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In vivo Antidiabetic Property of *Artemisia Pallens* Using Albino Rats as Model

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Abstract
Diabetes mellitus (DM) is a world's fastest growing metabolic disease with high prevalence worldwide; associated with markedly increased morbidity and mortality rate and reduces the quality of life. It is the third most common disease in the world affecting every 2.8% of the global population. Hence the oral administration of methanolic extract of Artemisia pallens has been studied on Wistar strain albino rats. The diabetic rats were treated with a methanolic extract of the whole plant with low and high dose for 28 days. Test extract seems to show a decrease in the glucose levels in the Wistar rats. There seems to be an increase and decrease in the 200 mg & 400 mg levels of methanolic extract of *A.pallens* of SGOT, SGPT, ALP, cholesterol, triglycerides, complete blood count levels, and the enzymatic parameters and the total protein. The Histopathological studies seems to show improvement with repeated administration of the methanolic extract of *A.pallens*. These data suggests that A.pallens may be used as a choice in producing potent antidiabetic drugs.

Keywords
Artemisia pallens, antidiabetic parameters, Histopathology, methanol extract, Wistar rats.

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INTRODUCTION
Diabetes, the chronic disease that occurs either when the pancreas does not produce insulin or when the body cannot effectively use the insulin. The global prevalence of diabetes is said to double since 1980. Diabetes, (2014) Artemisia *pallens* Wall. ex Dc is an aromatic herb belonging to the *Asteraceae* family. Artemisia (2009). It is commercially utilized for its fragrance, Artemisia (2009). The *A.pallens* seems to possess various properties like antipyretic, antidiabetic, antifungal, antibacterial, antimicrobial, antioxidant and analgesic activities, Praveen *et al*., (2010). Oral intake of *A.pallens* plays a role in lowering the effect of blood glucose level Subramoniam *et* al., (1996). The study analysed the various biochemical parameters like glucose, SGOT, SGPT, alkaline phosphatase, total cholesterol, triglycerides, HDL, LDL, CBC, SOD, catalase, glutathione peroxidase and glutathione using the methanolic extract of *A.pallens* in Wistar rats.

MATERIALS AND METHODS
PLANT MATERIAL
A. pallens was collected from Coimbatore, Tamil Nadu. It was later taxonomically identified by the Botanical Survey of India, Coimbatore.

PREPARATION OF PLANT EXTRACT

The plant was cut into small pieces and shade dried. Later it was macerated in a mortar and pestle. The powder was extracted with methanol kept overnight. The extract was filtered and kept for further use.

ANIMALS

Adult male albino Wistar rats of 6 weeks weighing up to 150 to 200 g were used. The animals were kept in clean propylene cages with a good ventilation and a constant 12 hrs. light and dark schedule. They were fed with standard rat pelleted diet and clean drinking water was provided at regular intervals. The animal procedures were carried out after prior approval from the ethical committee with the recommendations for the proper care and use of laboratory animals by the CPCSEA.

EXPERIMENTAL INDUCTION OF DIABETES MELLITUS

The animals were separated into 5 groups of six animals each. They were then put for overnight fasting and to check the initial fasting blood glucose from the tip of the rat tail vein. Streptozotocin, an antibiotic was dissolved in citrate buffer with a pH of 4.5 and nicotinamide was also dissolved in normal saline. Non-insulin dependent diabetes mellitus was induced to the overnight fasting rats by a single intraperitoneal injection of 60 mg/kg Streptozotocin. After about 15 mins, the intra peritoneal administration of 120 mg/kg of nicotinamide was given to the animals. Hyperglycemia was confirmed by the elevated levels of blood glucose levels at 72 hrs. The animals having blood glucose levels of more than 250 mg/dl were used for the study according to Pellegrino *et al*., (1998). The methanol extract was evaporated using an evaporator.

STUDY DESIGN

The animals were divided into five groups:

Group I: Control group was fed with normal saline.

Group II: Streptozotocin (60 mg/kg/b.w) given intraperitoneal injection and Nicotinamide infused rats 120 mg/kg given orally.

Group III: Streptozotocin (60 mg/kg/b.w) given intraperitoneal injection, Nicotinamide infused rats 120 mg/kg given orally and the standard Glibenclamide drug 20 mg/kg given orally.

