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"ANTI-DIABETIC PROPERTIES OF *KALANCHOE PINNATA* (LAM.) PERS. IN ALLOXAN INDUCED DIABETIC MICE"

N. Sunayana[2], Vinay B. Raghavendra[1*], M. Uzma[2], S.T. Girish[3]

[1*]P.G. Department of Biotechnology, Teresian College, Siddarthanagar, Mysore, Karnataka, India.

[2]Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore, Karnataka, India.

[3]Department of Microbiology and Biotechnology, Bangalore University, Bangalore, Karnataka, India.

ABSTRACT

Kalanchoe pinnata is a succulent perennial plant which is commonly known as 'air plant'. The anti-diabetic activity of *Kalanchoe pinnata* leaf extracts was investigated in alloxan induced diabetic swiss albino mice. A comparison was made between the action of different extracts of *K. pinnata* and a known antidiabetic drug glibenclamide. Hypoglycemic activity, determination of blood glucose by glucose oxidase method and total cholesterol was performed in experimental diabetic mice. Dose selection was made on the basis of acute oral toxicity study as per OECD guidelines. *K. pinnata* ethyl acetate extract (500 mg) and aqueous extract (500 mg) blood glucose levels of these extracts on 14th day of the study were 114 and 121 mg/dL. In alloxan-induced model, blood glucose levels of these extracts on 14th day of the study were 355 mg/dL and 98.67 mg/dL in comparison of diabetic control. In blood serum glucose level extracts of ethyl acetate (500 mg) of *K. pinnata* exhibited 321 mg/dL at initial day and finally the blood serum glucose reduces to 117 mg/dL. Whereas alloxan treated showed a maximum blood serum glucose level of 333, 346 and 367 on 1st, 7th and 14th days respectively. These ethyl acetate and aqueous extracts of *K. pinnata* also showed decreased cholesterol levels when compared to alloxan treated diabetic mice. In all these experiments, extracts of *K. pinnata* proved to be the potential antidiabetic drug.

KEYWORDS

Kalanchoe pinnata, Anti-diabetic and blood glucose levels.

Author for Correspondence:

Vinay B. Raghavendra,
P.G. Department of Biotechnology,
Teresian College,
Siddarthanagar, Mysore, India.

Email: viragh79@gmail.com

INTRODUCTON

The use of plants for treating diseases is as old as the human generations. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties. India is the gateway of medicinal plants in the world with a rich source for food and medicine[1]. From past several decades more importance are given to the research on medicinal and some plants were already tested for

anthelmentic, immunosuppressive, wound healing, hepatoprotective, antidiabetic effects, and hence several other plants remain intact to other diseases and has to be investigated[2]. In recent years, several authors have reported the antidiabetic potential of several medicinal plants using animal models[3,4].

Diabetes mellitus is a serious complex chronic condition that is a major disorder all over the world. Lack of hormonal insulin and hyperglycemia was characterized by the disturbances in proteins, carbohydrates and fat metabolisms[5]. The burden of the disease is comparatively high in India and treatment with the chemicals may have lots of side effects. Furthermore people in the rural areas are deficit of cost and death ratio was higher because of unavailability of treatment. Diabetes mellitus is one of the most dangerous disorder affecting over 2.8% of the world's population and expected to cross 10.4% by the year 2025.The rate of diabetic patients varies from 1.4% to 9.7% with annual mortality, which is linked to diabetes is now estimated more than one million all over the world[6]. Most patients with type 2 diabetes will ultimately require multiple anti-diabetic drugs to maintain adequate glycaemic control[7].

From several decades herbal medicines are the esteemed source of medicine therefore, Ayurveda and other traditional medicinal system are using for the treatment of diabetes reporting a number of medicinal plants which is used as herbal drugs. Hence, they play an important role as alternative medicine due to less side effects and low cost. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance[8]. Hypoglycemic herbs containing hypoglycemic agents like sulphonylureas and biguanides are the major compounds which increase insulin secretion, enhances glucose uptake by adipose tissues and also inhibit glucose absorption from intestine and glucose production from liver[9].

