International Standard Serial Number (ISSN): 2319-8141
International Journal of Universal Pharmacy and Bio Sciences 7(1): January-February- 2018
INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES
*IMPACT FACTOR 4.018****
*ICV 6.16****
Pharmaceutical Sciences **RESEARCH ARTICLE ……!!!**

ANTI-DIABETIC POTENTIAL OF *TRIANTHEMA DECANDRA* EXTRACT STUDIED IN ALLOXAN INDUCED DIABETIC MICE

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KEYWORDS:

Trianthema decandra, SGOT, SGPT, Bilirubin, Alloxan.

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ABSTRACT

Diabetes mellitus is one of the most common endocrine disorders accompanied with many metabolic syndromes. Use of herbal medicines has always been an option to treat a great number of diseases such as diabetes and its complications. The aim of the present study is to investigate the liver protective effects of *Trianthema decandra linn* extract in alloxan induced diabetic Swiss albino mice. Nine Swiss albino mice (weighing 28-32g) were randomly divided into control, alloxan treated and *Trianthema decandra linn* treated mice group. Diabetes was induced in mice by injecting intraperitoneally alloxan monohydrate at dose of 150 mg/kg body weight. Aqueous extracts of *Trianthema decandra linn* at dose of 250 mg/kg body weight were given orally in diabetic mice daily for three weeks after established LD_{50} value. In diabetic mice, the SGOT, SGPT, Bilirubin and serum glucose levels were significantly increased in comparison with the control groups. Statistical analysis ($p<0.05$) of the data indicated that aqueous extract of *Trianthema decandra linn* were significantly decrease serum contents of liver enzymes (SGOT, SGPT and Bilirubin) as well as serum glucose in treated groups. The results suggested that aqueous extracts of *Trianthema decandra linn* possesses liver protective effect against alloxan induced diabetic mice.

INTRODUCTION:

OXIDATIVE STRESS

The term oxidative stress is a state of unbalanced tissue oxidation refers to a condition in which cells are subjected to excessive levels of molecular oxygen or its chemical derivatives called reactive oxygen species (ROS). Under physiological conditions, the molecular oxygen undergoes a series of reactions that ultimately lead to the generation of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and H_2O. Peroxynitrite ($OONO^-$), hypochlorus acid (HOCl), the hydroxyl radical (OH^-), reactive aldehydes, lipid peroxides and nitrogen oxides are considered among the other oxidants that have relevance to vascular biology. Mild, chronic oxidative stress may alter the anti-oxidant systems by inducing or repressing proteins that participate in these systems, and by depleting cellular stores of anti-oxidant materials such as glutathione and vitamin E [1]. Free radicals and other reactive species are thought to play an important role oxidative stress resulting in many human diseases. Establishing their precise role requires the ability to measure them and the oxidative damage that they cause [2]. Oxidative stress is involved in the process of aging [3] and various chronic diseases such as atherosclerosis [4], diabetes [5] and eye disease, whereas fruit and vegetable diets rich in antioxidants such as polyphenols, vitamin C, and carotenoids are correlated with a reduced risk of such chronic diseases [6]. An excessive amount of reactive oxygen/nitrogen species (ROS/RNS) leading to an imbalance between antioxidants and oxidants can cause oxidative damage in vulnerable targets such as unsaturated fatty acyl chains in membranes, thiol groups in proteins, and nucleic acid bases in DNA [7]. Revelation of the mechanism of action of antioxidants and their true antioxidant potential can lead to identifying proper strategies to optimize the antioxidant defense systems in the body.

MATERIALS AND METHODS

CHEMICALS

ANIMAL MODEL

Male albino mice (100±160g), (*Rattus novergcus*) were procured from Tamil Nadu University for Veterinary and Animal Sciences, (TANUVAS) Chennai, India were used for the study. Animals were fed with commercially available standard rat pelleted feed (M/s Pranav Agro

Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided *ad libitum*. The rats were housed under conditions of controlled temperature (22±2°C) and acclimatized to 12-h light, 12-h dark cycle. Animal experiments were conducted according to the guidelines of institutional animal ethical committee. All the drugs (standard and test as well as vehicle were administered per-orally using insulin syringe.

PREPARATION OF PLANT MATERIAL

The *Trianthema decandra linn* were procured as a gift from the Siddha Maruthava Shop, Vellore. Aqueous extract was made by dissolving it in double distilled water using by mortar and pestle. The dose was finally made to 250 mg/kg body weight for oral administration after the LD_{50} estimation.

