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COMPARISION OF ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF SELECTED MEDICINAL PLANTS

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ABSTRACT

In this study area, herbal remedies are considered convenient for management of type 2 diabetes with postprandial hyperglycemia due to their traditional acceptability and availability, low costs and lesser side effects. The present study involves comparative screening of leaves of Taraxacum officinale, Piper betle and Leucas aspera *for anti-diabetic activity on alloxan induced diabetic and antioxidant activity by DPPH method. Wister rats are fed with 250mg/kg b.w and 500mg/kg b.w ethanolic extracts of Taraxacum officinale, Piper betle and* Leucas aspera *in alloxan induced diabetic rats,using glibenclamide as standard. Among all ethanolic extract,* Leucas aspera *shown more significant reduction in blood glucose level in alloxan induced diabetic rats, which are compared to control and glibenclamide (10 mg/kg b.w.).*

Keywords: *Antidiabetic, Blood glucose, Alloxan, Taraxacum officinale, Piper betle and Leucas aspera.*

INTRODUCTION

Diabetic mellitus (DM) is the condition arising due to abnormal metabolism of carbohydrate, proteins and fats. It is caused by insulin deficiency, often combined with insulin resistance. This disorder occurs worldwide and its occurrence is increasing quickly in most of the countries. Various complications develop as a consequence of the metabolic derangement in diabetes. The treatment of DM is based on parenteral insulin and oral anti-diabetic drugs. The knowledge of the system of diabetes mellitus, as the history reveals, existed with the Indians since prehistoric age. 'Madhumeha' is a disease in which a patient passes sweet urine and exhibits sweetness all over the body, i.e. in sweat, mucus, breathe, blood, etc. The practical usage of juices of various plants achieved the lowering of blood glucose by 10-20%. Diabetes mellitus is one of the common metabolic disorders. Almost 1.3% of the population suffers from this disease throughout the world [1] and number of diabetics is increasing by 6% per year [2].

Taraxacum officinale F. H. Wigg., commonly known as Dandelion (from the French dent-delion meaning lion's tooth) is thought to have evolved about thirty million years ago in Eurasia. The chief constituents of dandelion root are taraxacin, taraxacerin, and inulin (a sort of sugar which replaces starch in many of the Dandelion family, Asteraceae), gluten, gum and potash. Dandelions are one of nature's richest green vegetable sources of beta- carotene, from which vitamin A is created (14000 iμ/100 g leaves vs. 11 000 iμ/100 g in carrots). It is an important herb and is versatile in its nature, as being the whole plant can be used for medicinal as well as culinary purposes. Medicinally, dandelion is considered to be as anti-diabetic, detoxicant aperient, diuretic, stomachic, tonic, and stimulant Clarke CB [3].

Piper betle Linn. (Local name 'Paan') Piperaceae, a dioecious, annual creeper, climbing by many small adventitious rootless, grows to a height of approximately one metre, generally grown in hotter and damper parts of the country [4, 5]. It is generally found in damp forests and is propagated in India, Bangladesh, Vietnam and China. Plant part used is mainly leaves and roots. Oral administration of the water extract from the whole plant of *Piper betle* significantly lowered the plasma glucose levels in healthy rats. The water extract of *Piper betle* treatments lead to significant lowering of blood sugar level and reduction in serum lipids. It also has insulinomimetic activity [6].

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Leucas aspera belonging to the family Labiate is used as anti-inflammatory, stimulant, in jaundice, cough, asthma, conjunctivitis, diabetes, malaria, headache, otalgia, skin diseases, snake bite, toothache, and wound healing etc [7].

MATERIALS AND METHOD

Collection and Authentication of Plant Material: Fresh leaves of *Taraxacum officinale, Piper betle* and *Leucas aspera* were collected from the forests of Tirupathi in Andhra Pradesh. The plant material was identified and authenticated by Dr. K. Madhav Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University.