In view of the above aspects the present plant *Kalanchoe pinnata* that belongs to the Crussulaceae family is a smooth skinned more or less erect fleshy herb[10]. It grows up to about 1-2m tall. Its lower leaves are simple and the upper complex in formation. Its flowers are light green (and turn yellowish) and dull brownish red. The mucilaginous leaves of the plant are boiled and its juice used for healing ulcers. The juice of the crushed leaves is used to treat stomach ache and also applied to the forehead to relieve headaches. Furthermore it can also be used for treating common cold, hypertension and treatment of sore feet[11]. The leaves are boiled in water and the extract is used as a sedative for asthma and palpitation[12]. Moreover *K. pinnata* showed various pharmacological activities such as anthelmentic, immunosuppressive, wound healing, hepatoprotective, activities. Presence of flavonoids, polyphenols, triterpenoids and other chemical constituents were responsible for the antinociceptive and anti-inflammatory properties[13]. In order to scientifically apprise some of the ethno medical uses of *K. pinnata* leaves, a study was undertaken to investigate the antidiabetic properties of the *K. pinnata* leaf

MATERIAL AND METHODS

Preparation of extract

The leaves of *K. pinnata* were thoroughly washed in running water, air dried to 14 days and grounded to fine powder. The powder was passed through sieve no. 30 and stored in an airtight container for further use. Powder of *K. pinnata* was defatted with ethyl acetate (60-80 ºC) in a Soxhlet Apparatus by continuous hot-percolation. The defatted powder thus obtained was further extracted with ethanol (95% v/v), petroleum ether, methanol and acetone (data not shown). Fresh powder used for aqueous extraction by cold maceration method[14]. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

Procurement of experimental animals

Swiss albino mice (20-25 g) of either sex and of approximate same age are used in the present studies were procured from (DOS in Zoology, University of Mysore), Mysuru, India. The animals

were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. 150 mg of alloxan per kg body weight of mice was administered intraperitoneally after overnight fast (access to only water) of twelve hours to make them more susceptible to developing diabetes. The animals were kept under alternate cycle of 12 hours of darkness and light at 27 ± 2 ^{0}C and relative humidity 65^0 ± 10%. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

Acute oral toxicity study

Ethyl acetate and aqueous extracts of *K. pinnata* was checked for lethal dose 50 (LD50) by using OECD guidelines (423 guidelines). The LD50 of ethyl acetate extract and aqueous extract was found to be 2500 mg/kg therefore the LD50 value is 300mg/kg**.**

Induction of diabetes mellitus

The methods of[15] and[16] were used to induce diabetes in the mouse. After 12 hours from alloxan treatment, blood samples were obtained from the tail vein and blood glucose detected as described below. Alloxan treated mice with serum glucose level ranging between 150mg/dl or above were considered as diabetic. Treatment with *K. pinnata* extracts was started 24h after alloxan injection. After randomization into various groups, animals were acclimatized for period of 2 weeks under standard husbandry condition.

Experimental Design

Seven groups of mice, five in each received the following treatment schedule.

Group I: Normal control (saline).

Group II: Alloxan treated control (150 mg/kg.ip).

Group III: Alloxan (150 mg/kg.ip) + *K. pinnata* Aqueous plants extract (250 mg/kg, p.o),

Group IV: Alloxan (150 mg/kg.ip) + *K. pinnata* Aqueous plants extract (500mg/kg, p.o),

Group V: Alloxan (150 mg/kg.ip) + *K. pinnata* Ethyl acetate extract (250mg/kg, p.o),

Group VI: Alloxan (150 mg/kg.ip) + *K. pinnata* Ethyl acetate extract (500mg/kg, p.o),

Group VII: Alloxan (150 mg/kg.ip) + Standard drug, Glibenclamide (5mg/kg, p.o)

Whole plant extracts and standard drug glibenclamide (5mg/kg) and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline for 14 days. Group II are diabetic control mice. Group III and Group IV (which previously received alloxan) are given a fixed dose whole plants extract (250 mg/kg, p.o), (500 mg/kg, p.o), Group V and VI are diabetic and ethyl acetate extract (250 mg/kg, p.o), (500 mg/kg, p.o) and Group VII standard drug glibenclamide (5mg/kg) for 14 consecutive days.

Hypoglycemic activity

Blood glucose levels were checked at 0, 2, 4 and 6 hour intervals after administration of ethyl acetate and aqueous extracts and control drug, glibenclamide. Hypoglycemic effects of powdered *K. pinnata* extracts and glibenclamide in alloxan-induced normal Swiss albino mice. In all groups, blood glucose levels were estimated up to 6 hrs intervals after drug administration[17].