Group IV: Animals were treated with Streptozotocin, (60 mg/kg/b.w) given intraperitoneal injection Nicotinamide infused rats 120 mg/kg given orally and 200 mg of the methanolic extract.

Group V: Animals were treated with Streptozotocin, (60 mg/kg/b.w) given intraperitoneal injection Nicotinamide infused rats 120mg/kg given orally and 400 mg of the methanolic extract.

The standard drug, saline, and the extracts were administered for 28 days. During the study period the standard drug and the extract was dissolved in normal saline and distilled water. In the fasting animals, blood sugar levels were estimated on the 1st, 7th, 14th, 21st and 28th day from the tip of the rat tail vein.

BIOCHEMICAL ASSAYS

The animals were anaesthetized by ketamine hydrochloride and the blood was collected from retro-orbital sinus by using capillary tube into a centrifugation tube containing EDTA for haematological parameters and without EDTA for serum biochemical assays. The collected blood was allowed to clot at room temperature. The serum was separated and collected by centrifuging at 10000 rpm for about 10 mins. The collected serum was used for various biochemical assays. The animals were sacrificed by cervical dislocation. The liver and kidney were excised and washed in ice-cold saline and 0.15 M Tris-HCl with a pH set at 7.4. It was later blotted, dried, and weighed. 10% of the homogenate was prepared by using 0.15 M Tris-HCl buffer. It was processed for the estimation of lipid peroxidation. Part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used to estimate glutathione. Rest of the homogenate mixture was centrifuged at 10000 rpm for 10 min at 40^{o}C. The supernatant obtained was used to estimate catalase, SOD, reduced glutathione, glutathione peroxidase and lipid peroxidation assays. The blood and other body fluids were removed washed in normal saline and transferred to ice-cold containers with 10% formalin solution. These were then sent to the histopathological studies. The remaining tissues were cleaned with normal saline and was used for other in vivo and enzymatic parameters.

BLOOD GLUCOSE ASSAY

Blood was collected from the tip of the rat tail vein. It was later checked for blood sugar level using the glucose oxidase-peroxidase reactive strips and a glucometer made by Accu-chek, USA.

ESTIMATION OF SGOT, SGPT & ALKALINE PHOSPHATASE

Estimation of SGOT was done according to the IFCC kit (International Federation of Clinical Chemistry and Laboratory Medicine). Estimation of SGPT was done according to the IFCC kit.

ALP was determined using the method of King Kit method.

ESTIMATION OF CHOLESTEROL, TRIGLYCERIDES, HDL & LDL

Determination of Cholesterol was done according to CHOD/PAP method. Determination of triglycerides was done according to GPO/PAP kit. Estimation of HDL cholesterol was done according to Russell *et al*., (1982). The homogenate obtained after treatment with Tris buffer contained the LDL, VLDL and

chylomicrons. These were precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 min and was centrifuged for 10 min at 4000 rpm. The supernatant obtained had the HDL fraction. The cholesterol concentration in the HDL fragment was determined. The value of HDL-C was expressed in the unit of mg/dL. Estimation of LDL cholesterol was done according to William *et al*., (1972). The original plasma samples obtained from the experimental animals were mixed with EDTA. Total plasma cholesterol and triglycerides were obtained by precipitation procedures. In the chylomicron free samples, the VLDL was obtained by directly measuring the cholesterol from the supernatant fraction after ultracentrifugation of plasma for about 16 hours and by indirectly subtracting the cholesterol content from the sum of HDL and LDL. The values obtained were measured by mg/dL.

ESTIMATION OF RBC, WBC, PC AND HB

The RBC was enumerated according to Raminik (2007). The WBC was enumerated according to John *et al*., (2008). The differential platelet count was carried out according to John *et al*., (2008). The total number of lymphocytes and neutrophils per 100 cells were noted. From the different WBC and lymphocyte count, absolute lymphocyte and neutrophil count was calculated by the formula.

$$\text{Absolute neutrophil count} = \frac{\text{Number of neutrophils}}{100} \times \text{TWBC}$$

$$\text{Absolute lymphocyte count} = \frac{\text{Number of lymphocytes}}{100} \times \text{TWBC}$$

Estimation of Hb was done according to John *et al*., (2008)

ESTIMATION OF ANTIOXIDANT ENZYMES

SOD assay was determined by Kakkar *et al*., (1984). Catalase was carried out according to Roland *et al*., (1952). The Glutathione peroxidase was estimated according to Rotruck et al., (1973). The reduced glutathione was carried out according to Ellman (1959). Protein assay was performed by Lowry et al., (1951). The total amount of protein was expressed as mg/g tissue or mg/dl.