Preparation of plant extract: 100grams of *Taraxacum officinale,* 100grams of *Piper betle* and 100grams of *Leucas aspera leaves* was powdered, dried and continuously extracted for 48hrs with ethanol in a Soxhlet apparatus at 60°C. The collected extract was stored at 0-4°C until used.

Preliminary Phytochemical Screening:

Preliminary phytochemical investigation was carried out on hydroalcoholic extract of *Taraxacum officinale, Piper betle* and *Leucas aspera* for detection of various phytochemicals by following standard methods described in practical Pharmacognosy by C.K. Kokate and R.K. Khandelwal.

Experimental Animals:

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into 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. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiment were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee) with registeration number.

Acute Toxicity:

Rats selected by a random sampling technique were used in the study. Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD) 423 guidelines. Three male Wistar rats weighing between 150-200gm were used for each dose. The dose levels of 5mg, 50mg, 500mg, 1000mg, 2000mg and 3000mg/kg/body weight, were selected. The Lethal dose LD-50 value of the extracts was determined.

Evaluation of Antioxidant Potentials by DPPH method: [8]

To 1 ml of DPPH dissolved in methanol (0.33%), 1 ml of (1.25-10 µl/ml) essential oil/ascorbic acid was added. After the incubation for 30 min, at 37°C, the absorbance at 517 nm was measured using UV-spectrophotometer. Corresponding blanks were taken for the same. The experiment was performed in triplicate. The absorbance of DPPH as control was obtained at 518 nm. Lower absorbance of the reaction mixture was an indication of higher radical scavenging activity of essential oil/standard antioxidant. DPPH become a stable diamagnetic molecule by accepting an electron. The methanolic solution of DPPH (violet colour) has got a strong UV absorbance at 517 nm. The presence of a reducing environment in the solution pairs the odd electrons of DPPH radical and the solution in turn losses its colour stoichiometrically and also decreases the absorbance at 517 nm.

The DPPH scavenging activity (%) was measured using the followinf formula

DPPH radical scavenging activity (%)

$$\text{DPPH radical Scavening Activity (\%)} \; [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \text{ ----- (1)}$$

Where,
$Abs_{control}$ is the absorbance of DPPH radical + methanol
Abs_{sample} is the absorbance of DPPH radical + essential oil/standard

Anti-Diabetic Activity:

Experimental Animals: Alloxan induced diabetic model: [9, 10]

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (140 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

Experiment design:

Total of 54 rats were divided in to 9 groups (n=6) as follows

Group I - Served as normal control and did not receive any treatment.
Group II - Served as diabetic control and received alloxan monohydrate (140 mg/kg) and vehicle
Group III - Alloxan monohydrate + *Taraxacum officinale* (250 mg/kg, p.o.)
Group IV - Alloxan monohydrate + *Taraxacum officinale* (500 mg/kg, p.o.)
Group V - Alloxan monohydrate + *Piper betle* (250 mg/kg, p.o.)
Group VI - Alloxan monohydrate + *Piper betle* (500 mg/kg, p.o.)
Group VII - Alloxan monohydrate + *Leucas aspera* (250 mg/kg, p.o.)
Group VIII - Alloxan monohydrate + *Leucas aspera* (500mg/kg, p.o.)
Group IX - Alloxan monohydrate + Glibenclamide (10 mg/kg p.o.) served as standard

Group-I nondiabetic animals: received only 1% gum acacia (1 ml/kg/day, p.o.) for four weeks, and served as control. Group-II to IX was rendered diabetic by single intraperitonial dose of alloxan monohyderate 140 mg/kg, in citrate buffer (pH 4.5). Group II received 1 % gum acacia (1 ml/kg/day, p.o.) for 30days and served as diabetic control. Group-III and IV received two different doses of extracts of *Taraxacum officinale* (250 and 500 mg/kg/day, p.o.), Group-V and VI received two different doses of extracts of *Piper betle* (250 and 500 mg/kg/day, p.o.) and Group-VII and VIII received two different doses of extracts of *Leucas aspera* for 30days respectively. While Group-IX received glibenclamide (10 mg/kg/day, p.o.) for 30days.

