Evaluation of Antidiabetic Activity From the Root Extracts of Pavonia Odorata Wild in Alloxan Induced Diabetic Rates

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ABSTRACT: *Pavonia odorata wild belongs to the family Malvaceae. It is a herb. Its roots have musk like aromatic odour. The roots yield an essential oil that contains isovaleric acid, isovaleraldehyde, aromadendrene, pavonene, a-terpinene, azulene and pavonenol. The roots are aromatic, and possess refrigerant, antipyretic, stomachic and astringent properties. Based on ethno pharmacological information , Pavonia Odorata Wild has been used to treat diabetes by the tribals in and around tropical and subtropical areas. But there are no more scientific reports available about the antidiabetic activity of this plant. Hence the study was carried out to ascertain the activity. The toxic effect of MeOH extract of Pavonia odorata root was studied at a dose level up to 2000 mg/kg b.w. The plant extracts were also evaluated for antidiabetic activity at a dose levels of 100 mg/kg of $CHCl_3$, 100 mg/kg of EtOAc and 200 mg/kg of MeOH in Alloxan induced diabetic rats. The plant extracts have not produced any toxic symptoms within the treated animals. In the high light of the results, our study indicates that Pavonia odorata root extracts have antidiabetic activity.The lowest blood glucose(157.00±4.36) levels was observed at 15th day after oral administration of 100 mg/kg of $CHCl_3$ extract of Pavonia odorata root .From the observations, it was concluded that the reduction of blood glucose levels in diabetic rats were found to be dose dependent and also dependent on duration of action. So it might be useful in the treatment of diabetes without toxicity.*

KEY WORDS*: Antidiabetic activity , Hyperglycemia ,   Malvaceae ,Pavonia odorata wild, Root extracts*

I. Introduction

Use of herbal remedies is on rise globally. According to an estimate of World Health Organization (WHO) an approximately 85–90% of the world's population consumes traditional herbal medicines. The reason for this seems to be their better tolerance and negligible adverse drug reactions [1].Nature has provided a complete store-house of remedies to cure all ailments of mankind. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. A number of plants have been documented for their medicinal potential, which is in use by the traditional healers, herbal folklorists and in Indian system of medicine namely Siddha, Ayurveda and Unani. The use of herbal medicine has become increasingly worldwide popular and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Standardized extracts from them or pure phyto pharmaceuticals need to be studied extensively for their quality, purity, potency, safety and efficacy[2].

Pavonia odorata wild belongs to the family Malvaceae. The roots and shoots of this plant are extremely aromatic. Ayurveda, the oldest of all healing sciences has recorded the use of Sugandhabala herb and its extract as cooling, demulcent, arminative, diaphoretic, and diuretic, fever [4]. Previously reported from this plant, the presence of sesquiterpene alcohol called as pavonenol. The roots yield an essential oil that contains isovaleric acid, isovaleraldehyde, aromadendrene, pavonene, α-terpinene, azulene and pavonenol[3,4]. It has anti-diabetic activity and also used in a number of ayurvedic formulations. Hence the present investigation was undertaken by In Vitro Evaluation of antidiabetic activity of $CHCl_3$, EtOAc and MeOH extracts of Pavonia odorata wild root.

Diabetes mellitus is recognized as a syndrome, a collection of disorders that have hyperglycemia and glucose intolerance as their hallmark, due either to insulin deficiency or to the impaired effectiveness of insulin's action, or to a combination of these. Inorder to understand diabetes it is necessary to understand the normal physiological process occuring during and after a meal. Food passes through the digestive system, where nutrients, including proteins fat and carbohydrates are absorbed in to the blood stream. The presence of sugar, a carbohydrate, signals to the endocrine pancreas to secrete the harmone insulin. Insulin cause the uptake and storage of sugar by almost all tissue types in the body, especially the liver, musculature and fat tissues. The cause of diabetes continues to be anonymity, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles.

