

## Phytochemical evaluation and pharmacological screening of ethanolic and aqueous leaf extracts of *Pupalia lappacea* for antihyperlipidemic and antihyperglycemic activities in alloxan induced diabetic Albino wistar rats

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### ABSTRACT

**Background:** Diabetes is associated with damage to the liver, pancreas and kidney. The damage may vary among patients. This study assessed the hypoglycaemic and antihyperlipidemic activities of leaves ethanolic and aqueous extracts of *Pupalia lappacea* in alloxan induced diabetic albino wistar rats.

**Methods:** There were Fifty four rats divided into nine groups containing six rats each. Group 1 consists of normal rats that were given only normal saline and served as a control group. Group 2 consists of normal rats that were given alloxan monohydrate (150mg/kg b.w). Group 3 consists alloxan induced diabetic rats that were given daily sterile solution. glibenclamide+simvastatin (5mg/kg) Group 4 consists of alloxan induced diabetic rats that were given daily sterile solution, AEPL extract (100mg/kg). Group 5 consists of alloxan induced diabetic rats that were given daily sterile solution, drug extract AEPL (200mg/kg). Group 6 consists of alloxan induced diabetic rats that were given daily sterile solution, drug extract AEPL (400mg/kg) Group 7 consists of alloxan induced diabetic rats that were given daily sterile solution, drug extract EEPL (100mg/kg), Group 8 consists of alloxan induced diabetic rats that were given daily sterile solution, drug extract EEPL (200mg/kg) Group 9 consists of alloxan induced diabetic rats that were given daily sterile solution, drug extract EEPL (400mg/kg) respectively for 21days by an intragastric tube with free access of food and water.

**Results:** Chemical composition of the plant was estimated by GCMS technique several biochemical parameters were assessed. Oral administration of the extract resulted in significant reduction in mean values of blood glucose, triglycerides, total cholesterol, cholesterol ratio, LDL, VLDL, accompanied by increase in the mean value of the HDL in diabetic rats and histopathology of liver, pancreas, and kidney showed significant changes.

**Conclusions:** The effects produced were closely similar to standard antidiabetic and antihyperlipidemic drug. It can thus be concluded that the ethanolic and aqueous extract of *Pupalia lappacea* exhibit antihyperlipidemic and antihyperglycemic activities in alloxan induced diabetes in rats.

**Keywords:** Antidiabetic, Antihyperlipidemic, Alloxan, *Pupalia lappacea*

### INTRODUCTION

Research has shown that over 40% of plants comprise key ingredients which can be used for prescription drugs, and researchers look to traditional medicine as a guide to help them.

There are as many as one hundred and twenty compounds which have been taken from the higher plants, and 80 percent of those which are used in modern medicine have a positive connection between their There has been a resurgence of interest in herbal medicine interest in the past few years.<sup>1</sup> There are a number advantages associated

with using herbal medicines as opposed to pharmaceutical products. Reduced risk of side effects, Effectiveness with chronic conditions, Lower cost, Widespread availability.<sup>2</sup> *Pupalia lappacea* is a very variable plant. It can be annual or perennial. The plant is sometimes harvested from the wild for local use, mainly as a medicine but also as a food. Widespread through Africa but avoiding the wettest regions; through tropical Asia to Philippines, New Guinea and Polynesia. Savannah and woodland localities and forest path sides.

The plant has a number of medicinal uses. The leaves are antitussive, diuretic and febrifuge. They are taken in various ways in the treatment of coughs. They are also used in various ways to treat dysentery, diarrhoea and oedema. The leaves are used in an enema to treat constipation. Applied externally, the leaves are mixed with palm-oil or butter for the treatment of boils. The leaves are also used in topical applications to treat cuts.

The crushed seeds are considered to be a good remedy for infected sores and ulcers. The root is antidote and purgative. It is used in the treatment of snakebites and syphilis.