INDUCTION OF HYPERGLYCEMIA WITH ALLOXAN

The selected mice were weighed, marked for individual identification and fast for overnight. The alloxan monohydrate at the rate of 150 mg/kg body weight [8]was administered intraperitoneal (i.p) for making the alloxan induced diabetic mice model. Blood glucose level of these mice were estimated 72 hours after alloxan administration, diabetes was confirmed by blood samples collected from the tip of the tail using a blood glucometer (Accu Sure, Taiwan). Animals with blood glucose level equal or more than 200 mg/dl were declared diabetic and were used in entire experimental group [9].

EXPERIMENTAL DESIGN

The Mice's were divided into three groups, with three (3) mices in each group, as follows: (i) group I - control mice, (ii) group II - alloxan-induced diabetic control mice, (iii) group III diabetic mice given *Trianthema decandra linn* extract (250 mg/kg) in aqueous solution daily for 21 days through Gavage's method.

EXPERIMENTAL DESIGN

SEGGREGATION OF GROUPS

Experimental animals were divided into three groups of three mice each as follows.

Group I **:** Served as Control mice

Group II **:** Served as alloxan induced diabetic control mice received 250mg/kg

aqueous extract of *Trianthema decandra linn* extract orally intra gastric tube gavage for 21 days.

Group III : Diabetic mice given *Trianthema decandra linn* extract orally intra gastric tube gavage for 21 days.

ANIMAL SACRIFICE AND SAMPLE COLLECTION

After the last dose, animals were fasted for 12 hours and sacrificed. Blood samples were collected by orbital sinus puncture method [10]. Serum was prepared following procedure. Briefly, blood samples were withdrawn from orbital sinus using non heparinised capillary tubes, collected in dried centrifuge tubes and allowed to clot. Serum was separated from the clot and centrifuged at 3000 rpm for 15 min .at room temperature. The serum was collected carefully and kept at 20ºC until analysis Glucose [11]. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalacetate transaminase (SGOT) activities were measured according to the method described by King *et al.*, 1965a, while bilirubin (Jendrassik L, *et al*., 1938) activity was measured.

HISTOPATHOLOGICAL STUDIES

A portion of the liver was cut into two to three pieces of approximately 6 mm 3 sizes and fixed in 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5 mm thickness of liver tissue were cut and stained with haematoxyline eosin. The thin sections of liver were made into permanent slides and examined [12], under high resolution microscope with photographic facility and photomicrographs were taken.

STATISTICAL ANALYSIS

Results were presented as Mean±S.D and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). Difference among means had been analysed by applying Tukey's multiple comparison test at 95% ($p<0.05$) confidence level. Calculations were performed with the Graph Pad Prism Program (Graph Pad Software, Inc., San Diego, USA).

RESULTS AND DISCUSSIONS

SERUM GLUCOSE LEVEL

The effect of aqueous extract of *Trianthema decandra linn* on blood glucose levels is shown in Fig. 1. The mean level of glucose in the control group of mice was evaluated to be 74.33 ±7.31 mg/dl (range 65-85) whereas it was 222.5±22.52 mg/dl (range values 198-250) in alloxanized group. After the treatment of mice with the extract of *Trianthema decandra linn* the glucose level decreased down to 91±7.82 mg/dl having a range of 82-99 mg/dl. These variations in glucose concentrations are evident from Graph 1. The significant increase in glucose concentration in the diabetic animals than that of the control mice is evident on alloxanization. However, the oral administration of aqueous extract of *Trianthema decandra linn* significantly reduced the glucose level in serum when compared with alloxan induced diabetic mice.

SERUM GLUTAMATE OXALOACETATE TRANSAMINASE (SGOT)

In Control group of mice SGOT activity was found to be 25±5.06 IU/ml having the range of 20-32 IU/ml. In diabetics, its activity got raised to 50±6.87 IU/ml with values ranging from 40 to 59. However, extract treatment of this group for three weeks resulted in decrease of SGOT activity to 35.83±5.98 having values ranging from 25 to 41 IU/ml. These variations are depicted by the box-plot in Graph 2.