Diabetes mellitus is a serious health problem with continuously increasing rates of incidence and mortality [5]. Unfortunately, there is no cure for diabetes   yet , but by controlling blood sugar levels through a healthy diet, exercise and medication the risk of long–term diabetes complications can be decreased.(cataracts, retinopathy, kidney failure, neuropathy, ulcers, feet infections, hardening of arteries ,heart disease and stroke). As a very common chronic disease, diabetes is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases, because of its high prevalence, morbidity and mortality. Therefore once diagnosed, it is well regulated by means of various therapeutically effective drugs. Besides, the therapy based on chemotherapeutic agents, the present century has progressed towards naturopathy. Thus, medical plants have an ever emerging role to play in treatment or management of life long prolonging diseases like diabetes mellitus, especially in developing countries where resources are meagre[6].

In recent years, researchers turned attention specifically to oxidative stress and the key role it plays, as a common element in the pathogenesis of diabetes complications. Hyperglycemia generates reactive oxygen species which, in turn, causes membrane lipid peroxidation and degradation. Many of the complications of diabetes, including vascular atherosclerosis, major cause of mortality in DM, are closely related to oxidative stress and, thus, antioxidants play an important role in the treatment of diabetes. Herbal remedies contain large amounts of antioxidants such as flavonoids, polyphenolic acids, carotenoids, vitamins C and E; experimental research has shown that the antioxidant activity may be an important property of medicinal plants used for their hypoglycemic effect in the treatment of D.M.[7].

II. Materials and Methods

1. Collection of Plant Material
The plants have selected on their wide medicinal uses in the traditional literature. The root part of the *Pavonia odorata* was used as the test plant which has collected from Sannanallur near Kumbakonam, Thanjavur District in Tamilnadu in the month of October and authenticated by Prof. N.Ramakrishnan, Head and Associate Professor and voucher specimens (Department of Botany) and voucher specimens (GACBOT-232) were deposited at the Herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

2. Preparation of Extracts
The dried roots of Pavonia odorata extracted with 90% methanol (MeOH) (4 X500 ml) under reflux. The methanol extract (84 g) has subjected to column chromatography with silica gel (60-120mesh) as the stationary phase. The charged column has then eluted with different solvents of chloroform (4 x 250 ml) and ethyl acetate (4 x 250 ml) to yield several sub fractions. The fractions have collected and the solvent recovered by simple distillation and has concentrated *in vacuo* and left in an ice-chest for a week. The residue from chloroform and ethyl acetate fractions (24.3 & 18.6 g) have taken up in Me_2CO and left in an ice-chest for two days when a brown solid separated and recrystallized from hot methanol. Different dose levels of plant extracts were prepared in 1% sodium carboxyl methyl cellulose solution and were used for studies.

3. Acute Toxicity Studies
Toxicity studies were conducted as per internationally accepted protocol drawn under OECD(Organization for economic co-operation and development)-423 guidelines in Wistar albino rats (130–170 g) of either sex were used for this study at a dose level of extracts up to 200 mg/kg b.w. The toxic effect of the $CHCl_3$, EtOAc and MeOH extracts of Pavonia odorata root part were studied at a dose level up to 2000 mg/kg b.w. The animals were also closely examined for signs of intoxication, lethargy, behavioral modification and morbidity[8,9]

4. Design of Experiment
In this experiment, thirty six rats were randomly divided into six groups of 6 animals each. The different doses of extracts were administered orally to the Alloxan induced diabetic rats. All the extracts were suspended in 1% sodium CMC suspension.
Group-I: Served as normal control. Control rats received only normal saline.
Group-II: The Second group of rats with diabetes was induced by intraperitonial injection of alloxan for 2 days.
Group-III: Alloxan treated rats were administered the Glibenclamide (10mg/kg) and served as standard.
Group-IV: Alloxan treated rats were administered the $CHCl_3$, root extract of Pavonia odorata (100mg/kg)
Group-V: Alloxan treated rats were administered the EtOAc , root extract of Pavonia odorata (100mg/kg)
Group-VI: Alloxan treated rats were administered the MeOH, root extract of Pavonia odorata (200mg/kg)
The drug treatment was given to the animals and was fasted for 12 hr before estimating the bloodglucose level.

