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In Vitro and in Vivo Evaluation of Antidiabetic Activity of Leaf Essential Oil of *Pulicaria inuloides*-Asteraceae

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Abstract The essential oil of leaf of *P. inuloides* was extracted by using hydrodistillation process and the volatile components investigated with the help of gas chromatography coupled with mass spectrometry. Diabetic rats were treated with a 400 mg/kg dose of essential oil of *P. inuloides* orally for 3 h (acute) or 21 days (chronic). In acute model blood glucose levels were monitored at specific intervals. In the chronic model blood samples were collected from overnight fasted diabetic rats on day 21 to estimate blood glucose level. And the body weight, serum lipid profile and activities of liver and kidney enzymes were measured. Histopathological observations of liver sections were also studied. Total seventy compounds were identified as 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)- 58.19%; Oxirane, 2-ethyl-2-methyl- 8.25%; 1,2-Benzenedicarboxylic acid, diisooctyl ester 13.18%; n-Hexadecanoic acid 2.12% by gas chromatography coupled with mass spectrometry. Treatment of diabetic rats with 400 mg/kg of *P. inuloides* proved to have significant ($P < 0.05$) antihyperglycemic effect and have the capacity to correct the metabolic disturbances associated with diabetes. Histopathological studies showed that the essential oil of *P. inuloides* reinforced the healing of liver.

Keywords: *diabetes, rats, essential oil, Pulicaria inuloides, Histopathology*

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1. Introduction

Diabetes mellitus is abnormally high blood sugar level of the body's inability to use, and the conversion of glucose into energy. Diabetes happen as a result defects in the metabolism of carbohydrates to the lack of secretion of the pancreas to insulin, which leads to higher percentage of sugar in the blood and urine [1].

Recent years have experienced a sharp increase in the incidence and prevalence of diabetes mellitus, ranking it as the fourth or fifth leading cause of death in the world. It is associated with serious complications including coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness [2]. The current rapid global rise in diabetes rate is attributed to rapid rise in unhealthy life styles, urbanization [3]. Global estimate of the number of diabetics within the past three decades showed an increase from 153 million in 1980 to 347 million in 2008. Currently the prevalence of diabetes is estimated at 382 million people in 2013 and is expected to rise to 592 million by the year 2035. With this high prevalence, the highest mortality due to diabetis mellitus occurs in low and middle income countries [2]. Today, remarkable progress has been achieved in using traditional remedies in worldwide. There are many clinical and experimental evidences indicating involvement of oxidative stress in pathogenesis of diabetes [4]. Increased oxidative stress is due to excessive reactive oxygen species and inadequate antioxidant defenses [5]. Thus, it is necessary to reduce the oxidative stress in diabetics to reduce the severity of the disease. As plants produce significant amount of antioxidants to prevent oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Most of the traditional herbal remedies employed for treatment of diabetes also exhibits potential antioxidant activity. Plant aromatic medicinal have low toxicity levels and several therapeutic properties, it is more safer than synthetic drugs. Natural plant and food sources provide α-amylase and α-glucosidase inhibitors, which plays a role in offering techniques to control hyperglycemia with minimal side effects. Natural plant and food sources provide α-amylase and α-glucosidase inhibitors, which plays a role in offering techniques to control hyperglycemia with minimum side effects [5]. Plant materials used as traditional medicine for the treatment of diabetes are considered as one of the good sources for new drugs or a lead to make a new chemical entity (NCE). folk plants have been accepted by the users for the treatment of diabetes and any other diseases in many countries especially in the third world countries. Currently more than 400 plants are used in different forms for hypoglycaemic effects since all the claims of users are

baseless nor absolute. Therefore, a proper scientific evaluation and screening of plants by pharmacological tests followed by chemical investigations is of utmost importance. Genus Pulicaria belonging to the tribe Inuleae of the Asteraceae family consists of ca. 100 species distributed in Europe, North Africa and Asia and five species of this genus reported from Yemen [6]. This plant has been used as a traditional/herbal medicine among natives of these nations [7]. However, The main purpose of the present study was:

Firstly, evaluate the presence of various phytochemicals in leaves of *Pulicaria inuloides*.

Secondly, evaluation of essential oil of *P. inuloides* on diabetes

2. Materials and Methods

2.1Chemichals

All chemicals reagents were of analyzied grade and all solvents were HPLC grade. The chemicals were purchased from Sigma Al-drrich St. Louis, MO, USA.

2.2. Collection and Identification of Plant Material

Fresh matured whole leaves of *Pulicaria inuloides* were collected August 2014 during the flowering stage in the Sana'a area of Yemen. Botanical identification and authentication were performed in the Biology Department in the Faculty of Agriculture at Sana'a University. The identities, parts used and voucher specimen numbers of the investigated plants are SANB N. 196. They were sun-dried for 3 days. The dried leaves of the plant were pulverized by means of milling.

