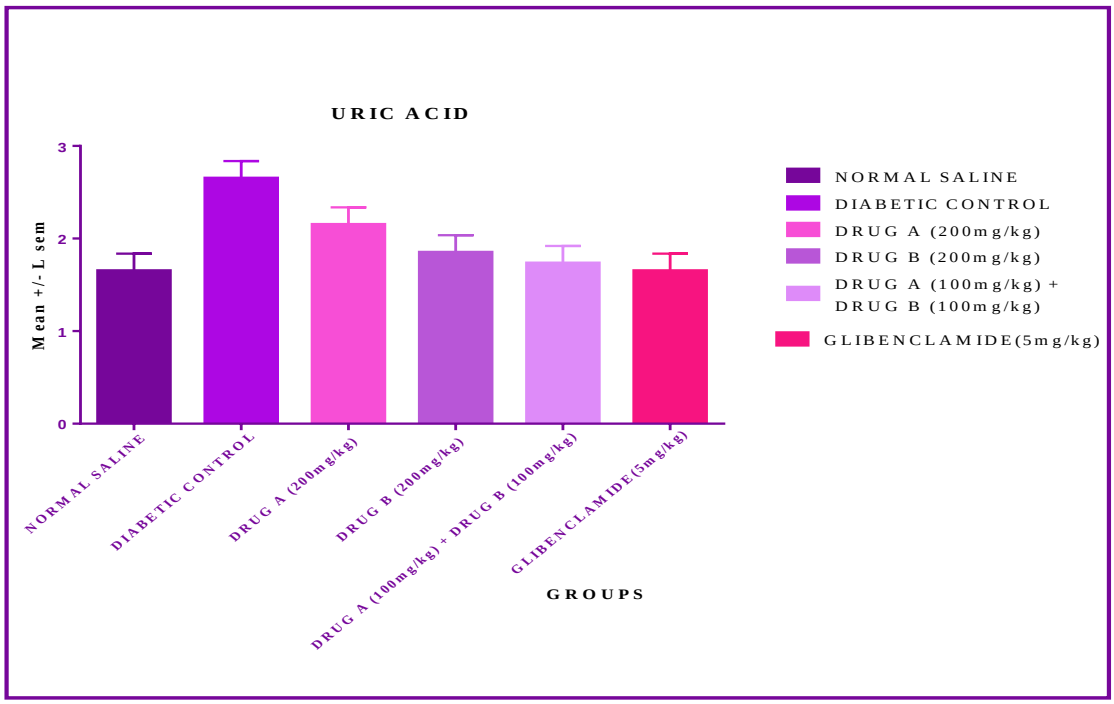


ANOVA followed by Dunnett's t-test

**P<0.05, was considered significant comparing to diabetic control group.

GRAPH 11:

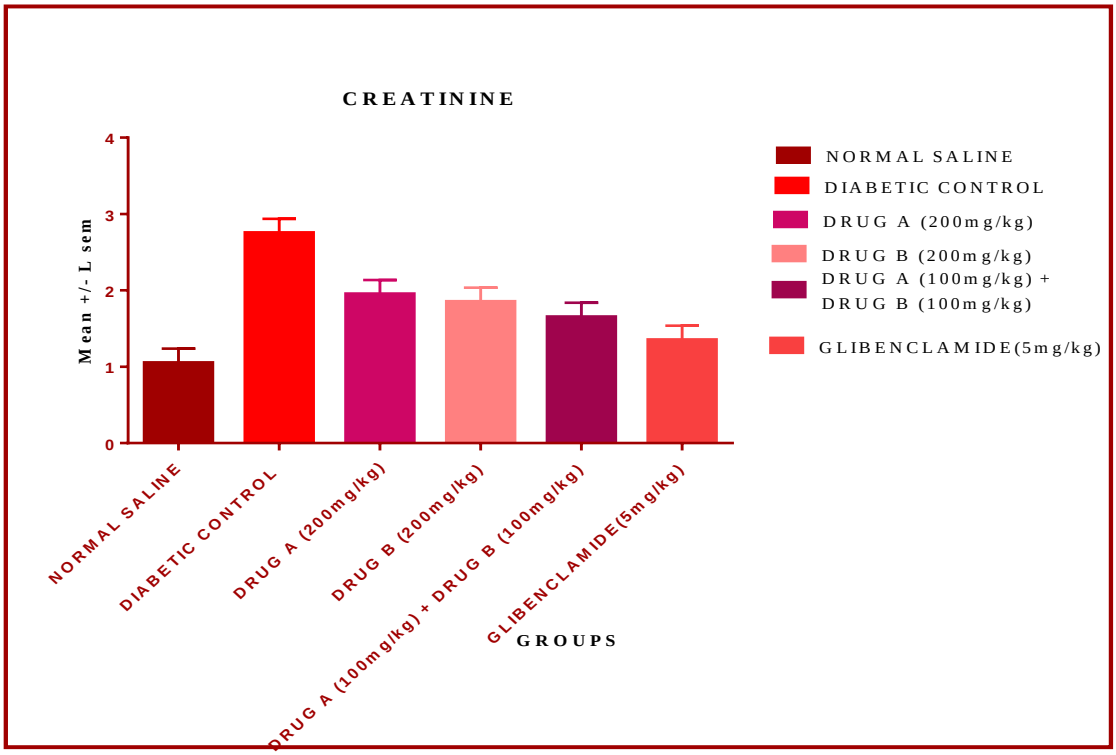
Effect of ethanolic extracts of the leaves of medicinal plants on uric acid