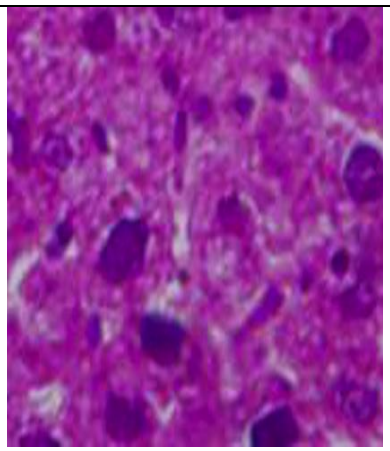
SERUM GLUTAMATE PYRUVATE TRANSAMINASE (SGPT)

In control mice group SGPT activity was found to be 20.71±4.96 having range values between 15 and 26.54 IU/ml which got raised to 53.83±6.70 (range values 45-63) IU/ml in diabetic mice. However, after the treatment of mice with the extract of *Trianthema decandra linn*, the activity decreased down to 30.83±4.87 (ranging between 25 and 38) IU/ml. These values are compared by the box-plot as evident in Graph 3.

BILIRUBIN LEVELS IN VARIOUS GROUPS

Bilirubin level of control mice was observed to be 0.53±0.054 mg/dl (values ranging between 0.44 and 0.60) which got increased to 0.82±0.093 mg/dl in alloxan induced diabetic mice. Bilirubin contents ranged from 0.70 to 0.90 in diabetic mice. However, after the treatment of diabetic mice with the extract of *Trianthema decandra linn*, the bilirubin level decreased down to

the mean value of 0.65±0.053 having values ranging from 0.59 to 0.72 mg/dl. These variations along with statistical significance are depicted by box-plot as shown in Graph 4.



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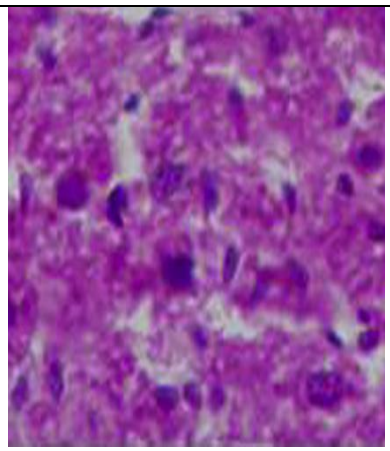

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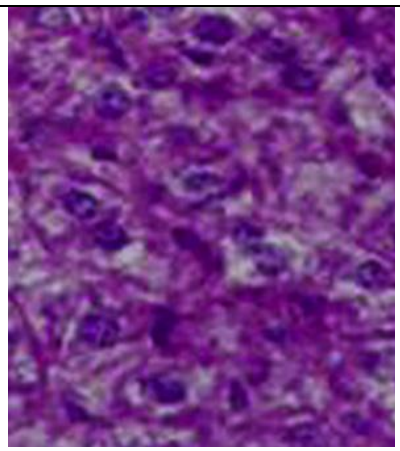
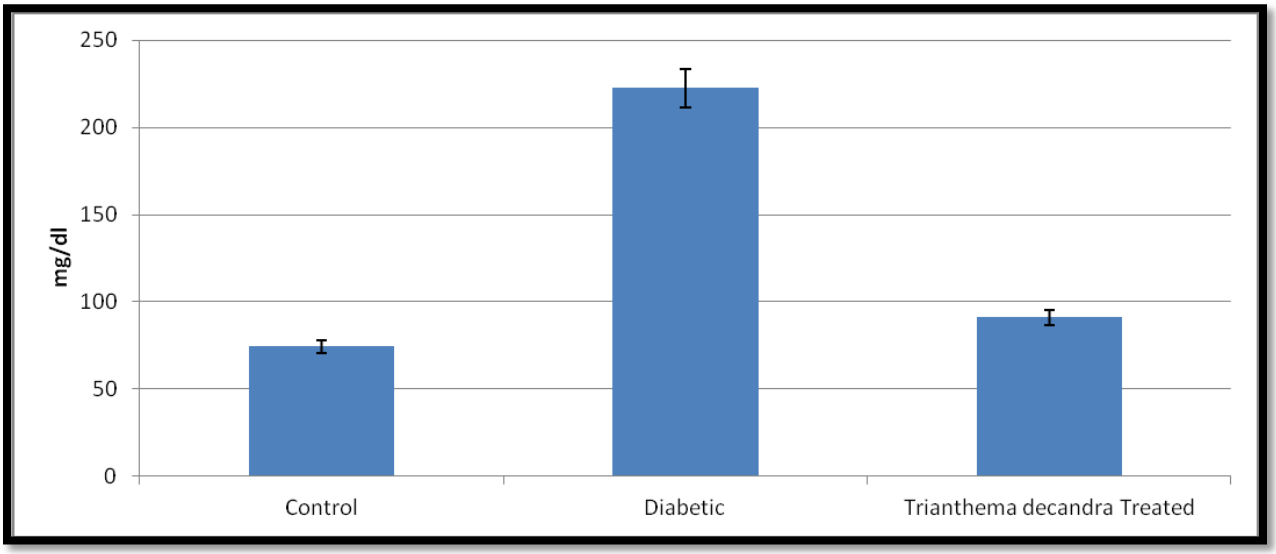