2.3. Isolation of Essential Oil

The essential oils of *P. inuloides* was obtained by steam-distillation of the plants using a pilot-scale system. Briefly, approximately 3 kg of fresh botanical material was loaded in the still. Steam was produced by a boiler and forced through the plant material to release the aromatic content. Steam containing the essential oils Eos was put through a cooling system for condensation. The Eos were spontaneously separated from the water solution and collected in a 50-mL Falcon tube containing 0.5 g anhydrous sodium sulphate (Na_2SO_4). The sample was vortexed for 30 s and centrifuged at 5000x g for 10 min at 5 °C. The Eos were then transferred to a screw-capped amber flask and stored at 10 °C±1. (As described in section 6. 2. 1).

2.4. Phytochemical Screening

Phytochemical screening was determined using methods variously described by standard procedures [8,9].

2.4.1. Test for Alkaloids

0.5 g of extract was diluted to 10 ml with acidified alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions and Mayer's reagent was added to one portion, while Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids**.**

2.4.2. Test for Anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H SO) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

2.4.3. Test for Steroids

In this test, 2 ml of acetic anhydride was added to 0.5 g of plant essential oil with 2 ml concentrated H_2SO4. The colour change from violet to blue or green is indication of steroids.

2.4.4. Test for Saponins

Approximately 0.5 g of extract was added 5 ml of distilled water in a test tube. Next in a water bath and filtered. The solution was shaken vigourously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion.

2.4.5. Test for Cardiac Glycosides

Keller-kiliani test was used to assess the presence of cardiac glycosides. 0.5 of extract diluted to 5 ml in water, 2 ml of glacial acetic acid containing one drop of 5 % ferric chloride ($FeCl_3$) solution. To this solution, 1ml of concentrated sulphuric acid was under-layered. The appearance of a brown ring at the interface of the two layers with the lower acidic layer turning blue green upon standing for a few minutes indicated the presence of cardiac glycosides.

2.4.6. Test for Reducing Sugar

To about 0.5 g of sample in the test tube was added 5 ml distilled water and the mixture boiled for 5 min. The mixture was filtered while hot and the cooled filtrate made alkaline to litmuspaper with 20 % sodium hydroxide solution. The resulting solution was boiled with an equal volume of Benedict qualitative solution on a water bath. The formation of a brick red precipitate depicted the presence of reducing compound.

2.4.7. Test for Terpenoids

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

2.5. Alpha-amylase Inhibition Assay

This assay was carried out using a modified procedure of McCue and Shetty [11]. A total of 250 µL of oil (1.25 – 10 mg/ml) was placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/ml) was added. The content of the tubes were pre-incubated at 25°C for 10 mins, after

which 250 µL of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals. The reaction mixtures were incubated at 25°C for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent and further incubated in boiling water for 5 min and cooled to room temperature. The content of each test tube was diluted with 5 ml distilled water and the absorbance measured at 540 nm in a spectrophotometer (Spectrumlab S23A, Globe Medical, England). A control was prepared using the same procedure except that the extract was replaced with distilled water. The α-amylase inhibitory activity was calculated as in Eq 1.

$$\% \text{ Inhibition} = \{(\text{Ac} - \text{Ae}) / \text{Ac}\}100 \quad (1)$$

where Ac and Ae are the absorbance of the control and extract, respectively.

The concentration of extract resulting in 50 % inhibition of enzyme activity (IC_{50}) was determined graphically using Microsoft Excel 2007.

7.2.6. α-Glucosidase Inhibitory Assay

The effect of the plant essential oil on α-glucosidase activity was determined according to the method described by Kim et al., [12], using α-glucosidase from Saccharomyces cerevisiae. The substrate solution p-nitropheynyl glucopyranoside (pNPG) (3.0 mM) was prepared in 20 mM phosphate buffer, pH 6.9. 100 µL of α-glucosidase (1.0 U/ml) was pre-incubated with 50 µL of the different concentrations of the extracts (ethanol) for 10 mins. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from pNPG at 405 nm. The results (% Inhibition) are expressed as percentage of the blank (control) as in Eq 2.

$$\% \text{ Inhibition} = \{(\text{Ac} - \text{Ae}) / \text{Ac}\}100 \quad (2)$$

where Ac and Ae are the absorbance of the control and extract, respectively. The concentration of extract resulting in 50 % inhibition of enzyme activity (IC_{50}) was determined graphically using Microsoft Excel.

2.7. In vivo Evaluation of Antidiabetic Activity of Essential Oil of Plant

2.7.1. Animals and Experimental Design

Forty eight of healthy adult male and female albino rats of Wistar strain (*Rattus norvegicus*), aged 50 days and weighing between 150 to 200 g were purchased from Shanghai Laboratory Animal Center, Chinese Academy Sciences. This work was carried out according to the international regulations for the use of laboratory animals. The animals were acclimatized to the laboratory conditions for ten days before the experimental work. The animals were housed in polypropylene cages, maintained under standard conditions (12-hours light/12-hours dark cycle; 25±3°C; 35%-60% humidity), were fed standard rat pellet diet and water *ad libitum*. Throughout the experiment, on a weekly basis, blood glucose and body weight were monitored and recorded.