The plant-ash is mixed with water and then drunk in the treatment of flatulence. The ashes mixed with water are applied to leprosy sores after they have been made to bleed.<sup>3,4</sup>

Diabetes mellitus (or diabetes) is a chronic, lifelong condition that affects your body's ability to use the energy found in food. There are three major types of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes. Normally, the body breaks down the sugars and carbohydrates you eat into a special sugar called glucose. But the cells need insulin, a hormone, in the bloodstream in order to take in the glucose and use it for energy.<sup>5,6</sup> With diabetes mellitus, either the body doesn't make enough insulin, it can't use the insulin it does produce, or a combination of both. Since the cells can't take in the glucose, it builds up in the blood. High levels of blood glucose can damage the tiny blood vessels in the kidneys, heart, eyes, or nervous system. That's why diabetes - especially if left untreated - can eventually cause heart disease, stroke, kidney disease, blindness, and nerve damage to nerves in the feet.<sup>7,8</sup>

Hyperlipidemia, hyperlipoproteinemia, is abnormally elevated levels of lipoproteins in the blood in the blood. It is the most common form of dyslipidemia. Hyperlipidemia is divided into primary and secondary subtypes. Primary hyperlipidemia is usually due to genetic causes while secondary hyperlipidemia arises due to other underlying causes such as diabetes.<sup>9,10</sup>

The present study of the selected medicinal plants has been studied for its antidiabetic and antihyperlipidemic activity. As the leaves of the plant are rich in flavonoids the idea to

do research about its anti-diabetic and hypolipidemic activity stemmed out.

The objective of the study includes:

- Collection and authentication of leaves of the selected plant.
- Preparation of ethanolic extract by maceration.
- Selection and grouping of animals.
- Induction of diabetes in rats by intraperitoneal injection of alloxan.
- Screening of antihyperglycemic and antihyperlipidemic activities of extracts of the medicinal plant.

Biochemical estimation of the selected plant on glucose level, triglycerides level, total cholesterol level, low density lipoprotein, high density lipoprotein, very low-density lipoprotein.

- Samples were analysed by GCMS analysis results were tabulated in Table 1.
- Histopathological studies of liver, pancreas and kidney were performed.

## METHODS

### *Collection of drugs*

Dried leaves of the plant were collected. The plant was taxonomically identified and authenticated by Dr.K. Madhava Chetty Assistant professor of Botany, Department of Pharmacognosy, Sri Venkateshwara University, Tirupati.

### *Chemicals*

Ethanol, Distilled water, Alloxan monohydrate 150mg/kg b.w, Glibenclamide, Simvastatin

### *Preparation of plant extract*

The leaves of the plant were subjected to shade drying. On complete drying the leaves were powdered and stored in air tight containers at room temperature. The powder of leaves was macerated with ethanol and distilled water for 7 days and then filtered. The filtrate was evaporated to obtain dried extracts. The extracts obtained were subjected for evaluation of anti diabetic and anti hyperlipidaemic activity. The plant extract was prepared by maceration. The filtrate thus obtained was ethanolic extract and aqueous extract respectively (Figure 1).

### *Preliminary phytochemical screening*

The extracts were tested for the presence of secondary metabolites like, flavanoides, alkaloides, saponins, glycosides, steroids, proteins etc. and the results were plotted in the Table 2.<sup>11</sup>



**Figure 1 (A to E): Preparation of extract by maceration technique.**

**Table 1: Phytoconstituents of the extract.**

Phytoconstituent	Amount
Steroids	+++
Glycosides	+++
Saponins	++
Alkaloids	+++
Sugar	++
Phenol	+
Tannins	-
Flavanoides	+++

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

### Toxicity study

#### Acute oral toxicity

The acute oral toxicity studies of all the three extracts were undertaken as per the (OECD) guidelines for testing of chemicals by up-and-down procedure. The rats were fasted overnight, and the weight of each rat used was recorded just before use. Animals were divided randomly into a control and three treatment groups for each extract, each group consisting of four rats (two males and two females). Control group received only the vehicle and each treatment group received orally the AEPL and EEPL of the studied plant in the limit test at a rate of 2000mg/kg body weight was conducted and terminated after four survivals out of four animals. Animals were kept under close observation for 4 h after administering the extracts, and then they were observed daily for 3 days for any change in general behavior and other physical activities and mortality.<sup>12</sup>

#### Experimental animals

Wistar albino rats (180-250 g) of both sexes were selected. Before and during the experiment rats were fed with standard diet. After randomization to various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle.