Collection of Blood Sample and Blood Glucose Determination

Blood samples were drawn from tail tip of mice at weekly intervals till the end of study (i.e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 1, 7, and 14 of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips.

On day 14, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted mice and fasting blood sugar was estimated[18]. Serum was separated and analyzed for serum cholesterol[19]. After seven days of administration of the extract to the Swiss albino mice, they were starved for a day (24 hours). Subsequently, blood samples were collected from them (Swiss albino mice) after decapitation. The blood samples were collected into sterile specimen bottles free of anticoagulant. The blood was centrifuged at 3000X g for 10 minutes and allowed to stand. The supernatant (serum) was decanted.

Determination of Blood Glucose by Glucose oxidase method

Glucose is the major energy source for the body processes. The energy that is necessary for much of the molecular synthesis and metabolic processes of the cells is provided by the metabolism of glucose. Therefore, the level of circulating glucose is of vital interesting. Fasting blood glucose level above or below the normal range usually indicate disease. Blood glucose determinations are, therefore used for diagnosis and follow-up of abnormalities of carbohydrate metabolism. Blood glucose concentration is measured using a modified glucose oxidase method[20]. The oxidation of sample glucose is catalyzed by glucose oxidase to form hydrogen peroxide and gluconate. The hydrogen peroxide is then determined by the peroxidase oxidative coupling of N,N-diethylaniline (N,N-DEA) with 4-amino-antipyrine (4-AAP).

$$\text{D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{D-Glucuronic acid} + H_2O_2$$

$$2\,H_2O_2 + \text{4-Aminoantipyrine} + \text{N,N-Diethylaniline} \xrightarrow{\text{Peroxidase}} \text{Purple dye (@ 553 nm)}$$

Estimation of blood cholesterol

The reaction depend on the colorimetric determination of a green colored compound resulting from the reaction of cholesterol with acetic anhydride-sulphuric acid (strong dehydrating and oxidizing agent) the absorbance of the green compound is then measured at 610 nm and calculated using the formula[21].

$$\frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times 300 = \text{mg/dl}$$

Statistical analysis

Analysis of variance technique (completely randomized design) was applied to test the significance difference at 5% significance level. Duncan's multiple range (DMR) test was applied using Minitab[22].

RESULTS

Hypoglycemic activity

Blood glucose levels of groups III and IV were significantly reduced at 2nd 4 and 6th h intervals. There was no significant change in blood glucose levels of group I which served as control. However, Blood glucose levels significantly reduced in 2nd and 4th hour blood glucose levels and maintained the same at 6th hour interval of Group VI respectively. Blood glucose levels in alloxan treated (group II) was significantly lowered at 2nd and 4th hour and reduces gradually at 6th hour intervals. Reduced blood sugar level in Group VI at 2nd and 4th hour interval which showed similar to (Group VII) treated with standard drug glibenclamide as shown in Figure No.1.

Blood Sample and Blood Glucose Determination

Fasting blood glucose estimation and body weight measurement were done on day 1, 7th, and 14th of the study. Blood glucose estimation was conducted using one touch electronic glucometer using glucose test strip as shown in Figure No.2 (A-E). In alloxan untreated mice showed 82 in the initial day and 100 mg/dL in the 14th day. Administration of ethyl acetate extract at 250 and 500 mg (group V and VI) decreases glucose levels in diabetic treated groups and showed equal blood glucose level of glibenclamide treated (group VII) animals as shown in Table No.1. Extracts of ethyl acetate (500 mg) of *K. pinnata* showed 321 mg/dL at initial day and finally the blood serum glucose reduces to 114 mg/dL. Whereas blood serum glucose was increased significantly in alloxan treated- diabetic mice which showed 305 in the initial day and gradually increased to 355 mg/dL.

Determination of Blood Glucose by Glucose oxidase method

The blood serum glucose was increased significantly in alloxan diabetic mice as compared to normal mice. Control mice showed 85.24 in first day and finally blood serum glucose showed 98.67 mg/dL at 14th day. Whereas alloxan treated (group II) showed a maximum blood serum glucose level of 333, 346 and 367 on 1st, 7th and 14th day respectively. Administration of ethyl acetate extract at 250 and 500 mg (Group V and VI) decreases glucose levels in diabetic treated groups and showed equal blood glucose level which is similar to glibenclamide treated animals (Group VII) as shown in Table No.2. Extracts of ethyl acetate (500 mg) of *K. pinnata* showed 321 mg/dL at initial day and finally the blood serum glucose reduces to 117

mg/dL. Whereas glibenclamide treated animals showed 298 in initial day and 103 mg/dL in the 14th day. Group III and IV showed reduced blood serum glucose level of 143 and 127 at 14th day as shown in Table No.2.

