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Effect of *Centella asiatica* (Linn) Leaves on Aminotransferases in Streptozotocin Induced Diabetic Rats

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ABSTRACT

This study was conducted to evaluate the therapeutic effect of methanol extract *Centella asiatica* leaves by assaying the activities of diagnostic markers (aminotransferases) in the streptozotocin induced diabetic rats. The rats were divided into five groups with 6 rats in each group. The methanol extract of *Centella asiatica* leaves was orally administered at a dose of 300 mg/kg body weight (b.w), and glibenclamide was administered at a dose of 5 mg/kg b.w. The effect of methanol extract of *Centella asiatica* leaves on total protein and the activities of diagnostic marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) were examined in the liver and kidney of control and experimental rats. The result showed a significant ($p < 0.001$) decrease in total protein concentration and increase in AST and ALT activities in the liver of diabetic rats whereas the levels were unaltered in the kidney of diabetic rats. The daily oral treatment of methanol extract of *Centella asiatica* leaves to diabetic rats for 30 days reversed the above changes in a significant ($p < 0.001$) manner. From our observations, we conclude that methanol extract of *Centella asiatica* leaves was able to restore the altered activities of aminotransferases. The obtained results were compared with glibenclamide, a standard antidiabetic drug. These results indicate that oral treatment with methanol extract of *Centella asiatica* leaves may be used to alleviate the liver functions impairment associated with diabetic condition.

Keywords: Aminotransferases, *Centella asiatica,* Diabetes mellitus, Streptozotocin.

INTRODUCTION

Diabetes mellitus is metabolic disorder and a major threat to the healthcare worldwide. World health organization project that, diabetes will be the seventh leading cause of death in 2030. [1] In 2012, an estimated 1.5 million deaths were openly caused by diabetes. [2] It is estimated that more than 80% of diabetes deaths occur in low and middle income countries. [2]

In diabetes along with the carbohydrates even the protein metabolism will be altered due to the insulin deficiency or insulin resistance. During diabetes negative nitrogen balance occurs when more nitrogen is lost from the body due to the altered protein metabolism. [3] Increased urea nitrogen production during diabetes may perhaps be accounted for enhanced catabolism of both liver and plasma proteins. [4] Enzyme activities in the tissues are often used as 'markers' to ascertain early toxic effects of administered alien compounds to experimental animals. [5-6]

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At present diabetes is controlled by handful of available drugs such as sulfonylureas, biguanides, α-glucosidase inhibitors insulin etc; however they have a large number of side effects. Herbal drugs are being used traditionally in many parts of the world in the treatment of diabetes mellitus where access to formal healthcare is limited. [7] The available literature reveals that treatment with the herbal extracts could possibly bring back the altered marker enzymes, like alkaline phosphatase, aspartate aminotransferase and alanine aminotransferases. [8-9]

Centella asiatica (L) Urban is used in traditional and alternative medicine due to the wide spectrum of pharmacological activities associated with secondary metabolites. [10] It is reported that traditionally *Centella asiatica* was used for diverse complaints including wound healing, bronchitis, asthma, urethritis, liver complaints, allergy, cancer, diuretic, and hypertension also to improve mental ability. [11] Recently we reported the anti-diabetic activity of *Centella asiatica* leaves in streptozotocin induced diabetic rats. [12] In the current study we have evaluated the therapeutic effect of *Centella asiatica* leaves by assaying the total protein concentration and the activities of diagnostic markers (aminotransferases) in the liver and kidney of streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Centella asiatica* were collected from Talakona forest, Chittoor district, Andhra Pradesh, India during November 2009. The taxonomic identification of *Centella asiatica* (L) plant was confirmed by a senior Botanist, Mr. Madhava Chetty, Department of Botany, S.V. University, and a voucher specimen was deposited in the S.V. University herbarium, Tirupati, Andhra Pradesh, India.

Animals

Wistar strain male albino rats, aged 3 months and weighing 160 ± 20 g, were purchased from the authorized dealer (M/S Raghavendra Enterprises, Bangalore, India). The rats were housed in clean polypropylene cages having 6 rats per cage and maintained under temperature controlled room (25 ± 2°C) with a photo-period of 12 h light and 12 h dark cycle, humidity 50 ± 10%. The rats were fed with water and a standard rat pellet diet *ad libitum* (purchased from Sai Durga Agencies, Bangalore, India). The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India (CPCSEA, 2003) and approved by the Institutional Animal Ethical Committee at Sri Venkateswara University, Tirupati, Andhra Pradesh, India (Resolution No. 10/(i)/a/CPCSEA/IACE/SVU/PSR-MRA/10.06.2010).