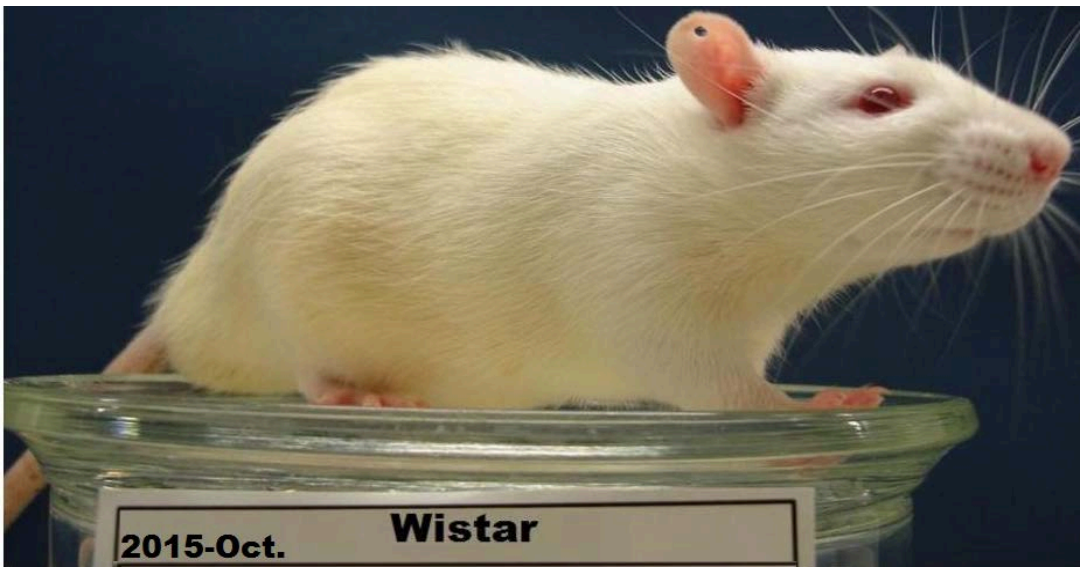

Figure 1. Wistar (*Rattus norvegicus*)

2.7.2. Acute Oral Toxicity Study

A limit dose of 2000 mg/kg body weight /oral was used. The signs of toxic effects and/or mortality were observed 3 h after administration then for the next 48 h. Since the essential oil of plant was found safe up to the dose level of 2000 mg/kg body weight, a dose of 400 mg/kg body weight of the different plant oil was selected for screening of the antidiabetic activity.

2.7.3. Induction of Diabetes Mellitus

Diabetes was induced by intraperitoneal injection of 55 mg/kg body weight of streptozotocin (STZ) (Sigma, St Louis, MO, USA) dissolved in freshly prepared citrate buffer (0.1 mol/L, pH 4×5). Fasting blood sugar for the animals was measured after 72 h using Medisafe Mini Blood Glucose Reader (TERUMO Corporation Ltd., Hatagaya, Tokyo, Japan). Rats with fasting blood sugar level 200 mg/dL were considered diabetic [13].

2.7.4. Oral Glucose Tolerance Test with Essential Oil in Diabetic Rats

The animals were divided into four groups with sex rats in each group. In group 1, Normal healthy rats (control) were treated with distilled water (5 mg/kg body weight) and used as the negative control. In group 2, Diabetic control rats were treated with distilled water (5 mg/kg body weight). In group 3, Diabetic rats were given standard drug glibenclamide (5 mg/kg body weight). In group 4, diabetic rats were treated with essential oil of *P. inuloides* leaves at dose of 400 mg/kg body weight. All the diabetic rats were fasted overnight (14 h) before the oral glucose tolerance test was done. Thirty minutes following the extracts or glibenclamide treatment, each rat was given an oral glucose load of 3 g/kg body weight. Blood samples were withdrawn from retro-orbital site at intervals of 60, 120 and 180 min of glucose administration.

Figure 2. Oral induced in Wistar (*Rattus norvegicus*)

2.7.5. Estimation of Fasting Blood Glucose

Blood glucose was investigated using Medisafe Mini Blood Glucose Reader. The glucose dehydrogenase present in the strip converts the glucose present in the blood sample to gulonolactone and this produces electrons which creates a proportional current which is displayed on the monitor of the meter 9.

2.7.6. Collection of Blood Samples and Estimation of Biochemical Parameters

At the end of the experimental period, day 21, the animals were fasted an overnight and the rats were dissected after treatment and dissected under ether anaesthesia. Pancreas and liver were collected immediately after dissection in tubes with heparin. For serum samples, blood was allowed to coagulate, followed by centrifugation at 3000 r/min for 15 min at 4 °C to separate serum. Sera were divided into aliquots and stored at -80 °C for biochemical assay.

Figure 3. collection of blood via cardiac puncture in Wistar (*Rattus norvegicus*)

2.8. Biochemical Analysis

For biochemical analysis we used standard commercial kits according to the manufacturer's protocol. Fasting serum glucose level was determined on day 21 by glucose

oxidase-peroxidase. method using the kit of RANDOX Laboratories Ltd, UK. Serum total cholesterol (TC), serum triglycerides (TG), and high density lipoprotein-cholesterol (HDL-C)] were also measured using kits (HDL-C) was calculated as follows: HDL-C= TC- TG / 5-HDL. Serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alanine Aminotransferase (ALT) were measured using kits from Randox Laboratory Ltd., UK. Serum urea was determined using a commercial kit from QUIMICA Clinica Aplicada, Amposta, Spain.