### Experimental design

- Group-I: Normal control rats received normal saline p.o
- Group-II: Diabetic control rats received alloxan 150mg/kg.b.w i.p
- Group-III: Diabetic rats treated with (glibenclamide+simvastatin) 5mg/kg.b.w p.o
- Group-IV: Diabetic rats treated with AEPL 100mg/kg.b.w p.o
- Group-V: Diabetic rats treated with AEPL 200mg/kg.b.w p.o
- Group-VI: Diabetic rats treated with AEPL 400mg/kg.b.w p.o
- Group-VII: Diabetic rats treated with EEPL 100mg/kg.b.w p.o
- Group-VIII: Diabetic rats treated with EEPL 200mg/kg.b.w p.o
- Group-IX: Diabetic rats treated with EEPL 400mg/kg.b.w p.o

The body weight gain and fasting BGL of all the rats were recorded at regular intervals during the experimental period. The BGL was monitored in the diabetic rats by tail tipping method.

### Preparation of drug solution

Ethanollic extract of the leaves of *Pedilanthus tithymaloides* and *Pupalia Leppacea* dissolved in normal saline to prepare dose level of 100, 200, 400mg/kg body weight for administration into rats.

### Induction of diabetes

The animals (male rats) were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of Alloxan monohydrate (150mg/kg) in ice cold 0.9% NaCl saline solution. The animals were given 5% dextrose solution to drink immediately after induction to overcome the drug induced hypoglycemia. Control rats were injected with normal saline alone. 48 hours later rats with blood glucose level

(BGL) above 200mg/dl were considered diabetic and selected for the experiment.