5 .Induction of Diabetes

Diabetes was induced by a single intraperitonial dose of 120 mg/kg of b. w of Alloxan dissolved in 0.1M fresh cold citrate buffer (pH 4.5) into 12 hr fasted rats. The blood samples were taken on fifth day from retro orbital plexus of the rats for the estimation of blood glucose levels by using the auto analyzer. After 18 hours of injection of Animals were maintained for five days in diabetic condition for well establishment of diabetes. Rats with diabetes having hyperglycemia (i.e. with blood glucose of 90 to 420 mg/dl) were taken for the experiment [10, 11].

6. Statistical Analysis

For *In-Vivo* experiments values are represented by Mean ± S.E.M. The mean values are analyzed by one way analysis of varience (ANOVA) followed by Dunnett's Multiple comparisons test. The statistical significance of difference between two independent groups was calculated for the determination of blood glucose levels. The p<0.05, 0.01 and 0.001were considered as statistically significant.

7. Assessment of Anti-diabetic Activity

7.1 Effects of consumed extracts on body weight of rats

The body weight of each group was estimated after the 0th , 5th , 10th and 15th days intervals and the results were mentioned in Table –1 and Fig. -1

7.2 Effects of consumed extracts on blood-glucose level of rats

The blood samples (0.5ml) were collected for every time intervels of 0th , 5th , 10th and 15th days in 1ml Eppendorf's tubes. Serum was separated by centrifuging at 6000 rpm for 20 minutes. 30 µl of serum sample and 3 ml of working glucose reagent were taken in to a dry and clean test tube and incubated for 10 minutes at 37°C. The pink colour developed was measured by using auto analyser [12-14] The results were mentioned in Table-2 and Fig. -2.

7.3 Serum analysis

On the twelfth day of experiment the animals were sacrificed and blood was collected from various groups by puncturing the retro-orbital plexus, kept aside for half an hour for clotting. Serum was separated by centrifuging the blood samples at 6000 rpm for 20 minutes and stored in the refrigerator until analyzed. The serum was analyzed for various biochemical parameters such as protein, cholesterol, triglycerides and total lipids[15-21]. The results were mentioned in Table-3 and Fig. -3.

III. Result and Discussion

The extracts did not produce any toxic signs during the observation period for 24 hours in any rats were tested. Hence it was concluded that the extracts are safe up to 2000mg/kg. The mean blood glucose levels of control and drug treated animals after oral administration of different extracts($CHCl_3$100mg/kg , EtOAc 100mg/kg and MeOH 200mg/kg) of Pavonia odorata root were at various day intervals (0th, 5th, 10th and 15th days) were shown in Table -2 and Fig. -2. The statistical significance of decrease in blood glucose levels was calculated with respect to initial blood glucose levels. Oral administration of 1% Sodium CMC suspension did not change the blood glucose levels of rats. The blood glucose levels of diabetic rats treated with Glibenclamide (10 mg/kg) showed significant decrease in blood glucose levels at 5&10th days and highly significant decrease in blood glucose levels on15th day. The $CHCl_3$ extract of Pavonia odorata root at a dose of 100 mg/kg showed significant decrease in blood glucose levels on 5th day and more significant decrease in blood glucose level on 10th day. However, oral administration of 100 mg/kg of EtOAc extract of Pavonia odorata root showed significant decrease in blood glucose levels on 5, and 15th days, and highly significant decrease in blood glucose levels on 10th day. Oral administration of 200 mg /kg of MeOH extract of extract of Pavonia odorata root showed significant decrease in blood glucose levels at 5 & 15th day and highly significant decrease in blood glucose levels on 10th day[22].Effect seems to reach maximum after 10 days of treatment and remained constant in third week. Vehicle control animals were found to be stable in their body weight while diabetic rats showed significant reduction in body weight during 10 days . Alloxan caused weight reduction, which was reversed by $CHCl_3$, EtOAc and MeOH extracts of the Pavonia odorata root after 5 days of treatment. Serum cholesterol, serum triglycerides, serum LDL, serum creatinine and serum urea, and levels were decreased significantly by glibenclamide in Alloxan-induced hyperglycemic rats without significant change in body weight. They also improved conditions of DM as indicated by parameters like body weight, and lipid profiles along with serum creatinine and serum urea [23]. In the high light of the results, our study indicates that Pavonia odorata root extracts have antidiabetic activity. The lowest blood glucose(157.00±4.36) levels was observed at 15th day after oral administration of 100 mg/kg of $CHCl_3$ extract of Pavonia odorata root .