Estimation of blood cholesterol

The blood cholesterol was increased significantly in alloxan treated diabetic mice as compared to normal mice. Administration of ethyl acetate extract at 250 and 500 mg (group V and VI) decreases cholesterol levels in diabetic treated groups and showed equal blood cholesterol level to standard drug glibenclamide treated animals (group VII) as shown in Figure No.2. Allaxon treated mice (groupII) showed blood cholesterol of 95.28 mg/dl. Extracts of ethyl acetate (500 mg) of *K. pinnata* showed blood cholesterol at 59.29 mg/dl compare to standard drug treatment 58.78 mg/dl. Whereas aqueous extract of 250 and 500 mg (group III and IV) showed blood cholesterol of 74.32 and 71.45 mg/dL as shown in Figure No.3.

DISCUSSION

K. pinnata has a novel account in terms of its medicinal use[23], reiterates a popular local quote of the Bengalis in the Western Himalayan region of India which translates as -A man cannot die of disease in an area where *K. Pinnata*, *Vitex negundo*, *Adhatoda vasica* and *Acorus calamus* are grown (provided that he knows how to use them). However there are many gaps which need to be filled by concurrent researchers in different disciplines. One must make the best use of the naturally available resources which provide valuable raw material for advanced research.

Diabetes mellitus, a common heterogeneous metabolic syndrome, is prevalent throughout the world and has been projected to become one of the world's main disablers and killers within the next 25 years. Blood glucose level, have been commonly measured to monitor the glycemic control mechanism. In the present study, fasting blood glucose estimation and body weight measurement were done for day 1, 7, and 14th of the study. Blood glucose estimation done by one touch electronic glucometer using glucose test strips gives a valuable method for screening of plant extracts for antidiabetic activities.

The leaves of *K. pinnata* were found to significantly reduce blood glucose levels of both normal and alloxan-induced diabetic mice. However, there was no significant changes in 2nd and 4th hour blood glucose levels and maintained the same at 6th hour interval of Group VI respectively. The glibenclamide which was used in the present study as a control drug also significantly reduced blood glucose which is similar to group VI. Previously, productions of hypoglycemic response in normal and diabetic mice have also been reported for many medicinal plants like *Berberis aristata, Acacia nilotica*, etc.[24]. Alloxan has been observed to cause a massive reduction of the β cells of the islets of Langerhans and induce hyperglycemia. Diabetes mellitus is a metabolic disorder, showing significant impact on lipid metabolism with alterations in blood lipids and lipoproteins profile[25].

In the present study, the serum glucose, and cholesterol in the diabetic animals were elevated to high levels during the study period. Treatment of *K. pinnata* extracts after 2 weeks in diabetic mice showed a significant reduction in all these parameters compared to control. Efficacy of this activity is appreciably good when compared to standard drug glibenclamide. Blood glucose showed significantly higher in alloxan treated (group II) of 333, 346 and 367 on 1st, 7th and 14th day respectively. Whereas *K. pinnata* leaf ethyl acetate (group V and VI) extracts showed 321 mg/dl at initial day and finally the blood serum glucose reduces to 117 mg/dl. Whereas only Whereas aqueous extracts of *K. pinnata* (Group III and IV) showed reduced blood serum glucose level of 143 and 127 at 14th day respectively.

High cholesterol is produced in both liver and intestine and increased blood cholesterol (95.28 mg' dl) significantly in alloxan diabetic mice. Whereas extracts of ethyl acetate (500 mg) of *K. pinnata* showed decreased blood cholesterol to 59.29 mg/dl compare to standard drug treatment 58.78 mg/dl, whereas aqueous extract of 250 and 500 mg (group III and IV) showed blood cholesterol of 74.32 and 71.45 mg/dL. The result indicates that the active

principle in *K. pinnata* leaf extract has similar to insulin treatment. Here the total cholesterol serves as an acceptor of lipids, especially free cholesterol from various extra hepatic cells to the liver for the ultimate excretion in the bile[26]. [27]Reported that ethanol extract of *K. pinnata* (500 mg/kg body wt. Shows reduction in both post prandial and streptozosin induced diabetes blood glucose levels, triglyceride levels. [28]reported that the comparison between the action of different extracts *Pongamia pinnata* was investigated for antidiabetic activity in alloxan induced diabetic albino rats.