Collection blood and serum samples: The glucose level is checked by glucometer from tail tip at different occasion, i.e., 0, 10th, 20th and 30th day. The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h).On 30th day, the blood was drawn from the retro orbital plexus of the rats, the blood samples were allowed to clot for 30mins at room temperature and then they were centrifuged at 3000 rpm for 10mins. The resulting upper serum layer was collected in properly labeled, clean and dry micro-centrifuge tubes. The serum samples were stored at -400 C and analyzed either immediately or within two weeks. The parameters studied were as follows: Biochemical parameters such as Serum total cholesterol [11, 12], Serum urea [13, 14], Creatinine [15, 16], Total protein [17-20], Body weight and Blood glucose.

Histopathololgy of pancreas:

The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5µthickness were cut and stained by haematoxylin and eosin (H & E) for histological examination.

Statistical Analysis:

Results were expressed as mean ± SEM, (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test by using Graph Pad Instat Software. P value less than 0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

RESULTS

Preliminary phytochemical screening:

Results of the preliminary phytochemical investigation of *Taraxacum officinale, Piper betle* and *Leucas aspera* are shown in Table No.1.

Table No. 1: Preliminary phytochemical screening

Sl. No.	Constituents	*Taraxacum officinale*	*Piper betle*	*Leucas aspera*
1	Alkaloids	-	+	+
2	Carbohydrates	+	+	+
3	Steroids	+	+	+
4	Protein	+	+	-
5	Tannins	+	+	+
6	Phenols	+	+	+
7	Flavonoids	+	+	+
8	Glycosides		+	+
9	Saponins	-	+	+
10	Terpins	+	-	-

+ = Present; -=Absent

Acute toxicity:

No acute toxicity was observed for ethanol extracts of *Taraxacum officinale, Piper betle* and *Leucas aspera* when it was administered orally at high dose level (3 g/kg body weight).

DPPH scavenging activities of *Taraxacum officinale, Piper betle Leucas aspera and* IC_{50} values:

The results for the free radical scavenging of *Taraxacum officinale, Piper betle* and *Leucas aspera* plant extracts and known antioxidants were presented in Table 5.5 and Fig 5.1. The results showed that ethanolic leaves extract of *Leucas aspera* exhibited the greatest free radical scavenging activity among the other plant extracts with a mean percentage of 88.93% at 100µg/ml. On the other hand, the scavenging activity of *Taraxacum officinale, Piper betle* extracts was 62.10% and 73.92% respectively at 100µg/ml (Table 2).

Table No. 2: DPPH scavenging activities

Conc.of extracts (µg/ml)	*Taraxacum officinale*	*Piper betle*	*Leucas aspera*
20	35.17	41.56	63.76
40	42.72	49.44	68.93.
60	47.26	52.17	74.67
80	50.12	63.89	85.01
100	**62.10**	**73.92**	**88.93**

IC_{50} values of *Taraxacum officinale, Piper betle* and *Leucas aspera:*

It is known that lower IC_{50} indicate higher antioxidant activity. Based on the screening results, it was indicated that the ethanolic leaves extract of *Leucas aspera* had the highest scavenging activity wth lower IC_{50} value 29.20 µg/ml. While *Taraxacum officinalea* and *Piper betle shows higher* IC_{50} values 52.48 and 40.66 µg/ml with low antioxidant activity when compared with *Leucas aspera.*

Anti-diabetic study of alloxan induced diabetic rats:

Body weight:

The Diabetic control showed significant decrease in the body weight during the treatment period. The diabetic animals treated with *Taraxacum officinale, Piper betle* and *Leucas aspera* (250mg/kg) showed slight reduction in body weight but not much when compared to control. The groups that received *Taraxacum officinale, Piper betle* and *Leucas aspera* 500mg/kg had shown significant results (Table 3 and Fig 1).