Table – 1: Effect of CHCl$_3$, EtOAc and MeOH extracts on Body weight of Alloxan-induced Diabetic rats.

S. No.	Treatment	Body weight (g)			
		0 day	5th day	10th day	15thday
1	Normal Control	162.67±1.53	157.33±6.43	152.67±2.08	155.67±3.21
2	Diabetic Control (Alloxan)	163.00±1.00	143.67±2.52	137.67±2.52	131.33±3.06
3	Standard Alloxan + glibenclamide (10 mg/kg)	162.33±2.89	147.67±4.16*	146.33±3.21**	145.00±6.25 ***
4	Alloxan + CHCl$_3$ Ext. (100 mg/kg)	162.00±1.00	150.67±7.09**	149.67±1.53*	152.33±2.52***
5	Alloxan + EtOAc Ext. (100 mg/kg)	164.00±1.00	157.33±1.15**	150.33±1.53**	153.00±2.65
6	Alloxan + MeOH Ext. (200 mg/kg)	162.00±1.53	142.67±3.06	136.67±2.08	137.33±2.89

Values are expressed in Mean $\pm$ S.E.M values. $p<0.05$, $p<0.01$ and $p<0.001$ (Dunnett's - test); diabetic control was compared with the extracts and standard treated groups . All values are recorded based on the six replications of tests and analysed statistically, where * ,** and ***represent the confident level at 95% ($p<0.05$) ,99% ($p<0.01$) and 99.9% ($p<0.001$) respectively.
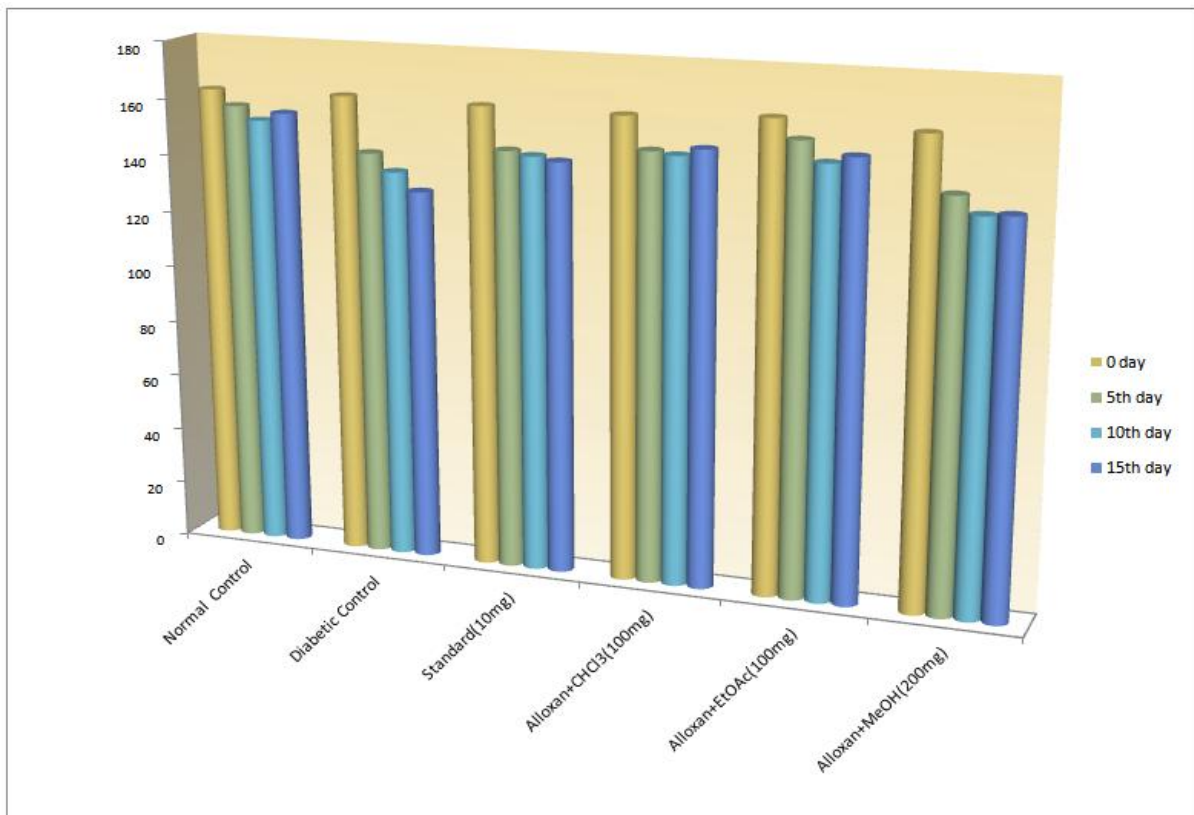