ANOVA followed by Dunnett's t-test
**P<0.05, was considered significant comparing to diabetic control group.

GRAPH 12:

Effect of ethanolic extracts of the leaves of medicinal plants on total creatinine.



ANOVA followed by Dunnett's t-test
**P<0.05, was considered significant comparing to diabetic control group.

Histopathology study of pancreas:

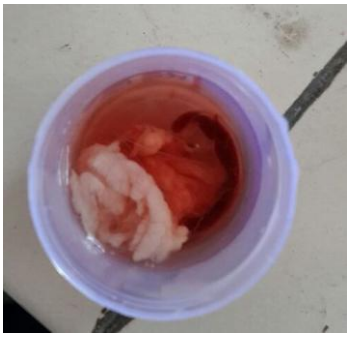


Figure no.6 : Isolated pancreas of rat.

The histopathological changes of pancreas of diabetic rat are shown in fig no. 1 which indicates serve necrotic changes of pancreatic islets, nuclear changes with dilation and congestion of blood vessels. Treatment with drug extracts EEM and EPL shown restoration of architecture of pancreas that was earlier affected with alloxan.

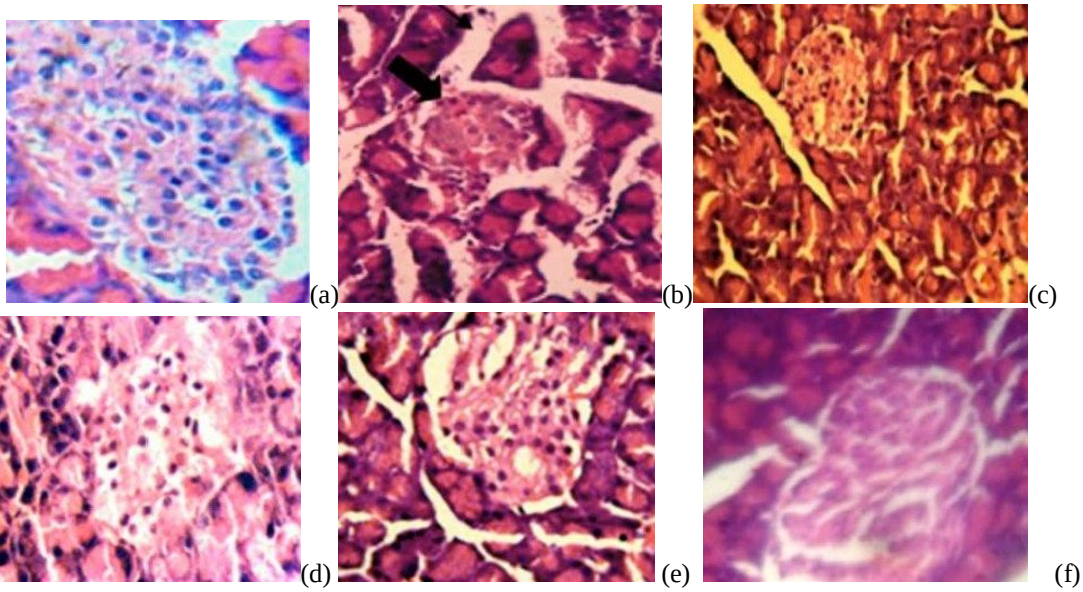


Figure no.7: Microphotograph of pancreatic tissues

Histopathology of pancreatic sections of alloxan induced diabetic rats treated with drug extracts. Pancreatic sections were stained with haemotoxylin-eosin and observed under 40X magnification of digital microscope. NC: Normal control DC: Diabetic control EEM: ethanolic extract of erythroxylum monogynum EPL: ethanolic extract of pupalia lappaceae GL: glibenclamide ALX: Alloxan.