**Table 2: Chemical composition of *Pupalia lappacea* by GCMS analysis.**

Retention time (min)	Active constituent	Molecular weight	Area %	Therapeutic uses
1.2	2,4-Thiazolidinedione	117	32.97	Anti diabetic, anti hyperlipidemic, anti convulsant, cardiogenic
1.490	3-Pentanone, 2,4-dimethyl-	114	2.10	
1.538	1-Propanol, 2-methyl	74	2.29	Hypertension, anxiety, migraine, headache, angina pectoris, myocardial infarction, cardiac arrhythmias, heart attack.
1.686	1-Butanol	74	0.21	
1.776	2-Pentadecyl-4,4,5,5-tetrahydro-1,3-dioxolane	284	0.23	Antioxidant activity
1.875	2-Propanol, 1,1'-oxybis	134	0.12	Hypertension, anxiety, migraine, headache, angina pectoris, myocardial infarction, cardiac arrhythmias, heart attack.
1.925	Acetone, ethyl methyl acetal	118	0.13	Fragrance, flavoring agent, solvent.
2.027	Ethane, 1,1-diethoxy	118	24.65	Anti knocking agent for carbureted fuels.
2.126	2-PENTENE, 3-METHYL	84	1.46	Anesthetic
2.301	Silane, diethoxydimethyl	148	0.89	Adhesive.
2.358	Propane, 2,2-diethoxy	132	3.57	stabilizer, additive.
2.683	3-Penten-2-one, 4-methyl	98	0.32	Synergistic for herbicide, insecticide.
2.834	Propane, 1,1-diethoxy-	132	0.23	Fragrance in cosmetic, perfume, detergent industries.
2.915	Ethane, 1,1-diethoxy	118	3.51	Anti knocking agent
3.117	Formic acid, 1-methylethyl ester	88	0.66	Anti microbial activity
3.495	Propane, 1,1-diethoxy-2-methyl	146	0.50	Anti malarial activity
4.192	4-Ethoxy-2-pentanone	130	14.49	Anti bacterial, anti viral agent,
4.525	CH <sub>3</sub> CH(OH)CH <sub>2</sub> C(O)CH <sub>3</sub>	102	0.03	Catalytic activity.
5.344	Butane, 1,1-diethoxy-3-methyl	160	0.41	Spasmolytic, coronary dilation, local anesthetic
17.534	Hexadecane	226	0.45	Emulsifying agent.
20.042	Heptadecane	240	0.75	Solvent
22.666	Heptadecane	240	2.97	Solvent
24.567	Undecane, 5-methyl-	170	0.06	Anti asthmatic, anti cancer
25.627	Heptadecane	240	2.21	Solvent
25.810	Pentadecane, 2,6,10,14-tetramethyl-	268	0.51	
27.117	Cyclohexane, 1,1'-[1-(2,2-dimethylbutyl)-1,3-propanediyl]bis-	292	0.23	Plant growth regulating effects in crop and non crop plants
27.208	2,6,6-Trimethyl-bicyclo	153	0.12	Insecticides, pesticides.
28.270	Eicosane	282	2.07	Anti bacterial and anti fungal in pharmaceutical cosmetic and food industries.
30.689	Nonadecane	268	0.28	Additive, arthritis, rheumatism, gingivitis, periodontal.
32.077	Oxiranecarboxamide, 2-ethyl-3-propyl	157	0.11	Hyperlipidemia by lipogenesis inhibition.
32.882	Hexadecane	226	0.49	Emulsifying agent.
35.921	Cyclohexane	292	0.22	
36.125	Beta-Ionone Epoxide	208	0.16	Flavoring agent, fragrance agent in pharmaceutical and agricultural industries
36.300	Cyclohexanol, 2-methyl-, cis	114	0.03	Centrally acting analgesic, trigeminal neuralgia.
41.933	2-Nonynoic acid	154	0.03	
42.956	1,2-Benzenedicarboxylic acid, dioctyl ester	390	0.42	Radio labeling agent, synthesis of dyes, perfume and other organic compounds, anti androgen.
43.058	5-cis-Methyl-1R,3-cis-cyclohexanediol	130	0.07	Highly active pesticides.

**Grouping treatment**

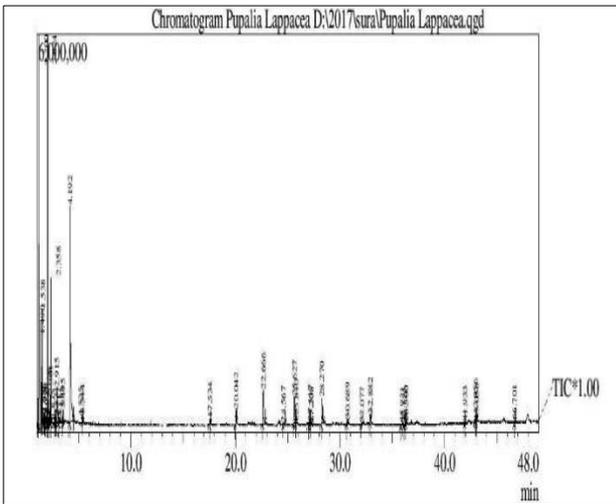
Groups 2, 3, 4, 5 and 6 will be induced diabetes by i.p route using alloxan monohydrate (150mg/kg b.w). The diabetic state of animals is then checked and confirmed. Animals becomes diabetic if their blood glucose level is 217±17mg/dl.

Collection of blood for determination of blood glucose level

Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i.e., 3 weeks). Fasting blood glucose estimation and body weight measurement were done on day 1, 7, and 14 and 21 of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips. On day 21, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated. Serum was separated and analyzed for following parameters. Blood glucose level, Total cholesterol, Triglycerides, High density lipoprotein (HDL), Low density lipoprotein (LDL), very low density lipoprotein (VLDL), cholesterol ratio were estimated by standard methods.