2.9. Tissue Preparation for Histology

The animals were sacrificed and liver tissues were collected, washed in normal saline and fixed by using fixative (picric acid, formaldehyde 40% and glacial acetic acid) for 24h and dehydrated with alcohol. All tissues were cleaned and embedded by using xylene and paraffin (melting point 55-60°C). Tissues were stained by double staining process. To differentiate the nucleus and cytoplasm, the basic due haematoxylin and the acid dye eosin were used. Electron micrographs were performed using transmission electron microscope and photographed by photomicrography.

2.10. Statistical Analysis

All the values were expressed as mean ± S.E.M. Data were analyzed by using ANOVA and Tukey/ Duncan post hoc test. The results were considered significantly different at $P<0.05$.

3. Results

3.1. Phytochemical Analysis

The results obtained from the phytochemical screening conducted on the *Pulicaria inuloides* leaf extracts are presented in Table 1. Anthraquinone was absent in the oil while Alkaloids, steroids, saponins, glycosides and reducing sugar were common.

Table 1. Phytochemical constituents of essential oil of *Pulicaria inuloides*

Compounds	Leaves essential oil
Alkaloids	+
Anthraquinones	-
Steroids	+
Saponins	+
Glycosides	+
Reducing sugar	+
Terpenoids	+

(+) = present and (-) = not detected.

3.2. In Vitro α-Amylase Inhibition Study

The maximum inhibition of *Pulicaria inuloides* essential oil was 85.50% at a concentration 250 µg/mL. The percentage inhibition ranged from 85.50% - 30.83 %. The IC_{50} values are tabulated.

Table 2. α-Amylase inhibitory activities of *Pulicaria inuloides* essential oil with IC_{50} values

Sample	Concentration (µg)	% inhibition	IC_{50}
	50	30.83±0.6	
	100	42.0±0.1	
Pulicaria inuloides	150	51.1±0.2	140.49±0.33
	200	63.8±0.1	
	250	85.50±0.2	

3.3. In Vitro α-glucosidase Inhibition Study

The in vitro α-glucosidase inhibitory studies demonstrated that *Pulicaria inuloides* had α-glucosidase inhibitory activity. The highest concentration 250 µg/ml tests showed a maximum inhibition of nearly 86.52 %. The percentage inhibition varied from 86.52 - 33.89 % from the highest concentration to the lowest concentration of 50 µg /ml. Table 3 represents the inhibitory activity of leaves of *Pulicaria inuloides* essential oil.

Table 3. α-glucosidase inhibitory activities of *Pulicaria inuloides* essential oil with IC_{50} values

Sample	Concentration (µg)	% inhibition	IC_{50}
	50	33.89±0.4	
	100	46.33±0.0	
Pulicaria inuloides	150	55.61±0.4	128.33±0.12
	200	70.48±0.2	
	250	86.52±0.4	

4. In vivo Antidiabete Activity of *Pulicaria inuloides* Leaf Essential Oil

4.1. Effect of *Pulicaria inuloides* on Body Weight (g) in Normal and Diabetic Rats

Results of the effect of leaves of *Pulicaria inuloides* oil at 400 mg/kg/day dose on body weight of STZ induced diabetic rats after 3 weeks of treatment are presented in Table 4. By the end of third week, diabetic rats gained less body weight with significant reduction ($P < 0.05$) to the normal healthy control rats. The essential oil of *Pulicaria inuloides* improved the body weight of diabetic rats with significant increase ($P < 0.05$) compared to the diabetic control rats.

Table 4. shows the effect of *Pulicaria inuloides* on body weight in normal and diabetic rats

		Body weight			
Groups	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
Group 1	Healthy control	153.11 ±0.22	162.66±0.88	178.24±0.22	210.22±0.98
Group 2	Diabetic control	140.11±1.44	115.73±0.78	100.00±1.63	70.30±1.7
Group 3	Standard	148.00±1.42	158.73±1.10**	174.23±1.07**	203.00±1.42**
Group 4	Leaves oil	145.40±0.12	159.00±1.22**	171.15±1.44**	200.44±1.13**

Data represent means ± SEM., n = 6 in each group, *p<0.05, **p<0.01, as compared to control group

4.2. Effect of *P. inuloides* on Serum Glucose Level in Normal and Diabetic Rats

Blood glucose level of the normal control rats, STZ induced diabetic control rats and diabetic rats treated with glibenclamide and Leaves oil dosed at 400 mg/kg at different time points (0, 7, 14 and 21 days) after oral administration of glucose (2 g/kg) is shown in Table 5. In the diabetic control rats, the results increase in blood glucose level was observed after day 7 and remained high over the next day 21. The essential oil of *Pulicaria inuloides* reduced ($P < 0.05$) the blood glucose level at day 7 and remained low over the next 21day when compared with the diabetic control rats.