Chemicals

All the chemicals used in the present study were Analytical Grade (AR) and were obtained from Sigma (St. Louis, MO, USA), Fisher (Pitrsburg, PA, USA), BDH Chemicals (England), Merck (Mumbai, India), Ranbaxy (New Delhi, India), and SISCO Research Laboratories (India).

Plant extract preparation

Fresh leaves of *Centella asiatica* were washed thoroughly, shade dried for 3 weeks and milled into fine powder using a mechanical grinder. The powdered material (2 kg) was soaked in 6 L methanol for 48 hours and filtered. The above filtrate was collected and evaporated in a rotavapour at 40-50°C under reduced pressure. The yield (9.86 % w/w) was dried in vaccum desiccator and greenish material obtained was stored in refrigerator at 4°C until use.

Induction of experimental diabetes mellitus

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution 50 mg/kg body weight (b.w) [13-14] dissolved in 0.1 M cold citrate buffer, (pH 4.5). After injection, animals had free access to food and water. The animals were allowed to drink 15% glucose solution for 72 hours to counter hypoglycemic shock. The rats were considered as diabetic if their blood glucose values reached above 250 mg/dl on day 3 after STZ injection. The blood glucose levels were measured by using one touch glucometer (Johnson & Johnson). After diabetes confirmation, rats were allowed for 7 days to acclimatize to diabetic condition, and rats with hyperglycemia (blood glucose > 250 mg/dl) were chosen for the study. Treatment was started on day 8 after STZ injection which was also considered as the first day of treatment and continued further until end of the study period.

Experimental Design

Rats of the same age group (3 months old) were divided into 5 groups, six rats in each group:

Group I (Normal Controls): This group of rats received distilled water for equivalent handling.

Group II [Normal rats treated with methanol extract of *Centella asiatica* leaves (CALEt)]: This group of rats received CALEt daily for a period of 30 days at a dose of 300 mg/kg b.w.

Group III (Diabetic untreated rats): In this group, diabetic rats are untreated for a period of 30 days which served as diabetic controls and received distilled water for equivalent handling.

Group IV (Diabetic rats treated with CALEt): After diabetic confirmation test, this group of rats received CALEt for a period of 30 days at a dose of 300 mg/ kg b.w.

Group V (Diabetic rats treated with Glibenclamide): In this group, diabetic rats were treated with the standard diabetic drug, glibenclamide 5 mg/kg b.w [15-16], for the same period as in group IV. This group was maintained for better comparison of the protective effect of *Centella asiatica* against diabetic induced complications.

After completion of 30 days of treatment, the animals were sacrificed by cervical dislocation then the liver and kidney tissues were excised at 4°C. The tissues

were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at -80°C for further biochemical analysis.

Biochemical Analysis

The protein content was estimated by the method of Lowry *et al.* [17] Alanine aminotransferases and aspartate aminotransferases enzyme activities were determined using the method of Reitman and Frankel [18] as described by Bergmeyer and Bruns. [19]

Statistical Analysis

The mean, standard deviation (SD) and probability test (Analysis of variance- ANOVA) were carried out according to Steel and Torrie [20] using BASIC programming techniques on SPSS PC for different parameters. The *p* value of more than 0.05 was considered as not significant.

RESULTS

In this study the results showed a significant ($p < 0.001$) decrease in total protein concentration (Fig. 1) in the liver of diabetic rats whereas in the kidney of diabetic rats the protein levels (Fig. 2) were unaltered. The daily oral administration of CALEt to diabetic rats for 30 days restored the protein concentration to the normal levels in a significant ($p < 0.001$) manner in the liver of treated rats.

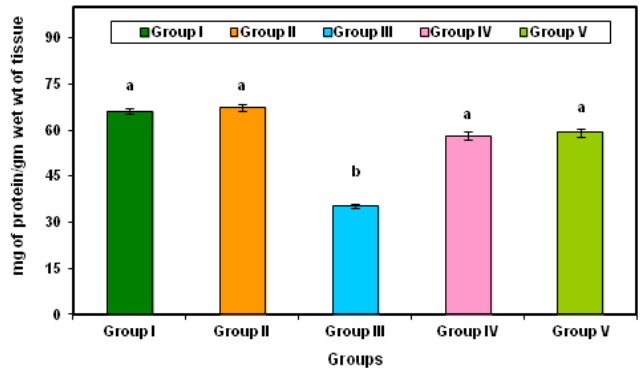

Fig. 1: Effect of CALEt on total protein in the liver of control and experimental group of rats after 30 days treatment

Values are given as mean ± S.D of six individuals

Mean values that do not share same superscript differ significantly from each other at $P < 0.05$