Biochemical parameters:

Diabetic animals treated with *Taraxacum officinale, Piper betle* and *Leucas aspera* showed significant decrease in serum creatinine, serum cholesterol and urea, and significant increase in serum albumin and total protein when compared with diabetic control (Table 5).

Histopathology:

Histopathological studies showed normal acini and normal cellular population in the islets of Langerhans in pancreas of control rats (Group I). Extensive damage to the islets of Langerhans and reduced dimensions of islets (Group II), restoration of normal cellular population size of islets were also shown (Group III-IX).

Table No. 3: Effect of *Taraxacum officinale (TO), Piper betle (PB)* and *Leucas aspera (LA)* on body weight in alloxan induced diabetic rats

Groups	Body weight of the animal (gms)			
	Initial	10th day	20th day	30th day
Normal	152±3.18	155±3.02	164±1.28	173±2.48
Alloxan +vehicle	189±2.10	178±4.20	169±2.62	145±4.21
Alloxan+ *TO* 250mg/kg	172±2.5	169±2.10*	172±4.36*	173±2.24*
Alloxan+ *TO* 500mg/kg	159±3.6	160±4.24**	162±7.10**	164±3.20**
Alloxan+ *PB* 250mg/kg	164±6.8	159±3.20*	160±2.80*	165±1.42*
Alloxan+ *PB* 500mg/kg	153±4.6	155±3.62**	158±6.20**	161±1.10**
Alloxan+ *LA* 250mg/kg	162±1.4	158±1.48**	159±2.80**	163±4.48**
Alloxan+ *LA* 500mg/kg	161±4.6	163±2.71***	170±4.30***	178±4.10***
Alloxan+ Glibenclamide 10mg/kg	**163±2.10**	**164±4.42*** **	**173±4.6*** **	**182±2.24*** **

Values are Mean ±S.E.M; n=5; ns= non-significant, *P <0.05, **P < 0.01 and ***P<0.001 vs. Diabetic Control

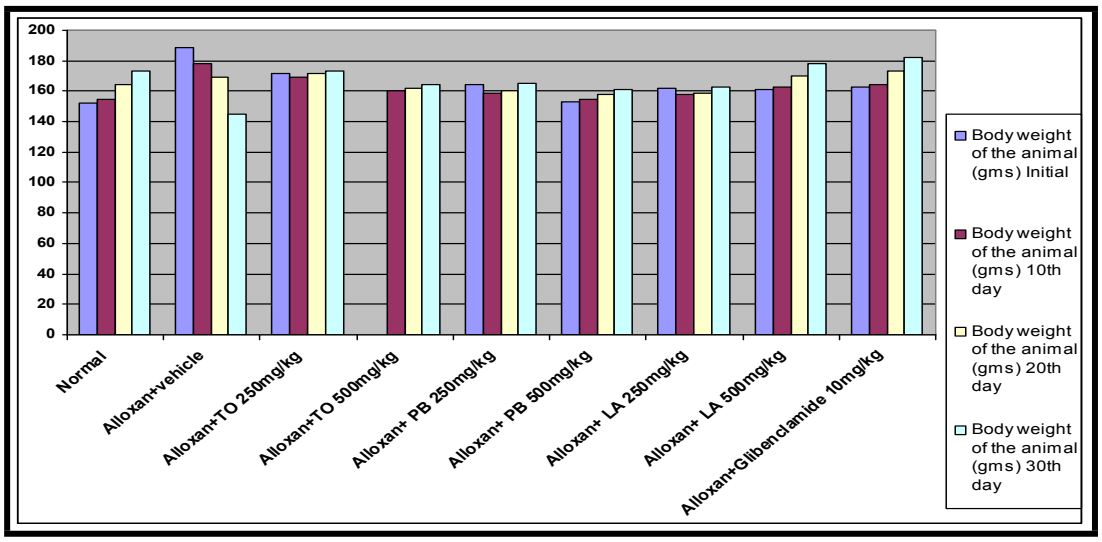


Fig. 1: Effect of *Taraxacum officinale, Piper betle* and *Leucas aspera* on body weight in alloxan induced diabetic rats