III

Group I. In control mice liver section showed normal structure of central vein, sinusoid and well arranged hepatocyte in sinusoid (H and E 3200).

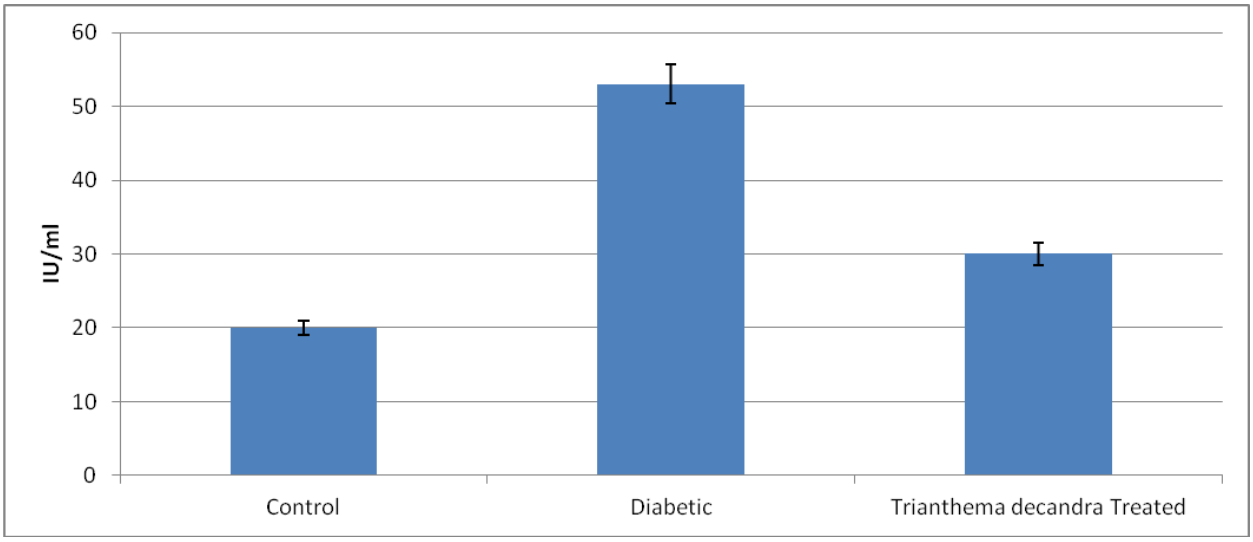
Group II. In diabetic mice liver section showed abnormal structure of central vein, sinusoid and hepatocyte are not arranged in sinusoid.

Group III. In *Trianthema decandra linn* mice liver section showed towards normal structure of central vein, sinusoid and also arranged hepatocyte in sinusoid.