a) Normal rat showing normal acini and normal cellular population in islets of langerhans and absence of both damage to islets and hyperplasia,
b) Diabetic control rat showing damaged islets and reduced islets size,
c) Diabetic rat treated with EEM (200mg/kg) showing restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia,
d) Diabetic rat treated with EPL (200mg/kg)showing restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia,
e) Diabetic rat treated with combination of EEM (100mg/kg) and EPL (100mg/kg) showing restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia,
f) Diabetic rats treated with GLB (5mg/kg) showing restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia.

Histopathology study of liver:

Figure no.8 : Isolated liver of rat.

Histopathological sections of liver of diabetic rats showed hepatocellular injury of diabetic rats showed hepatocellular injury pronounced in loss of normal architecture of the liver, there was severe fibrosis and leucocytic infiltration around the portal veins which appeared congested with blood with dilation in blood vessels.

Microscopic examination of liver of control group rats (figure a) showed normal histology with sinusoidal cards of hepatocytes with central vein and portal tracts. The portal tracts showed portal triad with portal vein, hepatic artery and bile duct. The liver of diabetic rats (figure b) showed distoration in the arrangement of cells around the central vein, enlargement and thickening of the walls of veins and capillaries, development of fibrosis with necrosis of hepatocytes, slight congestion of liver, and periportal fatty infiltration. The liver of ethanolic extracts treated diabetic rats (figure c,d & e) showed well rejuvenated normal cellular arrangement around the central vein and reduced necrosis and normal blood vessels. The liver of glibenclamide (figure f) treated diabetic rats showed normal cellular arrangement around the central vein and reduced necrosis with mild to moderate intrahepatic haemarrhage and few erythrocytes in central vein.

The treatment with EEM & EPL showed improvement in histological structure of liver sections of diabetic rats, pronounced in normalized appearance of liver lobules with strains of hepatocytes comparing with section of diabetic rat liver as shown in fig

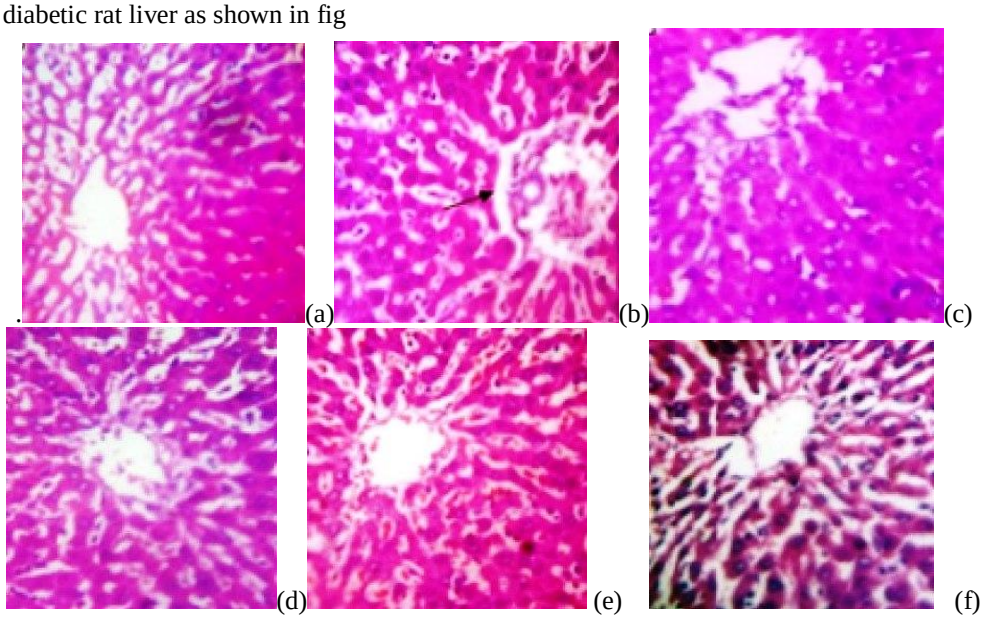


Figure no.9: Microphotograph of liver tissues.