Table No. 4: Effect of *Taraxacum officinale (TO), Piper betle (PB)* and *Leucas aspera (LA) on* blood glucose level in alloxan induced diabetic

Groups	Blood glucose level (mg/dl)			
	Initial	10th day	20th day	30th day
Normal	65.4±2.2	66.1±2.6	67.2±2.6	66.4±4.2
Alloxan+vehicle	242.4±2.4	279.4±2.6	290.2±2.4	312.4±3.4
Alloxan+*TO* 250mg/kg	251.6±3.7	201±3.4*	164±6.3*	95.4±9.2*

Alloxan+*TO* 500mg/kg	262.8±6.4	175.1±3.6***	126.4±9.8***	76.5±2.8***
Alloxan+ *PB* 250mg/kg	251.5±3.5	196.4±8.1**	162.6±1.6**	93.6±5.3**
Alloxan+ *PB* 500mg/kg	248.8±5.0	172±5.4**	122.5±3.6***	74.4±5.6***
Alloxan+ LA 250mg/kg	255.2±4.6	192.2±4.2**	152.2±5.4**	89.4±2.6**
Alloxan+ LA 500mg/kg	260.3±2.4	162.5±3.1***	116.4±5.2***	69.2±4.8***
Alloxan+Glibenclamide 10mg/kg	**240.2±4.3**	**115.4±3.9*** **	**84.8±8.1*** **	**65.5±4.2*** **

*Values are Mean ±S.E.M; n=5; ns= non-significant, *P <0.05, **P < 0.01 and ***P<0.001 vs. Diabetic Control*

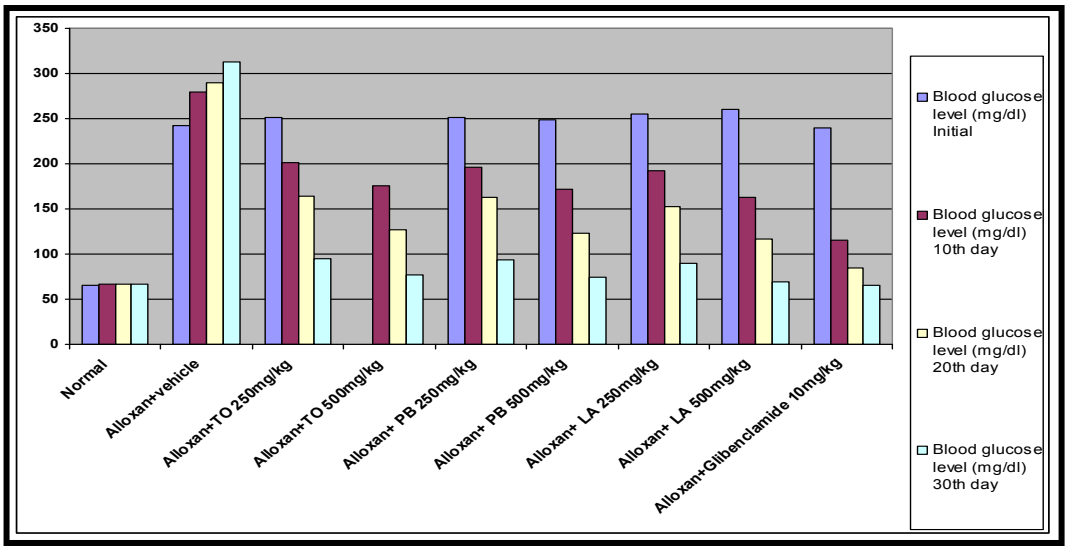
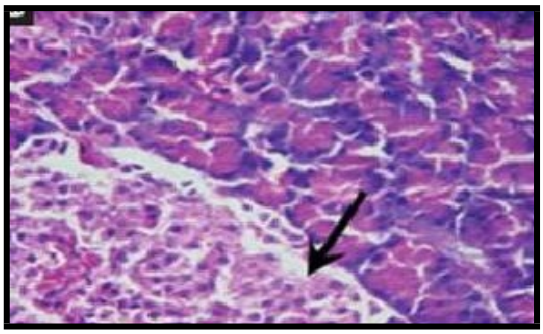