Table 5. shows the effect of *Pulicaria inuloides* on the blood glucose level in normal and diabetic rats

		Blood glucose level (mg/dl)			
Groups	Dose(mg/kg)	Day 0	Day 7	Day 14	Day 21
Group 1	Healthy control	73.24±0.84	74.04±0.13	75.50±1.51	76.07±0.21
Group 2	Diabetic control	184.10±1.88	188.02±1.07	192.00±1.22	198.5±1.33
Group 3	Standard	182.75±1.84	142.11±1.41	110.50±1.22 **	75.55±0.21**
Group 4	Leaves oil	186.30±1.34	146.31±1.20	115.11±1.13**	78.23±0.11**

Data represent means ± SEM., n = 6 in each group, *p<0.05, **p<0.01, as compared to control group.

4.3. Effect of *P. inuloides* on Lipid Profile in Normal and Diabetic Rats

The effect of *P. inuloides* essential oil was at 400 mg/kg/ day dose on serum lipid profiles of STZ-induced diabetic rats after 3 weeks**.** High cholesterol, high triglyceride (TG), high LDL cholesterol and low HDL cholesterol was observed in the diabetic animals of the present study (Table 6). Serum cholesterol, triglyceride high LDL cholesterol and low HDL cholesterol levels were decreased significantly by *P. inuloides* essential oil.

Table 6. Effect of *P. inuloides* essential oil on serum lipids profile in diabetic rats

		Serum lipid profile levels (mg/dL)			
Groups	Dose(mg/kg)	Total cholesterol	HDL-Cholesterol	Triglycerides	LDL
Group 1	Healthy control	148.01±1.11	44.11±1.01	84.78±1.04	13.23±1.24
Group 2	Diabetic control	**250.00±1.20**	**30.00±1.02**	**140.10±1.06**	**35.21±1.35**
Group 3	Standard	93.12±1.26 **	40.38±2.66 *	80.87±1.12 **	16.71±1.04 **
Group 4	Leaves oil	96.35±2.52 **	39.33±1.63*	85.01±1.22 **	15.00±2.04 **

N = 6, values are mean ±SEM, *p<0.05, **p<0.01, as compared to control group.

4.3.1. Changes on Total Cholesterol Level

Diabetic rats showed significantly high level ($P < 0.05$) of total cholesterol (**69**% compared to the healh control rats). A significant decrease ($P < 0.05$) 61.46% was observed in total cholesterol of diabetic rats given *P. inuloides* essential oil compared to the diabetic control.

4.3.2. Changes on High-Density Lipoprotein Cholesterol Level (HDL)

Diabetic rats showed insignificantly low level of HDL cholestrol. Treatment of diabetic rats with leaves oil of *P. inuloides* induced a significant increase ($P < 0.05$) 31.1% in HDL cholesterol compared to the diabetic control.

4.3.3. Changes on Total Triglycerides Level

Diabetic rats showed significantly high level ($P < 0.05$) of total trigycerides (65.25% compared to the normal control rats). Percentage reduction in total triglycerides after treatments ranged from 39.32% in *P. inuloides* to 42.28% in Standard. Thus, treatment of diabetic rats with leaves oil of *P. inuloides* enabled it to reduce the total triglycerides to levels comparable to that of normal controls.

4.3.4. Changes on Low-density Lipoprotein cholesterol level (LDL)

Diabetic rats showed highly significant ($P < 0.05$) HDL cholesterol level (166.14% compared to the normal control rats). Treatment of these rats with essential oil of *P. inuloides* and leaves and standard resulted in significant reduction in the level of HDL-C($P < 0.05$), corresponding to percentage reduction of 57.40% and 52.54% respectively.

4.3.5. Effect of Pulicaria Inuloides on Serum Biochemical Parameters in Normal and Diabetic Rats.

Results of the effect of *P. inuloides* leaf oil at 400 mg/kg/day on kidney function markers (Protein, Urea, creatinine, AST, ALT and ALP) in STZ- induced diabetic rats after 3 weeks are presented in Table 7.

4.3.5.1. Changes in Protein Level

Diabetic rats showed insignificant decrease ($P < 0.05$) protein level (50% compared to the normal control rats). Percentage reduction in total triglycerides after treatments ranged from 76.38% in *P. inuloides* to 78.31% in Standard**.**

4.3.5.2. Changes on Level of Urea

In diabetic control rats, the levels of urea was significantly increased ($P <0.05$) by 152.16% compared to normal control rats. When diabetic rats were treated with essential oil of *P. inuloides* leaves, the urea level was reduced by 56.78% compared to diabetic control rats and 36% below the normal level.

4.3.5.3. Changes on Level of Creatinine

In diabetic control rats, a significant increase in the level of creatinine was observed ($P <0.05$) (141.46% compared to level of normal control rats). However,

treatment of diabetic rats with essential oil of *P. inuloides* and standard significantly (P <0.05) reduced their creatinine levels by 74.75% and 80% respectively compared to the diabetic group, bringing the level closer to normal value.