Histopathology of liver sections of alloxan induced diabetic rats treated with drug extracts. Liver sections were stained with haemotoxylin-eosin and observed under 40X magnification of digital microscope. NC: Normal control DC: Diabetic control EEM: ethanolic extract of erythroxylum monogynum EPL: ethanolic extract of pupalia lappaceae GL: glibenclamide ALX: Alloxan.a)Normal control with typical histological structure of rat liver, b) Diabetic control group showing loss of normal architechture with distended central vein (→) c) DC +EEM (200mg/kg) d) DC +EPL(200mg/kg) e) DC + EEM(100mg/kg) +EPL(100mg/kg) f) DC+GL (5mg/kg) . All ethanolic drug extracts treated group showed significant improvement in liver histopathology as compared to DC.

Histopathology study of kidney:

Figure no. 10 : Isolated kidney of rat.

The histological changes in the renal specimen of normal and diabetic animals are showm in figure no. Diabetic glomeruli showed some areas of messengial matric expansion, messengial cell proliferation and thickening of glomerular basement membrane. The kidney of control rats (figure no.) a showed normal histology with well developed proximal and distal convoluted tubules and normal glomeruli and bowman's capsule. The kidney of diabetic rats showed (figure no.) b shrunken glomerulus, thickening of vesicles, glomerulus show some cellular proliferation with fibrosis and some congestion, marked tubular damage, hemorrhage in the Bowman's space. The kidney of drug extracts (figure no.) c, d, & e treated diabetic rats show well-rejuvenated proximal and distal convulated tubules with glomerulus, Bowman's capsule, and renal tubules. Also, tubules with no congestion and hemorrhage, no tubular damage and no fibrosis were observed. The kidney of glibenclamide treated rats (figure no.) f showed glomeruli, which appeared normal with milddilated tubules and degenerative changes of tubular epithelium with moderate haemorrhages in glomeruli.
The histological changes in the renal specimen of normal and diabetic animals are shown in fig. No. . diabetic glomeruli showed some areas of messengial matric expansion, messengial cell proliferation and thickening of glomerular basement membrane. Treatment with EEM (200mg/kg) , EPL (200mg/kg) and combination of drug extracts leads to regeneration of tissues of tissues that were earlier affected with alloxan.

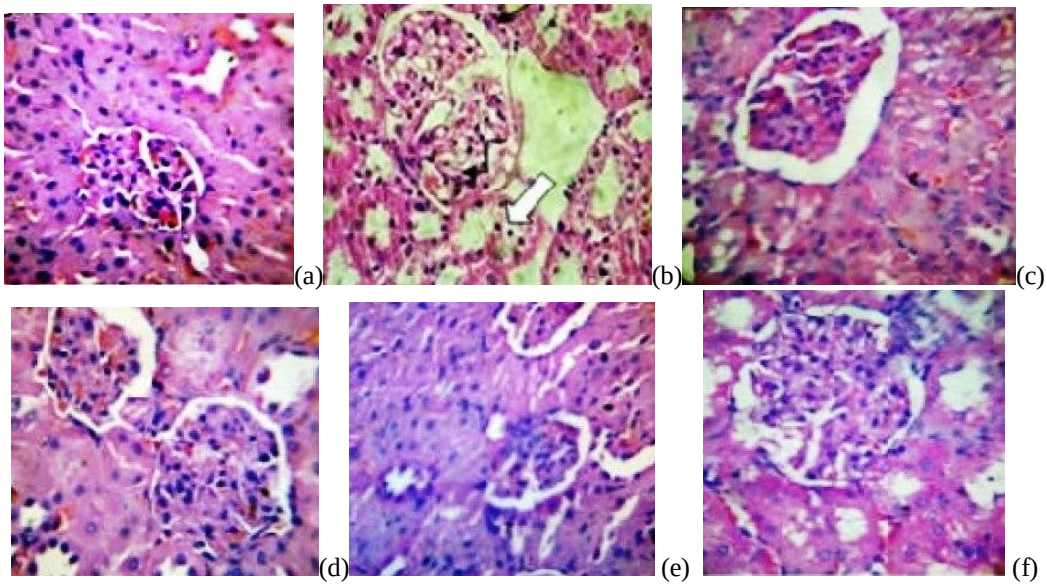


Figure no.11 : Microphotograph of kidney tissues.