**RESULTS**

In toxicity studies, ethanolic leaf extract of *Pupalia lappaceae* did not exhibit any mortality and abnormal behavioural changes upto the dose level of 200mg/kg b.w in rats. Hence the pharmacological studies were carried out at an oral dose of 100, 200 and 400mg/kgb.w.



**Table 3: Effect of ethanolic extracts of the leaves of medicinal plants on blood glucose levels (mg/dl) in alloxan induced diabetic rats on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of treatment.**

Groups	1st day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
I	87.50±0.309	87.30±0.264	89.50±.242	87.5±0.392
II	228.3±0.226	238.2±0.288	265.8±0.285	281±0.423
III	216.7±0.311	154.6±0.278	130.3±0.211**	120.7±0.274***
IV	218.5±0.777	205.3±0.275	196.5±1.086*	178.9±0.244*
V	226.3±0.784	195.8±1.875	185.5±0.243*	180.5±0.026**
VI	220.6±0.712	184.5±0.182	168.5±1.004**	165.5±0.06**
VII	219.5±1.013	179.8±0.512	175.3±0.250**	150.6±2.184**
VIII	220.9±0.137	159.5±0.615	138.5±1.999**	123.6±1.466**
IX	222.9±0.235	168.9±0.274	130.8±1.651**	145.5±2.249***

- Values are given as ± SEM for group of six animals in each group.
- \*P<0.05, \*\*P<0.001, \*\*\*P< 0.0001 was considered significant comparing to diabetic control group.

**Table 4: Effect of ethanolic and aqueous extract of *Pupalia lappacea* on serum lipid profile in alloxan induced diabetic rats after 21 days of treatment.**

Groups	LDL	VLDL	HDL
I	33.5±0.763	16.80±0.325	32.83±0.279
II	80.50±0.76	27.34±0.202	18.67±0.213
III	31.5±0.343***	16.5±0.303***	32.67±0.232***
IV	54.33±0.208*	21.69±0.300*	22.39±0.346*
V	45.36±0.212**	20.36±0.199**	25.09±0.149**
VI	40.96±0.385**	18.95±0.164**	28.5±0.297**
VII	38.6±0.251**	17.9±0.216**	30.1±0.150**
VIII	34.33±0.214**	17.3±0.211**	31.49±0.289**
IX	33±0.154***	17.2±0.129***	32.35±0.168***

\*P<0.05, \*\*P<0.001, \*\*\*P<0.0001 was considered significant comparing to diabetic control group.

**Histopathological studies of liver**

- Group I (Liver)

Individual hepatocytes appeared normal in portal, periportal and centri lobular region of liver - Arrow

- Group II (Liver)

Foci of inflammation noticed in centri lobular region of liver - Arrow

Moderate foci of inflammation noticed in Perivascular region of liver - Arrow

- Group III (Standard)

Mild sinusoidal hemorrhages noticed in the liver - arrow

- Group IV (EEPL 100mg/kg b.w)

Individual hepatocytes appeared normal in portal, periportal and centri lobular region

But mild sinusoidal hemorrhages noticed in the sinusoidal spaces in the liver - Arrow

- Group V liver (EEPL 200mg/kg b.w.)

Individual hepatocytes appeared normal in portal, periportal and centri lobular region - Arrow

- Group VI liver (EEPL 400mg/kg b.w.)

Individual hepatocytes appeared normal in portal, periportal and centri lobular region – Arrow

**Table 5: Effect of ethanolic and aqueous extract of *Pupalia lappacea* on TC, TRG, CR in alloxan induced diabetic rats after 21 days of treatment.**

Groups	Total cholesterol	Total triglycerides	Cholesterol ratio
I	81.84±0.164	83.5±0.483	2.35±0.189
II	126.83±0.623	132.33±0.590	6.44±0.294
III	82±0.186***	85±0.371***	2.5±0.150***
IV	101.39±0.23*3	108.45±0.33*8	4.702±0.158*
V	89.4±0.256**	101.8±0.249**	3.813±0.177**
VI	86.02±0.199**	94.75±0.224**	3.35±0.120**
VII	86.45±0.204**	89.5±2.220**	2.935±0.220**
VIII	81.5±3.047**	85.5±0.329**	2.54±0.659**
IX	84.3±1.699***	86.5±0.923***	2.78±0.529***

\*P<0.05, \*\*P<0.001, \*\*\*P<0.0001 was considered significant comparing to diabetic control group.