Fig.1: Effect of CHCl$_3$, EtOAc and MeOH extracts on Body weight of Alloxan-induced Diabetic rats.

Table – 2: Effect of Extracts ($CHCl_3$, EtOAc and MeOH) on Blood glucose level against Alloxan induced Diabetic rats.

S. No.	Treatment	Blood Glucose			
		0 day	5th day	10th day	15^{th}day
1	Normal Control	97.67±10.69	91.67±3.79	97.67±3.79	94.33±2.52
2	DiabeticControl (Alloxan)	388.67±6.11	401.67±3.51	405.67±2.08	411.33±2.52
3	Standard (Alloxan + glibenclamide 10 mg/kg)	374.33±4.04***	342.33±2.52***	273.33±4.04***	152.00±4.58***
4	Alloxan + $CHCl_3$ Ext. (100 mg/kg)	381.33±4.16*	348.00±3.00***	285.67±5.86***	157.00±4.36***
5	Alloxan + EtOAc Ext. (100 mg/kg)	389.67±4.51	320.67±7.37***	255.00±3.00***	135.33±2.52***
6	Alloxan + MeOH Ext. (200 mg/kg)	387.42±7.32	351.29±3.40***	291±2.57***	161.20±2.69***

Values are expressed in Mean ± S.E.M values. $p < 0.05$, $p < 0.01$ and $p < 0.001$ (Dunnett's - test); diabetic control was compared with the extracts and standard treated groups . All values are recorded based on the six replications of tests and analysed statistically, where * ,** and ***represent the confident level at 95% ($p < 0.05$) ,99% ($p < 0.01$) and 99.9% ($p < 0.001$) respectively.
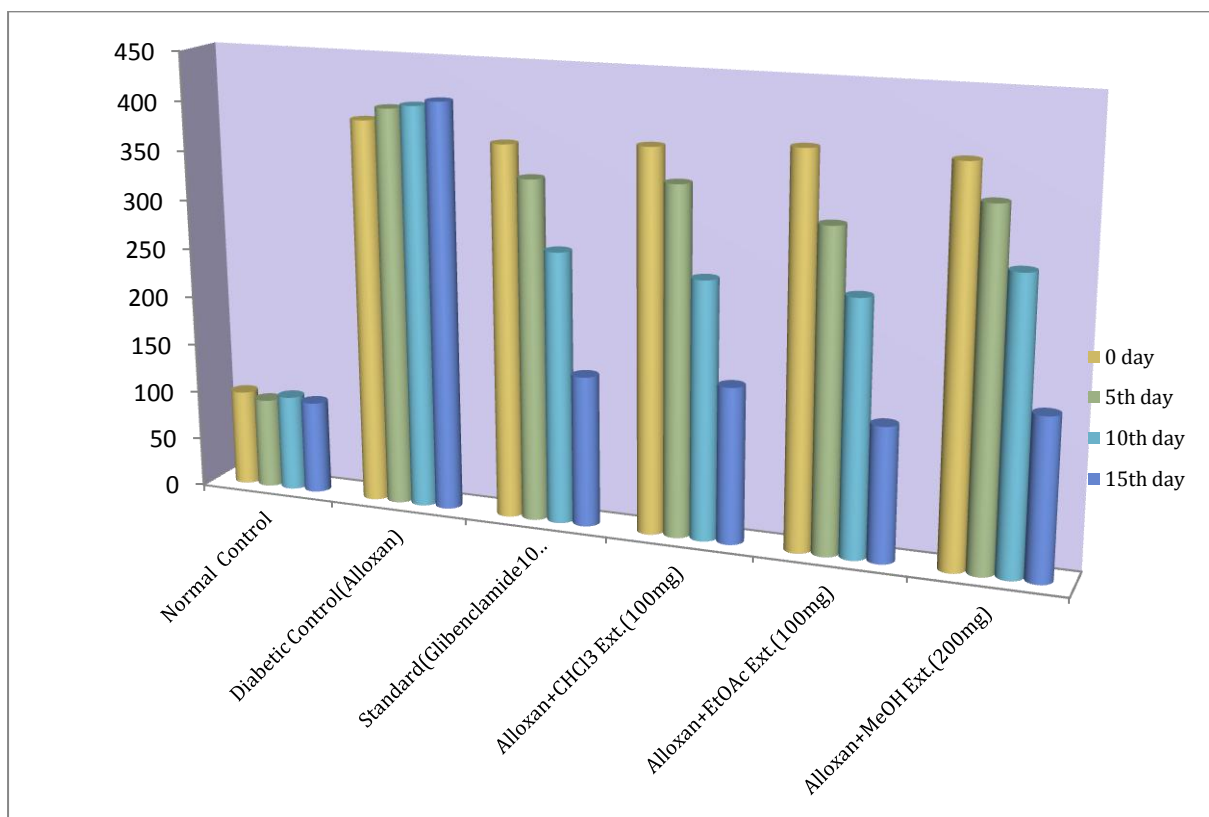