Histopathology of kidney sections of ALX treated diabetic rats treated with EEM & EPL. Kidney sections were stained with haematoxylin-eosin and observed under 40X magnification of digital microscope. NC: normal control ,DC: Diabetic control EEM: ethanolic extract of erythroxylum monogynum EPL: ethanolic extract of pupalia lappaceae GL: glibenclamide ALX: Alloxan.

a)Normal control with typical histological structure of rat kidney. b) diabetic control group showing glomerular basement membrane thickening (⟶), tubular dilation (⟶), and cell infiltration (⟶) c)DC+EEM (200mg/kg) d) DC + EPL(200mg/kg) e) DC + EEM(100mg/kg) + EPL(100mg/kg) f) DC + GL(5mg/kg).
All ethanolic drugs extract treated group showed significant improvement in kidney histopathology as compared to DC.

X. Discussion

Diabetes causes damage to the liver, kidney and pancreatic β-cells of patients. This damage varies in proportion and susceptibility from one individual patient to another. The liver is a vital organ in humans, which has a wide range of functions including detoxification of foreign substances in the body as well as serving as the power house for protein synthesis. Other functions of the liver includes, building complex molecules from simple substances absorbed from the digestive tract, neutralization of toxins, manufacture of bile which aids fat digestion and removal of toxins through the bowels.

On the other hand, the kidneys are vital organs that function to keep the blood clean and maintain chemical balance within. They process blood to extract waste products and extra water. These by products become urine to be ultimately excreted from the body. The kidney serves many other important functions, including, filtering out wastes to be excreted in the urine, regulating blood pressure via both urinary excretion of wastes and initiating the rennin-angiotensen hormone regulatory system, regulating an acid-base balance via the bicarbonate system and stimulating red blood cell production via release of the hormone erythropoietin.

The pancreas contain cells that produce juices to break down fats and proteins and a hormone known as insulin to balance blood sugar content in the human body. Any abnormality to these organs may lead to organ dysfunction and threat to human life. Diabetes is one of such causes of damage to these organs leading to organ dysfunction and endocrine related diseases which may be life threatening.

In the absence of reliable and kidney-protective drugs in medical practice, herbs have become reliable substitutes and have so far played significant role in the ethanopharmacological management of various liver disorders and the accompanying oxidative stress.

Various articles have been published regarding evaluation of different medicinal plants on the management of diabetes and its complications in diabetic animal models, an intensive literature review have been done on this articles[15].

In this study Erythroxylum Monogynum and Pupalia Lappacea was evaluated for its use in the management of complications of diabetes as hepatoprotective, nephroprotective and antihyperlipidemic.

Effect of ethanolic extract of the whole leaf of erythroxylum monogynum and pupalia lappacea in alloxan induced diabetic rats:

In this study groups 2,3,4,5 and 6 induced with diabetes using alloxan developed serum hypoglycaemia. This condition was either due to pancreatic β-cells necrosis mediated by alloxan which enhances ATP dephosphorylation resulting in the generation of free radicals into the blood circulation or simply due to decrease in serum insulin or an integration of both processes. Diabetes induction and the generation of free radicals such as superoxide, hydrogen peroxide and hydroxyl radicals, caused the destruction of hepatic, pancreatic and kidney cells and tissues.

This study demonstrated that continuous oral administration of ethanolic extract of erythroxylum monogynum and pupalia lappacea for 21 days significantly decreased blood glucose levels in diabetic rats by 43% compared with the decreases of 49% with the glibenclamide, a well known antidiabetic drug.