4.3.5.4. Changes in Activity of Plasma AST

In diabetic control rats the activity of plasma AST was significantly (P <0.05) increased (142.10% relative to normal levels). After treatment of diabetic rats with leaves of *P. inuloides*e oil and standard (Glibencalmide) , the level of AST was significantly (P <0.05) decreased by 33.39% and 55.22% respectively to reach values closer to normal level.

4.3.5.5. Changes in Activity of Plasma ALT

In diabetic control rats the activities of plasma ALT was significantly (P <0.05) increased by 218.45% relative to normal level. The treatment of diabetic rats with *P. inuloides* essential oil and standard significantly reduced (P <0.05) the level of ALT by 62.32% and 68.60% respectively compared to the diabetic control rats.

4.3.5.6. Changes in Activity of Plasma ALP

In diabetic control rats the activities of plasma ALP was significantly (P <0.05) increased by 75.08% relative to normal level. The treatment of diabetic rats with *P. inuloides* essential oil and standard significantly reduced (P <0.05) the level of ALP by 40.54% and 41.88% respectively compared to the diabetic control rats.

Table 7. shows the effect of *P. inuloides* on serum biochemical parameters in normal and diabetic rats

		Serum biochemical parameters					
Groups	Dose(mg/kg)	Total protein (g/dL)	Urea (g/dL)	Creatinine (IU/L)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group 1	Healthy control	8.30±0.10	21.03±0.11	0.41±1.02	9.50±0.10	15.00±1.14	241.11±0.35
Group 2	Daibetic control	4.15±1.22	62.77±0.30	0.99±0.12	23.00±0.30	47.77±1.82	422.13±0.34
Group 3	Standard	7.40±1.07 **	23.22±0.08**	0.20±1.11 **	.10.30±1.12 **	15.01±1.23**	245.33±0.51 **
Group 4	Leaves oi	7.32±1.12 **	27.13±0.05 **	0.25±1.12 **	15.32±0.11 **	18.00±1.24 **	251.25±0.31 **

N = 6, values are mean ±SEM, *p<0.05, **p<0.01, as compared to control group.

4.3.5.7. Histopathological Studies

Images of sections of liver are shown in Figure 1. Normal control rats (Figure 4A) showed more or less normal hepatic parenchymal organization, with normal portal tracks and sinusoids. Unlike other rats, the sinusoids of normal controls did not show hypertrophied Kupffer cells. Sections of the diabetic control rats (Figure 4B) seemed to reflect friable parynchemal architecture withintense infiltration of polymorph leucocytes in portal triads zones. Hypertrophied Kupffer cells ran along the sinusoids, and hepatocytesat different stages of necrosis could be detected; in addition, therewas hemorrhage into foci of lysed hepatocytes. Histopathological features in sections of rats treated with glibenclamide (Figure 4 C) showing normal hepatocellular architecture with normal nucleus, cytoplasm and distinct hepatic layer. Histopathological features in sections of rats treated with *P. inuloides* essential oil section liver shows normal hepatocellular architecture with normal nucleus and cytoplasm.