RESULTS AND DISCUSSION

Effects of extracts and Glibenclamide on body weight in rats

The body weight of rats belonging to the diabetic control, extract and Glibenclamide treated rats were found to be gradual. **(Table 1)** There was neither increase nor decrease in body weights among the diabetic control, extract and Glibenclamide treated rats. In (**Table 2)** the 1st day blood glucose level of 200 mg and the 400 mg extract was found to be increased than the diabetic control. The 7th day blood glucose level of 200 mg and 400 mg extract was found to be decreased than the diabetic control. The 14th day blood glucose level of 200 mg extract was found to be increased and 400 mg extract was found to be decreased than the diabetic control. The 21st day blood glucose level of 200 mg and 400 mg extract was found to be decreased than the diabetic control. The 28th day blood glucose level of 200 mg and 400 mg extract was found to be decreased than the diabetic control. In (**Table-3)**, there seems to be an increase and decrease in the SGOT values. There seems to be an increase and decrease in the values of SGPT. In ALP, total cholesterol and triglycerides there seems to be a decrease in the values than the diabetic control. In **(Table-4)**, the 200 mg extract and 400 mg extract values of RBC was found to be lower than the diabetic control. The 200 mg extract and 400 mg extract of WBC was found to be higher than the diabetic control. The 200 mg extract and 400 mg extract of Hb was found to be lower than the diabetic control. The 200 mg extract of polymorphs was found to be higher than the diabetic control and the 400 mg of extract of polymorphs was found to be lower than the diabetic control. The 200 mg extract of lymphocytes was found to be lower than the diabetic control and the 400 mg of extract of lymphocytes was found to be higher than the diabetic control. The 200 mg extract and 400 mg extract values of monocytes was found to be lower than the diabetic control. In (**Table -5**), the SOD, catalase, glutathione peroxidase, the glutathione value and the total protein value appears to be lower than the diabetic control.

Table-1 Changes in Body Weight

GROUP	CONTROL(NON DIABETIC)	ONLY STZ	STZ + STD	STZ + EXT 200 mg/kg	STZ + EXT 400 mg/kg
1st WK	151.5±1.784	162.7±1.333	162.7±1.978	165.8±1.641	161±1.915
2ndWK	158.3±2.092	96±30.55	148.3±3.63	124±24.91	146±2.828
3rd WK	173.3±3.639	107.3±34.06	159±3.606	105±33.57	162±3.933
4th WK	178±2.683	99.67±31.74	165.7±2.092	109.7±35.11	136.7±27.66

p values: $^{**}P< 0.05$

Table-2 Estimation of blood glucose

Group	Only Stz	Stz + Std	STZ + EXT 200 mg/kg	STZ + EXT 400 mg/kg
In blood glucose level	88.33±3.801	89.67±3.432	90.83±3.005	89.17±3.005
7th day blood glucose level	420±90.48	393.3±58.12	341.7±59.35	406.7±45.58
14th day blood glucose level	316.7±104	375±29.41	353.3±86.82	306.7±65.46
21st day blood glucose level	258.3±90.2	241.7±19.56	236.7±77.87	211.7±50.76
28th day blood glucose level	256.7±82.69	95±19.79	107.5±34.64	93.33±19.94

p values: $^{**}P< 0.05$

Table-3 ESTIMATION OF SERUM BIOCHEMICAL:

GROUP	ONLY STZ	STZ + STD	STZ + EXT 200 mg/kg	STZ + EXT 400 mg/kg
SGOT (U/L)	402.1±21.48	239±46.52	373.1±16.34	156.1±6.128
SGPT (U/L)	33.2±2.227	53.9±1.914	34.6±2.663	39.6±3.58
ALP (U/L)	128.1±4.05	95.47±4.53	111±2.43	97.67±2.71
TOTAL CHOLESTEROL (mg/dl)	86.57±3.386	66.73±2.63	73.4±6.099	64.9±4.321
TRIGLYCERIDES (mg/dl)	133.1±6.833	79.63±2.936	102.1±4.196	90.83±5.579

p values: $^{**}P< 0.05$

Table-4 Estimation of CBC:

GROUP	ONLY STZ	STZ + STD	STZ + EXT 200 mg/kg	STZ + EXT 400 mg/kg
RBC	4.12±0.5417	3.297±0.05239	4.05±0.1242	3.217±0.1985
WBC	10.43±0.3844	13.4±0.3215	11.47±0.5364	13.07±0.5207
HAEMOGLOBIN (g/dl)	14.47±1.2	9.9±0.1528	12.17±0.3756	9.633±0.6173
POLYMORPHS	9.333±1.453	5±1.32	11.33±1.764	7.667±0.8819
LYMPHOCYTES	84±1.528	88±1	82±2.082	85.33±0.6667
MONOCYTES	5.333±0.6667	4.333±1.202	4±0.5774	4.333±0.8819

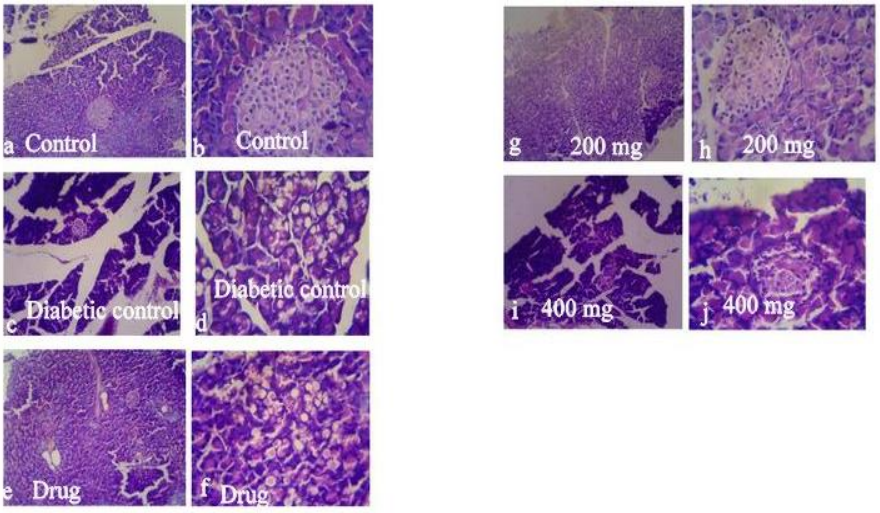
p values: $^{**}P< 0.05$

Table-5 ESTIMATION OF ENZYME PARAMETERS

GROUP	ONLY STZ	STZ + STD	STZ + EXT 200 mg/kg	STZ + EXT 400 mg/kg
SOD	0.3263±0.02033	0.2317±0.02167	0.287±0.00781	0.2103±0.05456
Catalase	0.3263±0.02033	0.247±0.0197	0.3137±0.0273	0.257±0.01206
GPX (glutathione peroxidase)	1.021±0.1229	0.759±0.04729	0.744±0.06901	0.5837±0.03522
GSH(reduced glutathione)	0.4013±0.02356	0.2277±0.009387	0.2587±0.0181	0.229±0.009074
Total Protein	1.374±0.288	0.7757±0.04883	1.077±0.2419	0.666±0.08924

p values: $^{**}P< 0.05$

Figure-1 Histopathological studies of Wistar strain Albino rats of the Artemisia *pallens*



HISTOPATHOLOGY

The Histopathological studies of the control showed the section of the pancreas to be normal with normal pancreatic acini. Islets are normal in number and size. The Histopathological studies of the diabetic control shows the pancreatic acini and islets to have cytoplasmic vacuolation and islets to be small and reduced in number. The Histopathological studies of the diabetic control with the drug shows the pancreatic acini to have mild focal cytoplasmic vacuolation and islets to be reduced showing small in size. The histopathological studies of the 200 mg extract show normal pancreatic acini and the islets are normal in size and has mild focal cytoplasmic vacuolation. The histopathological studies of the 400 mg extract show the normal pancreatic acini and the islets are normal in size with normal cytoplasmic vacuolation.

CONCLUSION

The methanolic extract of A.*pallens* seems to be good and can be used as the best choice for preparing some antidiabetic medicines since it has good antidiabetic properties.

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