Fig. 2 : Effect of extracts ($CHCl_3$, EtOAc and MeOH) on Blood glucose level against Alloxan induced Diabetic rats.

Table – 3: Effect of Pavonia odorata root extracts on biochemical parameters of rat's blood on 12th day.

S. No.	Biochemical parameter	Zero Day	12th day Treatment (M±SD)					
			Normal Control	Diabetic Control (Alloxan)	Standard (Alloxan + glibenclamide 10 mg/kg)	Alloxan + CHCl$_3$ (100 mg/kg)	Alloxan + EtOAc (100 mg/kg)	Alloxan + MeOH (200 mg/kg)
1	Haemoglobu lin (g/dl)	14±0.42	15.26±0.27	12.00±0.28	14.84±0.39	13.61±0.36	14.09±0.45	12.84±0.27
2	Albumin (g/dl)	3.8±0.05	3.82±0.07	3.10±0.05	3.90±0.07	3.43±0.07	3.66±0.06	3.59±0.05
3	Globulin (g/dl)	2.6±0.06	2.61±0.04	1.80±0.04	2.71±0.06	2.52±0.04	3.00±0.02	2.62±0.06
4	Serum Urea (mg/dl)	26±0.68	26.20±0.26	38.00±0.53	22.03±0.49***	30.41±0.37**	35.90±0.79	40.27±0.68
5	Serum Creatinine (mg/dl)	0.83±0.053	0.80±0.025	1.30±0.053	0.90±0.042	0.63±0.044	1.10±0.020	0.99±0.054
6	Serum Chloesterol (mg/dl)	168.0±2.08	168.33±1.53	98.00±2.65	144.00±2.08***	121.67±2.5***	138.33± 1.5 ***	137.52±2.68***
7	Serum triglycerides (mg/dl)	61.07±1.00	61.13±0.23	42.07±1.53	62.10±1.04***	47.50±0.50	63.33± 0.58***	54.60± 1.00***
8	Serum Protein (g/dl)	6.43±0.40	6.40±0.10	5.05±0.13	4.60±0.04	7.10±0.08	7.8±0.07	6.10±0.10
9	HDL (mg/dl)	34.67±1.53	34.04±0.07	28.17±1.53	35.10±0.72**	31.00±1.00	46.17± 1.04**	37.51±1.52*
10	LDL (mg/dl)	65.00±1.73	64.50±0.50	52.07±0.58	58.03±0.70*	54.33±0.58	58.70±0.61	55.10±1.76