Fig. 2: Effect of *Taraxacum officinale, Piper betle* and *Leucas aspera* on blood glucose in alloxan induced diabetic rats

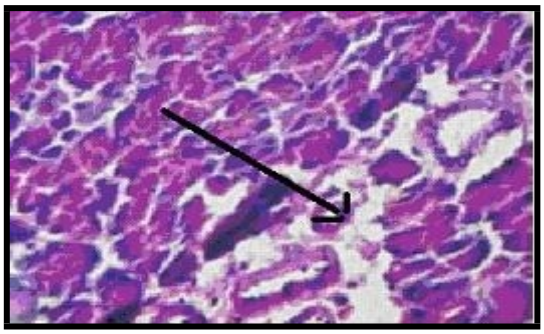
Table No. 5: Effect of *Taraxacum officinale, Piper betle* and *Leucas aspera* on biochemical parameters in alloxan induced diabetic rats.

Groups	Serum protein (mg/dl)	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Serum cholesterol (mg/dl)
Normal	7.0±0.2	37.2±0.1	0.71±0.01	**68.20±21.1**
Alloxan+vehicle	4.2±0.3	88.4±2.1	1.9±0.04	**135.25±1.3**
Alloxan+*TO* 250mg/kg	5.1±1.6	58.1±2.2*	1.34±0.08*	**102.2±6.8***
Alloxan+*TO* 500mg/kg	5.9±2.8	40.2±4.1**	0.82±0.03**	**76.40±4.2****
Alloxan+*PB* 250mg/kg	5.2±2.8	56.4±4.7**	1.24±0.06**	**94.06±0.4****
Alloxan+*PB* 500mg/kg	6.2±0.4	37.2±0.2***	0.79±0.02***	**74.26±3.4*****
Alloxan+LA 250mg/kg	5.4±0.2	54.5±6.3**	1.0±0.04**	**86.20±4.2****
Alloxan+LA 500mg/kg	6.6±0.4	36.8.8±2.8***	0.74±0.01***	**70.24±2.2*****
Alloxan+Glibenclamide 10mg/kg	**6.9±0.3**	**37.8±2.8*****	**0.72±0.02*****	**69.26±0.4*****

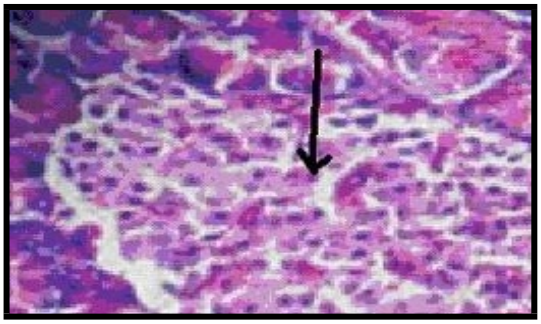
*Values are Mean ± S.E.M; n=6; * P<0.05, **P < 0.01 and ***P < 0.001 vs. Diabetic Control*