In all of the above mentioned assays antidiabetic compounds were found to be high in ethyl extract of *K. pinnata* when compare to other extracts this results will give better information for the biotechnologists to isolate newer molecules for developing new novel drugs.

Table No.1*:* Effect of treatment of different extracts of *K. pinnata* at the doses 250 and 500mg/kg b.w. for 2 weeks on blood glucose estimation in diabetic mice

S.No	Group/Treatment	Average Blood glucose estimation		
		Day 1	Day 7	Day 14
1	Group-I	82 ± 6.23	95 ± 5.2	100 ± 0.56
2	Group II	305 ± 5.28	336 ± 3.26	355 ± 4.25
3	Group II	280 ± 3.57	185 ± 0.67	125 ± 0.56
4	Group IV	308 ± 6.72	163 ± 5.25	121 ± 0.62
5	Group V	327±0.65	170±2.85	116±0.625
6	Group VI	321±4.86	170±3.85	114±0.625
7	Group VII	298±0.67	180±2.85	103± 0.56

Values are expressed as mean ±SEM; (n = 5)

Table No.2*:* Effect of treatment of different extracts of *K. pinnata* at the doses 250and 500mg/kg b.w. for 2 weeks on serum glucose concentration in diabetic mice

S.No	Group/Treatment	Average Blood Serum Glucose (mg/dl)		
		Day 1	Day 7	Day 14
1	Group-I	85.24±6.23	93.35 ±5.2	98.67±0.56
2	Group II	333±4.36	346±3.26	367±4.25
3	Group III	297±4.87	192±2.69	143±3.78
4	Group IV	325±4.36	167±3.25	127±0.62
5	Group V	330±4.86	172±3.85	121±0.625
6	Group VI	321±4.86	170±3.85	117±0.625
7	Group VII	294±4.87	176±3.85	105± 0.56

Values are expressed as mean ±SEM; (n = 5)

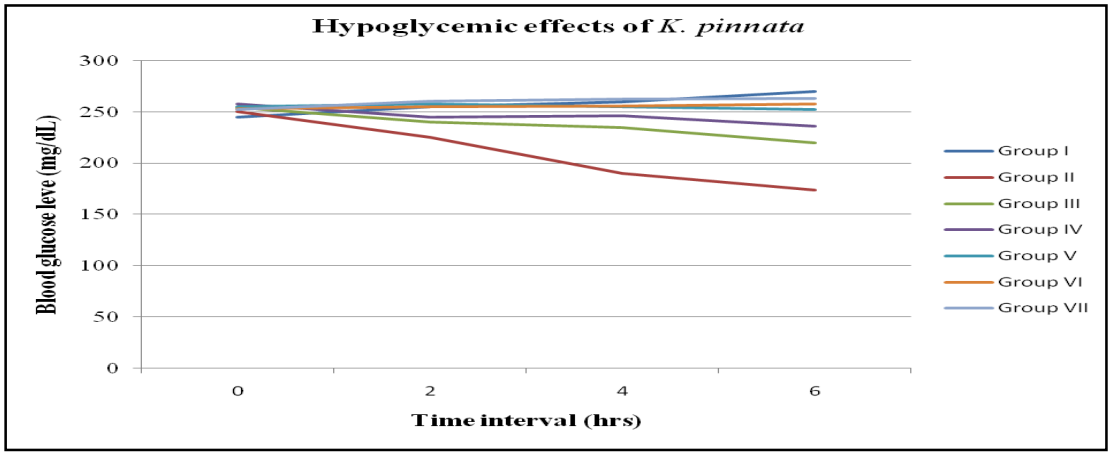

Figure No.1: Effect of treatment of different extracts at the doses 250 and 500mg/kg on blood glucose levels (mg/dL ± SEM) of normal and alloxan-induced diabetic mice at 0, 2, 4 and 6 h intervals

Values are expressed as mean ±SEM; (P≥0.05)