Effect of ethanolic extract of the whole leaf part of erythroxylum monogynum and pupalia lappacea on serum lipid profile in alloxan induced diabetic rats:

In addition to marked hyperglycemia, results revealed that the alloxan diabetic rats developed notable hyperlipidaemia. Diabetes induced hyperlipidaemia was observed in diabetic experimental animal models. This is very important, since elevated concentrations of cholesterol, triglyceride and LDL-C are important risk factors in the development of atherosclerosis in diabetes mellitus. The study revealed that this extracts normalized serum lipids (cholesterol, triglyceride, HDL-C, LDL-C) closely to the level the control or normal rats. The findings are consistent with a recent study by Bavarva and Narsimhacharya (2010) which reported that leaves of Leucascephalotes lowered both plasma and hepatic lipid profiles (total lipid, triglycerides and cholesterol) and LDL-C while elevating the HDL-C levels. They suggest that these improvements in lipid profiles are most likely due to its insulin-like actions of the leaf extract of Leucascephalotes. Similarly, a previous study done by Lopes-Virella et al. (1983) also reported that diabetic patients taking insulin injections exhibited both high lipoprotein lipase activity and low level of plasma triglyceride concentrations. Thus, it can be concluded that the enhancement of insulin secretion or level is accompanied by enhancement of glucose utilization as well as a reduction of lipid level in diabetic rats. It is possible to suggest that mechanism of antihyperlipidemic effect of erythroxylum monogynum and pupalia lappacea might be similar to some of those suggested for anti-diabetic plants exhibiting antihyperlipidemic activity, such as activation of lipoprotein lipase insulin-mediated lipolytic activity or inhibition of lipogenic enzymes or hormone sensitive lipase.

EFFECT OF ETHANOLIC EXTRACT OF THE WHOLE LEAF PART OF ERYTHROXYLUM MONOGYNUM AND PUPALIA LAPPACEA ON SERUM UREA, URIC ACID, CREATININE, PROTEIN AND ALBUMIN IN ALLOXAN INDUCED DIABETIC RATS:

The study also showed a significant decrease in serum total protein and albumin in untreated diabetic rats, whereas total protein and albumin significantly increased after the administration of this extract. Total

protein and albumin levels in blood can also be used as an indicator of liver function. Similar results were obtained when the glibenclamide were administered orally in alloxan diabetic. These results suggest that this extract can improve some biochemical parameters that are related to liver functions.

Hyperglycemia has also been recently implicated in initiation and development of various types of diabetic complications. Nephropathy is one of these serious microvascular complications that have been observed in diabetic complications. Blood urea and creatinine that has been observed in diabetic individuals. Blood urea and creatinine concentrations were increased among uncontrolled diabetic rats and this increase among uncontrolled diabetic rats and this increase could be a result of impaired renal function due to an increased blood glucose level.

It was reported that diabetic individuals had lower serum albumin concentrations as well as higher serum uric acid and urea levels than nondiabetic individuals. Thus, the reduction in urea and creatinine levels probably can be explained by a reduction in blood glucose level.

Further, DM is also considered as a risk factor for cardiovascular disease, and elevated serum uric acid has been linked to cardiovascular disease, especially if accompanied with high triglyceride and low HDL. Moreover, high levels of serum uric acid, urea and creatinine may act as a marker of kidney problems. Thus,it is possible to suggest that this extract might play an important role in reducing risk of kidney problems as well as cardiovascular diseases by lowering serum urea, uric acid, creatinine as well as improving lipid profile.

The study of the literature indicated that free radicals areone of the main contributors to development of DM as well as its complications. It is also worth mentioning that alloxan can induce rapid death of β cells of pancreas, resulting in partial or complete loss of insulin production and leading to the development of hyperglycemia and its complications in experimental animals; and this action of alloxan was mediated by formulation of free radicals. Moreover, a wide range of studies have strongly supported the notion that antioxidant compounds derived from plant extracts might play a vital role in the treatment of DM and prevent or delay its complications. Thus, it is possible to suggest that these therapeutic values of the drug extract could be attributed to this drug extracts of erythroxylum monogynum and pupalia lappacea.

The preliminary phytochemical screening of extract of erythroxylum monogynum shows the presence of alkaloids, flavonoids, steroids, glycosides, phenolic compounds, saponins, tannins and extract of pupalia lappacea shows the presence of alkaloids, flavonoids, steroids, glycosides, triterpenoids, saponins, amino acids, coumarins, starch and tannins.

Thus the ethanolic extract of leaves of erythroxylum monogynum at a dose of 200mg/kg, pupalia lappacea at a dose of 200mg/kg and combination of both (EM, 100mg/kg and PL, 100mg/kg) possess a glucose lowering effect, lipid lowering effect, decrease in uric acid, urea, creatinine and a significant increase in protein, albumin in alloxan induced diabetic rats. It was also found to be highly effective in managing complications associated with diabetes mellitus.