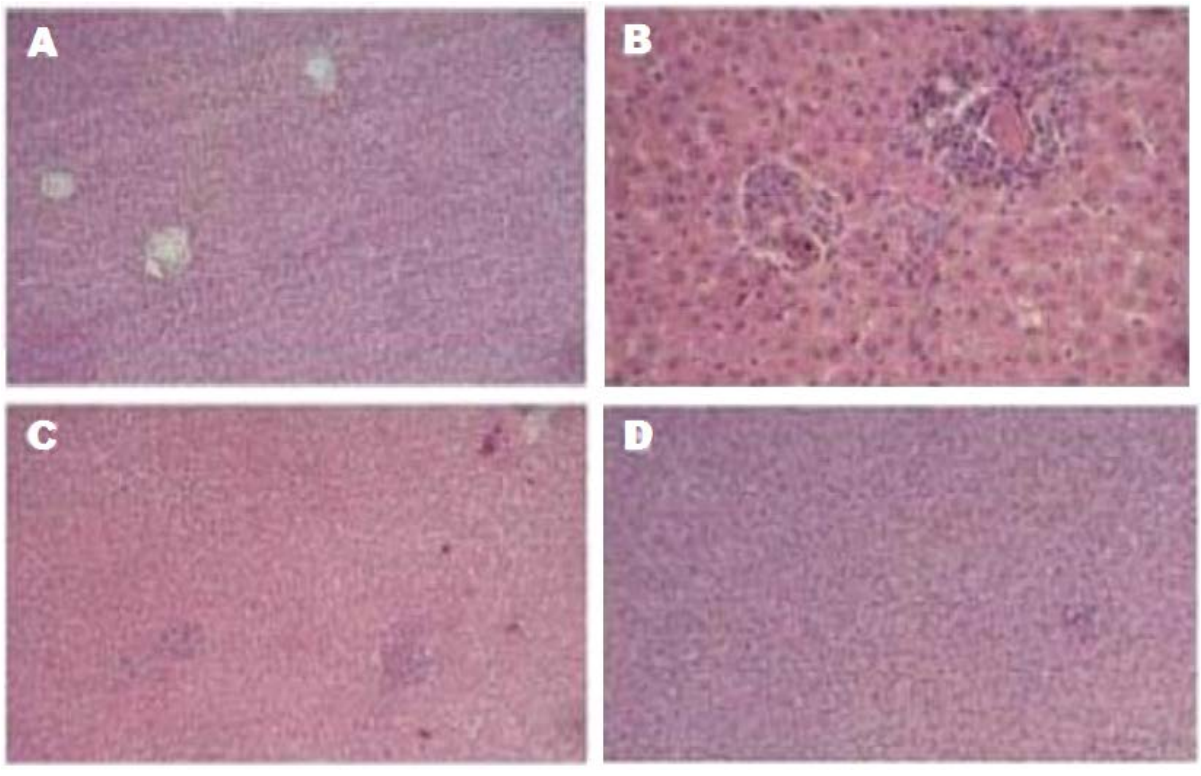

Figure 4. Histology of liver in experimental rats after 21days of treatment

5. Discussion

5.1. Phytochemical Analysis

Preliminary phytochemical screening of the *P. inuloides* essential oils were summarized in Table 1. Alkaloids, steroids, saponins, glycosides, raducing sugar and terpenoides were present in the essential oil of *P. inuloides*. These components were reported to exhibit a variety of biological activities including antiviral, antibacterial, anti-inflammatory and analgesic, all of which were relevant to the traditional uses of some species of the genus [15]. Terpenoids identified in primary phytochemical screening may contribute to this promising hypoglycemic activity [15,16]. Terpenes have been found to stimulate the secretion of insulin or possess insulin like effect isolated from anti-diabetic medicinal plants. Terpenoids type components mono-terpenes cause a restoration to normal glycogen metabolism when hepatic glycogen concentration is reduced [11]. Hypoglycemic effect of glycoside is also proved [18,19]. We are still not sure about how this plant essential oil effect on hypoglycemia. In the future, detecting the active biological compounds responsible for this hypoglycemic action may offer novel and safe antidiabetic compound.

5.2. Inhibition Assay for α-amylase and α- glucosidase

Diabetes mellitus is a dreadful disorder and leads to various other metabolic disorder. It is estimated that its annual incidence rate will continue to increase in the future worldwide. Diabetes involves with the development of micro and macro vascular diabetic complications [20]. In human, glucose tolerance impairs prior to maturity-onset of hyperglycemia [21,22] and is widely used as a clinical index to predict the potentiality of developing diabetes [23]. The objective of our study is to investigate the hypoglycemic effect in the ethanol extract of *Pulicaria inuloides*. In the present study, in vitro α-amylase and α-glucosidase inhibitory studies demonstrated that *Pulicaria inuloides* had inhibitory activity. The percentage inhibition at 50, 100, 150, 200, 250 µg/mL concentrations of *Pulicaria inuloides* on α-glucosidase and α-amylase showed a concentration dependent reduction in percentage inhibition. The highest concentration of *Pulicaria inuloides* (250 µg/mL) tests showed a maximum inhibition of nearly 85.50% and 86.52 % of α -amylase andα-glucosidase respectively. The antidiabetic effect of *Pulicaria inuloides* might attribute to its inhibitory effect against a-glucosidase that retarding the digestion of carbohydrate to delay the postprandial rise in blood glucose. Our in vitro studies demonstrated an appreciable α-glucosidase and α- amylase inhibitory activity present in *Pulicaria inuloides* where further experiments can be performed on animal models to confirm the hypoglycemic activity. It is a safe and effective intervention for diabetes.

5.3. Effect of Plant Essential Oil on Body Weight

Diabetic rats showed a significant reduction in the body weight, which reversed significantly ($p<0.05$) by *Pulicaria inuloides* essential oil treated groups (leaves) and glibenclamide (10 mg kg^{-1}) treated group during 21 days treatment, as shown in Table 4. There was significant loss in body weight of diabetic rats compared to normal rats. The loss of body weight associated with STZ Streptozotocin induced diabetes could be due to dehydration and catabolism of fats or breakdown of tissue proteins [24]. Normal body weight gain is indicator of efficient glucose homeostasis; but in diabetics, glucose is not available therefore the cells use alternatively proteins for energy; consequently due to excessive breakdown of tissue protein a loss in body weight occurs. Similar effect on body weight gain was reported with other plants, well known for their anti-diabetic activity [25]. This is also in agreement with the finding that normal controls that were given the extracts had a higher weight gain compared to the untreated normal controls. This could in part be explained also by the fact that extract treated normal controls had a higher food intake compared to untreated normal controls.