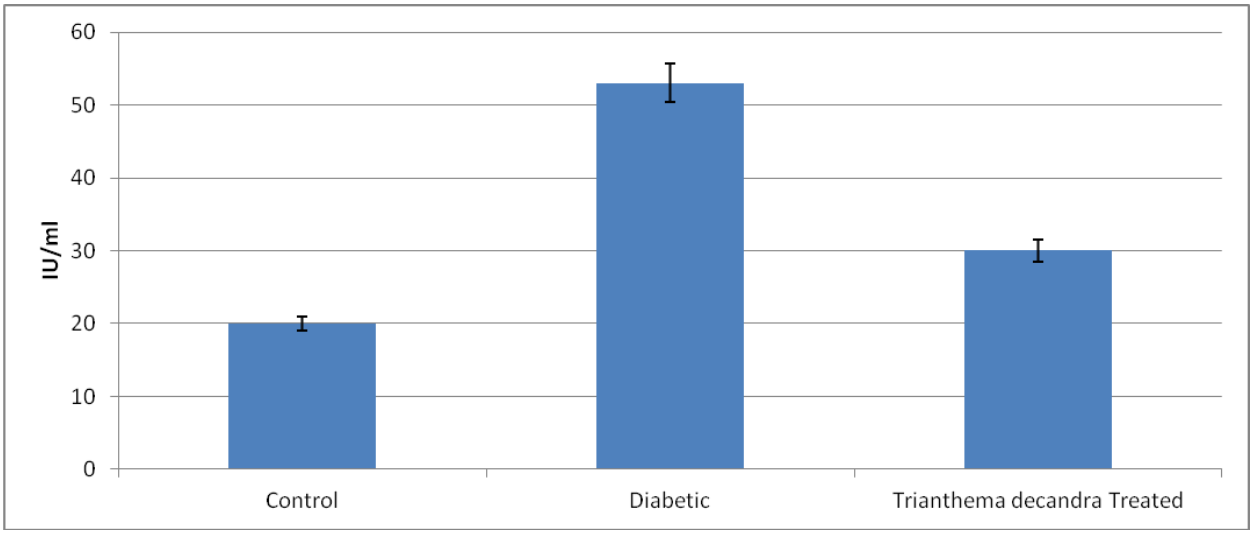


Graph 1. Effect of *Trianthema decandra linn* on diabetic induced group showing glucose levels (n=3, values are Mean ± S.D).

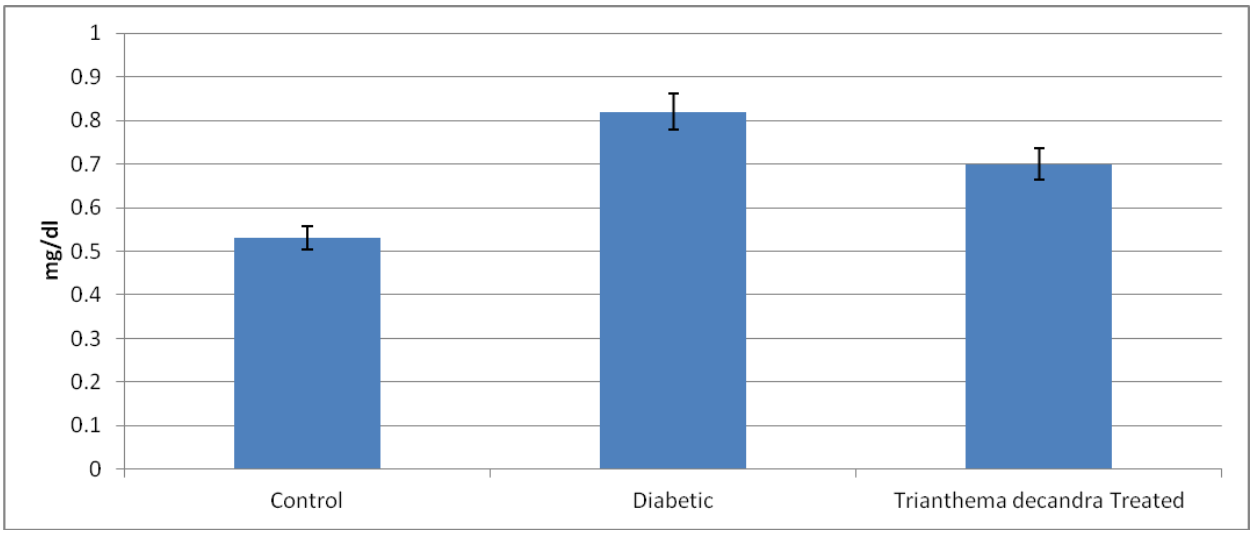
 International Standard Serial Number (ISSN): 2319-8141



Graph 2. Effect of *Trianthema decandra linn* on diabetic induced group showing SGOT levels (n=3, values are Mean ± S.D).



Graph. 3 Effect of *Trianthema decandra linn* on diabetic induced group showing SGPT levels (n=3, values are Mean ± S.D).



Graph 4. Effect of *Trianthema decandra linn* on diabetic induced group showing bilirubin levels (n=3, values are Mean ± S.D).