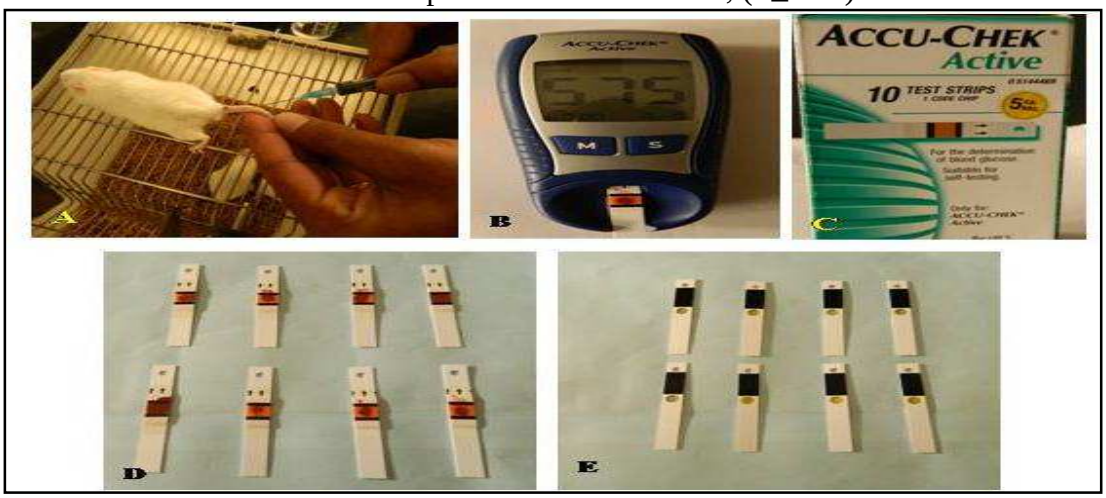

Figure No.2: A: Mice model B: Electronic glucometer C: Accu-Chek glucose test trips D: Showing glucose strips with blood samples E: Strips showing color changes

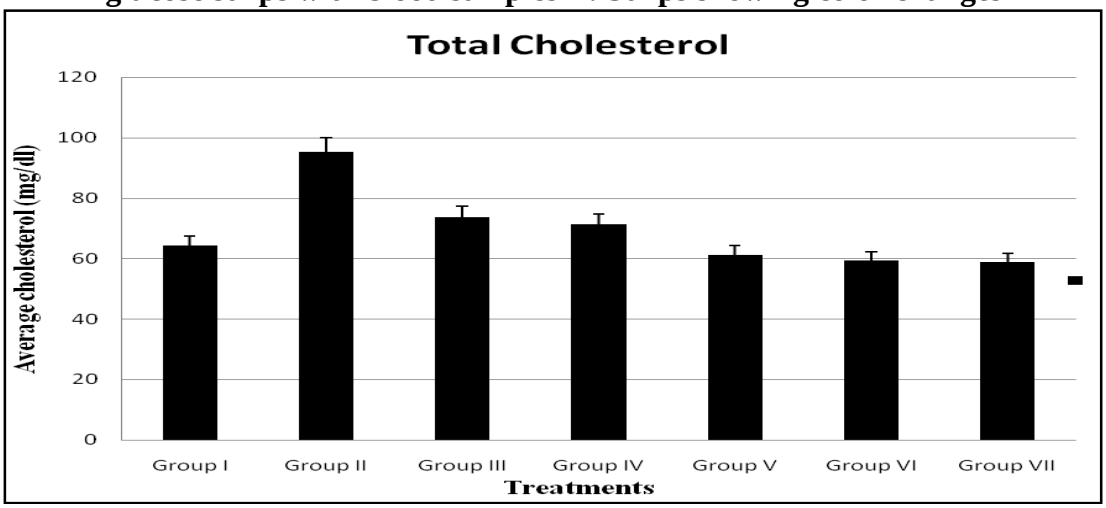

Figure No.3: Effect of treatment of different extracts of *K. pinnata* at the doses 250and 500mg/kg b.w. for 2 weeks on serum cholesterol (mg/dl) in diabetic mice

CONCLUSION

The present investigation has also opened avenues for further research, especially with reference to the different dose studies and development of potent formulation for diabetes mellitus from *K. pinnata* leaves. Activity guided fractionation, formulation and its evaluation is in progress and will be available in a short period of time.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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