In conclusion, this study revealed that oral administration of the ethanolic extract of erythroxylum monogynum and pupalia lappacea exhibit antihyperglycemic, nephroprotective, hepatoprotective, and anti hyperlipidemic activities via enhances insulin production in alloxan-induced diabetic rats.

XI. Summary & Conclusion

Diabetes mellitus is a disorder in which blood sugar (glucose) levels are abnormally high because the body does not produce enough insulin to meet its needs.

People with diabetes may experience many serious, long-term complications. Some of these complications begin within months of the onset of diabetes, although most tend to develop after a few years. Most of the complications gradually worsen. In people with diabetes, strictly controlling the levels of glucose in the blood makes these complications less likely to develop or worsen.

Overtime, elevated levels of glucose in the blood and poor circulation can harm the heart, brain, legs, eyes, kidneys,nerves and skin resulting in angina, heart failure, strokes, leg cramps during walking (claudication), poor vision, kidney failure, damage to nerves (neuropathy), and skin breakdown.

Various types of drugs available in the market for the treatment of diabetes and various complications that are associated with it, but these drugs are not completely effective and also have side effects. Medicinal plants have attracted considerable global interest in recent years. In the past few decades pioneer work in identification, documentation and recognition of traditional medicine has been done in india. Investigation of traditional medicine is very important for the welfare of rural and tribal communities for the treatment of conventional illness.

In this study erythroxylum monogynum and pupalia lappacea were evaluated for its use in the management of diabetes. Erythroxylum monogynum belong to the genus erythroxylum, represented by over 256 to 279 species worldwide. Genus erythroxylum is rich in tannins, glycosides, flavonoids, steroids and phenols. Traditionally plants belong to the genus are used for the treatment of of skin disorders, diaphoretic, diuretic and stomach problems. Leaf juice given internally as a cooling beverages and jaundice and stem bark decoction is

used for treat of hiccups. Stem bark decoction is managed for cure of itches. It is also used to hepatoprotective activity. Various parts of the plant are used in Indian traditional medicine for many therapeutic values. The infusion of bark and wood is used as stimulant and also in mild cases of dyspepsia and continued fever. E. monogynum have been noted for its antibacterial properties. The different parts of E. monogynum are used as medicine for many sickness and ailments.

Pupalia lappacea also evaluated for its use in the management of diabetes. It belongs to the genus pupalia which is rich in alkaloids, amino acids, glycosides, flavanoids, glycosides, saponins, tannins, starch, steroids, terpenoids and coumarins. The plant has been reported to have antiplasmodial & antipyretic activity. Dichloromethane extracts of P. lappaceae has shown moderate antiplasmodic activity (IC50-50.29 µg/ml) against P. falciparum. Ethanolic extracts of P. lappaceae was found to reduce the yeast elevated rectal temperature in a dose dependent manner and the effect was comparable to that of standard paracetamol.

The ethanolic extract of leaves of erythroxylum monogynum and pupalia lappacea were evaluated for in vivo hepatoprotective, nephroprotective, antihyperlipidemic and antihyperglycemic activity against alloxan induced diabetic rats. Alloxan causes selective destruction of beta cells which are involved in the production of insulin.

The results of the present investigation clearly indicates that the ethanolic extract of leaves of erythroxylum monogynum (200mg/kg), pupalia lappacea (200mg/kg), and combination of two (erythroxylum monogynum (100mg/kg) and pupalia lappacea (100mg/kg)) possess a glucose lowering effect, lipid lowering effect, decrease in uric acid, urea, creatinine and a significant increase in protein, albumin in alloxan induced diabetic rats. It was also found to be highly effective in managing complications associated with diabetes mellitus.

In conclusion, this study revealed that oral administration of the ethanolic extract of both the plants exhibit nephroprotective, hepatoprotective and antihyperlipidemic activities via enhances insulin production in the alloxan- induced diabetic rats. Thus, oral use of this extract might positively affect the functional capacities of various rat tissues, particularly kidney and liver against toxic action of alloxan compound (dose of 150mg/kg BW). Hence, the therapeutic potential of this plant material should also be seen in combination with other medicinal agents and further biochemical and pharmacological investigations are needed to isolate and identify active ingredients in the extract using other models.

The plant materials should be further investigated for use in humans to treat diabetes mellitus and the various complications of diabetes like damage to liver and kidney.

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