SUMMARY AND CONCLUSION

In the study, the level of SGOT, SGPT and bilirubin level were significantly increased [13]. Increased level of serum marker enzymes due to directly conversion of amino acids to keto acids are AST and ALT. Inflammatory hepatocellular disorders results in extremely elevated transaminase levels [14]. The increase in the activities of plasma AST and ALT indicated that diabetes may be induced hepatic dysfunction. Supporting our findings it has been found [15], that liver was necrotized in diabetic patients. Chronic mild elevation of amino transferase is frequently found in type 2 diabetic patients. Therefore, an increase in the activities of AST and ALT in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [16]. Further that, our results on the recovery after treatment with *Trianthema decandra linn* extract is in parity with findings with other plants reported by other workers [17-28]. In conclusion, the present study demonstrated that the treatment of diabetic mice with *Trianthema decandra linn* has exerted a considerable hypoglycemic effect. In addition, these herbs could be liver damage associated with alloxan diabetes. However, further biochemical studies should be conducted to promote using of these herbs as antidiabetic agents.

Diabetes mellitus patients in India are increasing day by day probably due to change in life style change in food pattern *i.e.* from traditional fibre rich diet to sugary fast food diet and also

because of genetic basis. The disorder being chronic in nature needs long term treatment to prevent the complications arising due to persistent high blood glucose level. Pharmacotherapy available for the treatment of diabetes in modern healthcare system includes insulin and oral 16 hypoglycemic drugs [29]. However due to economic constraints, it is not possible for majority of the diabetic patients in developing countries like India to use these drugs on regular basis. Moreover these synthetic antidiabetic drugs are associated with large number of adverse effects. Hence there is increase in the trend to use traditional indigenous plants widely available in India for the treatment of diabetes mellitus. Over 150 plant extract and some of their active principles including flavonoids, tannins, alkaloids etc are used for the treatment of diabetes [30]. During the present investigation, alloxan (150 mg/kg i.p) was used to induce diabetes in mice and their serum glucose levels were found to be significantly elevated as compared to normal mice. The increased levels of serum glucose may be due to the partial damage of the pancreatic β-cells. Alloxan, a β-cytotoxin, induces "chemical Diabetes" in a wide variety of animal species including rats by damaging the insulin secreting β-cells [31,32]. Similar results reported [33] shows that the administration of alloxan significantly increases the level of glucose when compared to control, which might account for the cytotoxic effect of alloxan on beta cells. Alloxan is relatively toxic to insulin producing pancreatic β-cells because it preferentially accumulates in β-cells through uptake *via* the GLUT-2 glucose transporter. This cytotoxic action is mediated by ROS source of generation of ROS is dialuric acid, a reduction product of alloxan. These radicals undergo dismutation to H_2O_2. The action of ROS with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells, thereby decreasing the secretion of insulin, which in turn increases the blood glucose level. Another result of alloxan, a β-cytotoxin, was preferred to produce the diabetic state in mice as it induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic beta cell resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues [34].

On the other hand, treatment of *Trianthema decandra linn* extract (250 mg/kg b.w) for 21 days, the elevated level of serum glucose level was significantly decreased. Our results are similar to

previous reports [35,17]. The antidiabetic activity of aqueous extract of *Trianthema decandra linn* may be its promote insulin secretion by closure of K^+ATP channels, membrane depolarization and stimulation of calcium influx, an initial key step in insulin secretion. In this context, number of other plants has also been reported to have antidiabetic and insulin stimulatory effects [18]. Flavonoids sterols, triterpenoids, alkaloids and phenolics are known to be bioactive anti-diabetic principles [19]. Flavonoids are known to regenerate the damaged β cells in the alloxan induced diabetic rats [20]. Phenolics are found to be effective anti-hyperglycemic agents. On this basis we have selected the glucose induced hyperglycaemic model to screen the anti-hyperglycaemic activity of the plant extracts. Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum amino transferases, alkaline phosphatase, bilirubin. Hepatocellular damage causes release of these enzymes into circulation. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitis, infarction, and muscular damages. ALT, which mediates conversion of alanine to pyruvate and glutamate, is specific for liver and is a suitable indicator of hepatic injuries [21].

ACKNOWLEDGEMENT:

We are thankful to the faculty members of the PG & Research Department of Biochemistry for their appreciativeness and making the necessary facilities available for the research. The authors would like to thank the Secretary & Correspondent, Principal of K.M.G college Gudiyattam for their encouragement, providing the necessary facilities and support in carrying out the work.

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