**Histopathological studies of pancreas**

- Group I pancreas

Ductular pancreas appeared normal - Arrow

- Group II pancreas (Diabetic control)

Individual beta cell necrosis or apoptosis leads to atrophy of glandular pancreas - Arrow

- Group III pancreas (Standard)

Beta cells in islets of pancreas or glandular pancreas appeared normal - Red arrow

- Mild proliferation of beta cells noticed

Acinar cells in non glandular region appeared normal - Green arrow

- Group IV pancreas (EEPL 100mg/kg b.w.)

Moderate proliferation of beta cells in islets of pancreas - glandular pancreas indicate the active regeneration of islets cells initiated - Arrow

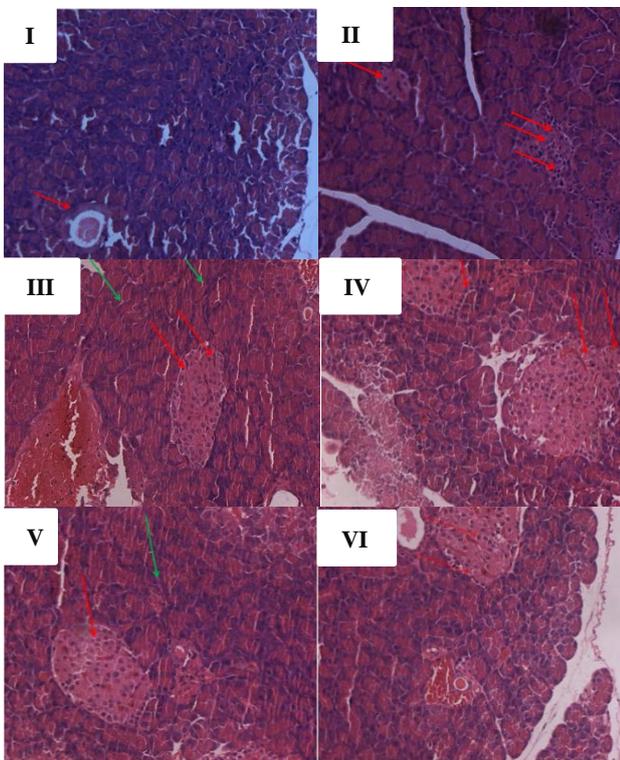
- Group V (EEPL 200mg/kg b.w.)

Moderate proliferation of beta cells in islets of pancreas - glandular pancreas indicate the active regeneration of islets cells initiated - Red arrow

Acinar cells in non glandular region appeared normal - green arrow

- Group VI Pancreas (EEPL 400 mg/kg b.w.)

Midl hemorrhages noticed in glandular pancreas - islet of pancreas but the islets cells appeared normal - Arrow.



**Figure 5: Histopathological studies of Pancreas [Group I (N.C), Group II (DC), Group III (STD), Group IV (EEPL 100mg/kg), Group V (EEPL200mg/kg), Group VI (EEPL400mg/kg)].**

### **Histopathological studies of kidney**

- Group I: Glomerulus appeared normal - Arrow

No degenerative and inflammatory changes noticed

- Group II: Nephrons were destroyed completely

- Group III: glomerulus was normal

- Group IV kidney (EEPL 100mg/kg b.w.)

Mild infiltration of lymphocytes noticed in Perivascular region of kidney - arrow

- Group V kidney (EEPL 200mg/kg b.w.)