Values are expressed in Mean ± S.E.M values. $p<0.05$, $p<0.01$ and $p <0.001$ (Dunnett's - test); diabetic control was compared with the extracts and standard treated groups . All values are recorded based on the six replications of tests and analysed statistically, where * ,**   and   ***represent the confident level at 95% (p<0.05) ,99% (p<0.01) and 99.9% (p<0.001) respectively.
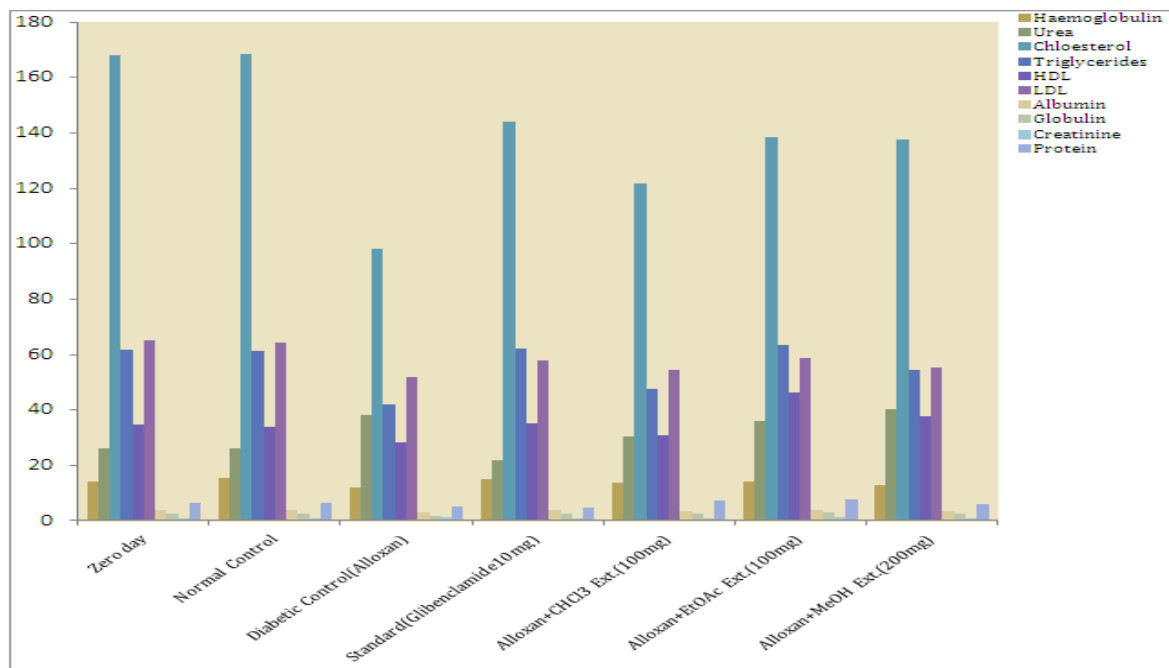

Fig.3 : Effect of Pavonia odorata root extracts on biochemical parameters of rat's blood on 12th day.

IV. Conclusion

From this study, it is concluded that the CHCl$_3$, EtOAc and MeOH extracts of the root part of Pavonia odorata root possess blood glucose lowering effect in alloxan induced diabetic rats. Thus, the folk use of the plant for the control of diabetes may be supported by this study. Further characterization of active principles flavonoids, alkaloids and tannins in Pavonia odorata root studies are in progress to isolate, identify and characterize such active components. The anti-hyperglycaemic activity of CHCl$_3$, EtOAc and MeOH extracts of the root part of Pavonia odorata may probably be due to the presence of several bioactive antidiabetic principles. It is thus apparent that CHCl$_3$, EtOAc and MeOH extracts possesses antihyperglycaemic effect.

Diabetes is a metabolic disorder which can be considered as a major cause of high economic loss which can in turn impede the development of nations. Moreover, uncontrolled diabetes leads to many chronic complications such as blindness, heart failure, and renal failure. In order to prevent this alarming health problem, the development of research into new hypoglycemic and potentially antidiabetic agents is of great interest. In the present review, interest is focused on the profile of herbal plants ,which have a hypoglycemic effect Medicinal plants in different oral formulations were recommended to the diabetic patient, but the mechanisms for hypoglycaemic activity still remained incompletely understood.

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