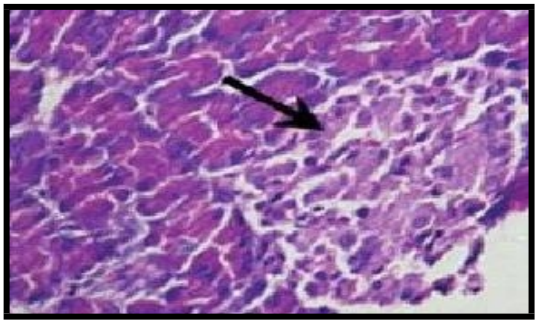
Group I: Normal



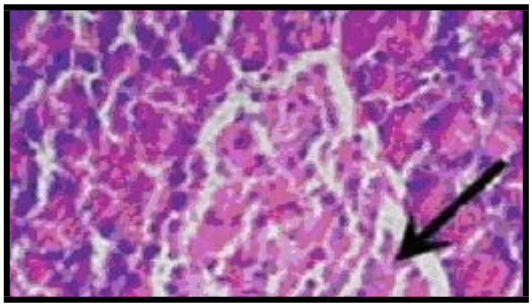
Group II: Diabetic rats



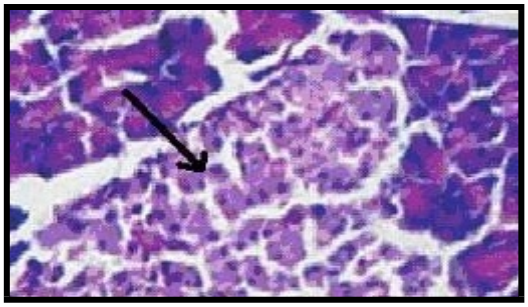
Group III: Alloxan+ TO 250mg/kg



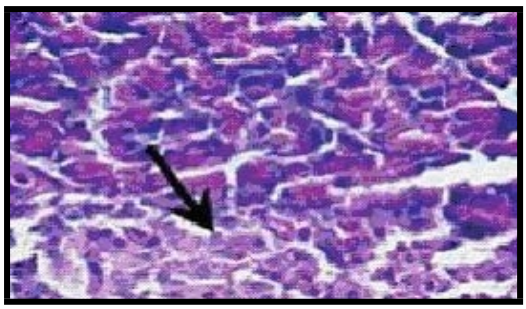
Group IV: Alloxan+ TO 500mg/kg



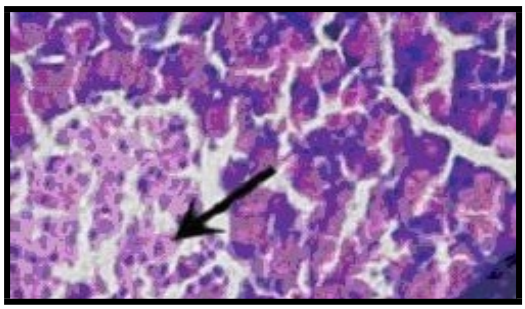
Group V: Alloxan+ PB 250mg/kg



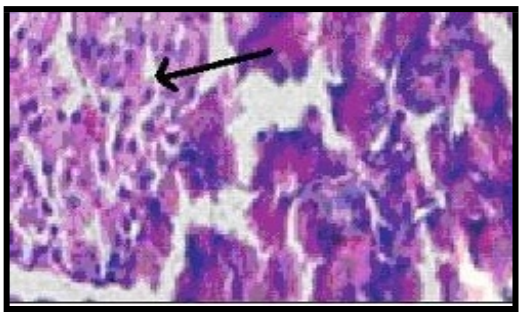
Group VI: Alloxan+ PB 500mg/kg



Group VII: Alloxan+LA250mg/kg



Group VIII: Alloxan+ LA500mg/kg



Group IX: Alloxan+ Glibenclamide 10mg/kg

DISCUSSION

Diabetes mellitus ranks highly among the top ten disorders which cause mortality throughout the world. Diabetes mellitus being chronic disorder, treatment without side effect for long term control is important. Present antidiabetic agent possess side effect as risk of hypoglycemia, anemia, choestatic jaundice [21]. There has been growing public interest in herbal medication for treatment of diabetes.

In the present study the periodic estimation of plasma glucose revealed that *Taraxacum officinale (TO), Piper betle (PB)* and *Leucas aspera (LA)* produced significant antihyperglycemic activity which began from 20nd day of treatment and it progressed throughout the study. The antidiabetic effect of the *Taraxacum officinale, Piper betle* and *Leucas aspera* could possibly be due to presence of glycosides, tannins and saponins. Substances like glycosides, alkaloids, terpenoids, tannins and saponins are frequently implicated as having antidiabetic effects [22].