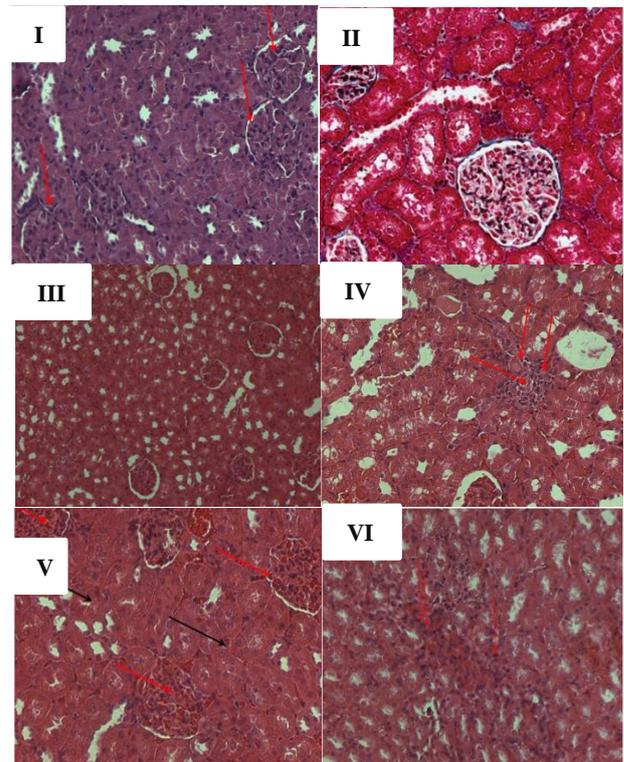
Glomerulus appeared normal - Arrow

No degenerative and inflammatory changes noticed

- Group VI kidney (EEPL 400mg/kg b.w.)

Glomerulus appeared normal - Red arrow

Tubular region appeared normal - Black arrow.



**Figure 6: Histopathological studies of kidney [Group I (N.C) Group II (D.C) Group III(STD), Group IV (EEPL100mk/kg) Group V (EEPL200mg/kg) Group VI (EEPL400mg/kg)].**

## DISCUSSION

In the present work, we investigated the hypoglycemic, anti hyperlipidemic property of the ethanolic and aqueous leaf extract of leaves of *Pupalia lappacea* in the alloxan induced diabetic animal model. Chemical substances also cause diabetes in experimental models. These includes substances such as alloxan and streptozotocin. In the present study alloxan is used as a diabetomimetic in mice. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas. The ethanolic and aqueous extracts of leaves of *Pupalia lappacea* was tested on laboratory animals.

In the present study, the administration of *Pupalia lappacea* leaf extracts to alloxan induced hyperglycemic rats demonstrated prominent reduction in blood sugar level the results are plotted in Table 3, normalization of serum biochemical profile including lipid content, cholesterol, as compared to alloxan control rats the results were plotted in Table 4 and Table 5. The phytochemical analysis has shown the presence of potent phytochemicals such as flavonoids, terpenoids, glycosides, steroids, saponin and phenols the results were tabulated in Table 1. This study revealed the presence of chemical composition of EEPL ethanolic extract by GCMS analysis. The results were tabulated in Table 2.

## CONCLUSION

The present study shows that the ethanolic leaf extract of *Pupalia lappacea* is effective in the treatment of diabetes. Diabetes was induced in rats by alloxan monohydrate at dose of 150 mg/kg body weight injected intraperitoneally. The animals were kept under observation. After 48hr the animal were tested for glucosurea using strips. The blood glucose level was checked before and after 72hr after alloxan injection to confirm the development of diabetes. At the end of the administration period, i.e 21 days, the rat was anaesthetized and dissect for the collection of blood and liver tissues. Serum was evaluated for the estimation of glucose, LDL, VLDL, HDL, cholesterol, triglycerides.

The results from this study indicated that ethanolic and aqueous leaf extract of *Pupalia lappacea* had hypoglycemic effects in alloxan induced diabetic mice, thus scientifically verifying its use in the management of diabetes mellitus. These actions were exhibited due to phytoconstituent present in the extract like flavonoids.

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*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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