5.4. Effect of Plant Essential Oil on Fasting Blood Glucose

The fasting blood glucose was significantly increased in the high fat and sucrose-induced diabetic group compared to control while the *P. inuloides* treatment to diabetic group was able to significantly lower the blood glucose level similar to the standard (glibenclamide) treated diabetic group. The antihyperglycemic effect of PIEO may be attributed to several mechanisms such as its ability to increase glucose uptake and oxidation in the peripheral tissues, impair absorption of glucose at the intestine α-glucosidase inhibition [26], enhance insulin sensitivity and thereby increasing the glucose transporters [27], control lipid metabolism thereby mending the putative inhibition of insulin signaling [28,29], scavenge the free radicals antioxidant activity [30] which disrupt the plasma membrane integrity, resulting in decreased plasma membrane receptors or transporters necessary to signal and uptake glucose from the blood stream [31]. In the present study, we have assessed the glucose uptake and oxidation in liver, one of the mainorgans necessary to maintain glucose homeostasis.

5.5. Effect of *Pulicaria inuloides* on Lipid Profile in Normal and Diabetic Rats

The elevated levels of serum cholesterol and triglycerides with reduced level of serum HDL cholesterol in diabetic condition, poses to be a rises of factor for developing microvascular complication leading to atherosclerosis and further leads to cardiovascular diseases like coronary heart disease. The abnormal high concentration of serum lipid found in diabetic rats was due to increased mobilization of free fatty acids from peripheral fat depots, since insulin inhibits the hormone sensitive lipase, insulin deficiency or insulin resistance may be responsible for dislipidimia [32]. In diabetic rats the administration of *Pulicaria inuloides* exhibited a very highly significant hypolipidemic effect ($p<0.01$) as shown in Table 4, when compared to control (healthy rats) serum. Total Cholesterol (TC), Triglycerides (TG) and LDL levels were increased and HDL-c decreased clearly in diabetic rats. The standard drugs as well as *Pulicaria*

inuloides (250 and 500 mg kg-1) plant oil used in the experimental study significantly decreased ($p<0.01$) the levels of serum cholesterol, triglycerides, LDL levels whereas HDL cholesterol level was improved in both standard and test drug, after 21 days treatment. The result of our study is in accord with the findings of other researchers who reported that many plants extracts have potential therapeutic value in combating artherosclerosis which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level [33]. This effect not only due to better glycemic control but could also been due to inhibition of the pathway of cholesterol synthesis and increased HDL/LDL ratio may be due to the activation of LDL receptors in hepatocyte, which is responsible for taken up LDL into the liver and reduce the serum LDL level [34].

5.6. Effect of leaves *P. inuloides* on Serum Biochemical

5.6.1. Parameters in Normal and Diabetic Rats

Diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids and this account for the atherosclerosis, arteriosclerosis and severe coronary heart disease which leads to increase in levels of transaminases, marker enzymes important in heart and liver damage. Studies have observed that the liver is necrotized in diabetic patients. Therefore, the increment of the activities of ALT, AST, and ALP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, as a result of the hepatotoxic effect of STZ [35]. Other studies have also shown that STZ results in a significant increase in serum levels of ALT, AST and ALP 1, 3 and 6 hours after treatment [35]. The study established that the level of increase of these enzymes was dependent on the dose of STZ administered with higher STZ doses resulting in a greater increase in enzyme levels. [35] also report that studies are needed to verify and clarify the relationship between different doses streptozotocin induced diabetes and biochemical parameters. The effect of *Pulicaria inuloides* on liver and kidney functions are represented in the Table 7. Levels of liver function marker enzymes ALT, AST and ALP were significantly elevated in STZ-induced diabetes. The rats treated with *Pulicaria inuloides* (leaves) showed significant ($p<0.01$) reduction in the elevated levels of these enzymes.

On the other hand, kidney function markers like creatinine and urea were elevated in the STZ-induced diabetic rats when compared with the normal rats. *Pulicaria inuloides* leaves reduced in both levels. Also, the lowered level of total protein in diabetic rats was significantly ($p<0.01$) elevated, after 21 days treatment (Table 7). Elevated serum levels of urea and creatinine are significant markers of renal dysfunction. These results thus indicate that diabetic rats did suffer renal dysfunction [36].

5.7. Effect of Leaves of *P. inuloides* Essential Oil on Histopathology

(A) Normal control section shows the normal liver showing normal hepatic cells and architecture.

(B) Diabetic control section shows the clear hepatocellular necrosis and extensive vacuolization with vanishing of nuclei and disordered liver structure in STZinduced rat.

(C) Diabetic + leaves of *P. inuloides* essential oil (100mg/kg) section liver shows normal hepatocellular architecture with normal nucleus and cytoplasm.

(D) Diabetic +glibenclamide (10mg/kg) –section of liver showing normal hepatocellular architecture with normal nucleus, cytoplasm and distinct hepatic layer.

6. Conclusion

This study revealed that *Pulicaria inuloides* essential oil exerts an inhibitory effect on α-glycosidase and α-amylase, while as bringing back the blood glucose and body weight to normal in diabetic rats. Moreover, the treatment of rats with essential oil of PI for 21 days was effective in controlling the hyperglycemia, hyperlipidaemia and the oxidative damage. This investigation is helpful for understanding mechanism of action of *Pulicaria inuloides* and also reveals its potential use as a natural oral agent with hypoglycemic, hypolipidemic and strong antioxidant effects. These results suggested the potential use of this plant as a dietary supplement or in the manufacture of drugs for the control of increased blood glucose level in the body.

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