Various reports suggest that there is reduction in the body weight in diabetic rats. Loss of body weight could be due to, dehydration and catabolism of fats and protein seen during diabetes mellitus [23]. It is reported that the recovery in body weight is far less in the poorly controlled diabetic rats as compared to well-controlled diabetic rats. In the present study diabetic control group rats showed significant loss of body weight. All animals treated with *Taraxacum officinale, Piper betle* and *Leucas aspera* showed significant prevention of the loss in body weight throughout the study. This prevention of loss in body weight by *Taraxacum officinale, Piper betle* and *Leucas aspera* may be due to increasing glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein or by glycemic control.

Diabetes produces qualitative and quantitative changes in the composition of the basement membrane and this altered material undergoes accelerated glycosylation and further rearrangement to form advanced glycation end-products (AGEs), which stimulate protein synthesis, further decrease degradability of the basement membrane, increase its permeability and cause endothelial dysfunction. Hyperglycemia increases the expression of transforming growth factor beta (TGFβ) in the glomeruli and of matrix protein specifically stimulated by cytokine. TGFβ may contribute to both the cellular hypertrophy and enhanced collagen synthesis is observed in diabetic nephropathy [24].

It has reported that inulin, a constituent in Taraxacum officinale, may act to buffer blood glucose levels and has experimental hypoglycemic activity in animals [25].

During diabetes, there is increased protein catabolism with inflow of amino acids to liver, which feed gluconeogenesis and accelerate ureagenesis, resulting in hypoproteinemia and hypoalbuminemia [26]. Diabetic hyperglycemia induces elevation of the levels of serum creatinine, urine total protein and urine albumin which are considered as significant markers of renal dysfunction [27].

In the present study, diabetic animals treated with *Taraxacum officinale, Piper betle* and *Leucas aspera* showed reduction in body weight, glucose levels, proteinurea and albuminurea and also showed improvement in the serum total protein and albumin level, of which *Leucas aspera found to be more effective than Taraxacum officinale* and *Piper betle.* Treatment with *Leucas aspera* also prevented the rise in serum creatinine levels more effective than *Taraxacum officinale* and *Piper betle* and. These results indicate that *Taraxacum officinale, Piper betle* and *Leucas aspera* attenuates the progression of renal damage in alloxan induced diabetic rats. The use of typical antioxidants alone or in combination may retard or even prevent the normal progression of diabetic complications [28].

The number of functionally intact β-cells in the islet organ is of decisive importance for the development course and outcome of diabetes. The renewal of β-cells in diabetes has been studied in several animal models. The total β-cell mass reflects the balance between the renewal and loss of these cells.

Histopathological studies showed normal acini and normal cellular population in the islets of Langerhans in pancreas of control rats (Group I). Extensive damage to the islets of Langerhans and reduced dimensions of islets (Group II), restoration of normal cellular population size of islets were also shown (Group III-IX).

Hence, the results obtained in the present study indicate that ethanolic leaves extracts of *Taraxacum officinale, Piper betle* and *Leucas aspera* has the potential to treat diabetes mellitus and prevent diabetes mellitus associated renal damage due to the antioxidant potentials present in the extracts.

CONCLUSION

In the present study the ethanolic extracts of *Taraxacum officinale, Piper betle* and *Leucas aspera* leaves shown better Anti-diabetic activities in experimental rat models, it may be due to the presence of flavonoids and other poly phenolic compounds. Hence, the research justifies that the extract of *Taraxacum officinale, Piper betle* and *Leucas aspera* leaves can be effectively used in treatment of diabetes by reducing the body weight and glucose levels. These extracts showed improvement in parameters like body weight and lipid profile as well as regeneration of β-cells of pancreas and so might be valuable indiabetes treatment. Further studies are needed to isolate and characterize the active component(s) responsible for the anti-diabetic properties of the test extract and findings should be confirmed by performing clinical studies.

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