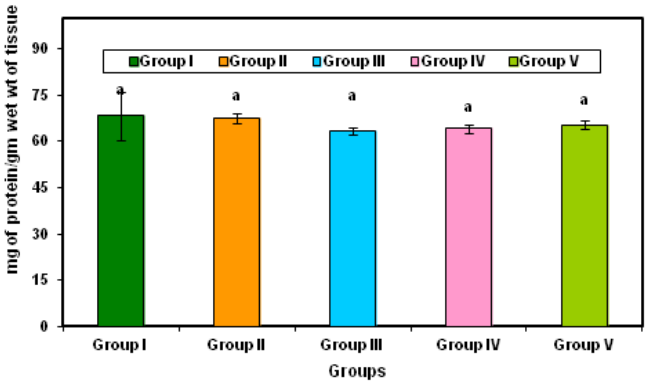

Fig. 2: Effect of CALEt on total protein in the kidney of control and experimental group of rats after 30 days treatment

Values are given as mean ± S.D of six individuals

Mean values that do not share same superscript differ significantly from each other at $P < 0.05$

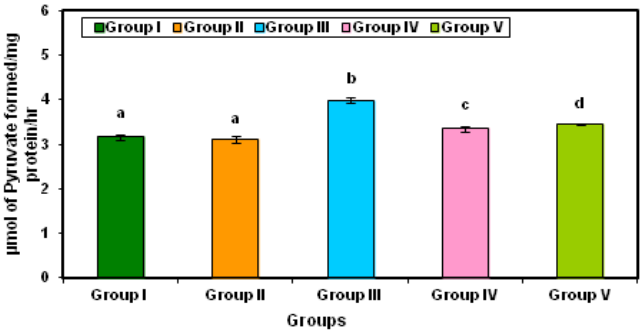

Fig. 3: Effect of CALEt on AST activity in the liver tissue in control and experimental group of rats after 30 days treatment

Values are given as mean ± S.D of six individuals

Mean values that do not share same superscript differ significantly from each other at $P < 0.05$

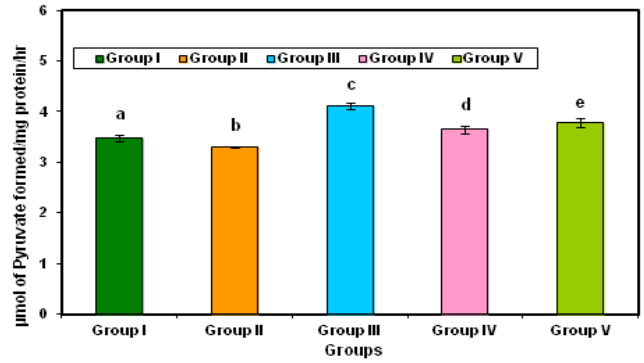

Fig. 4: Effect of CALEt on ALT activity in the liver tissue in control and experimental group of rats after 30 days treatment

Values are given as mean ± S.D of six individuals

Mean values that do not share same superscript differ significantly from each other at $P < 0.05$

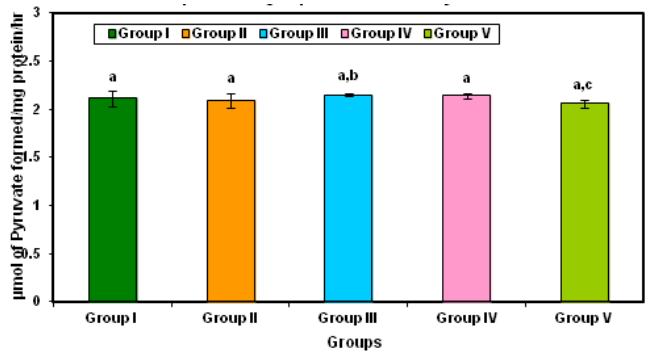

Fig. 5: Effect of CALEt on AST activity in the kidney tissue in control and experimental group of rats after 30 days treatment

Values are given as mean ± S.D of six individuals

Mean values that do not share same superscript differ significantly from each other at $P < 0.05$

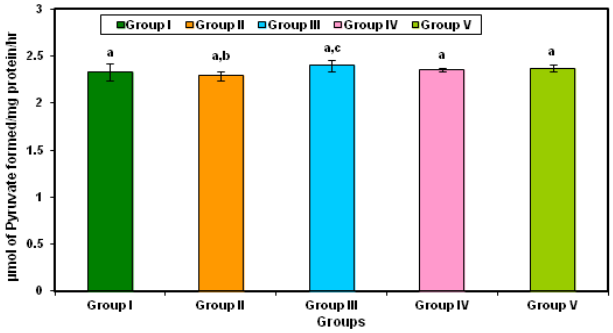

Fig. 6: Effect of CALEt on ALT activity in the kidney tissue in control and experimental group of rats after 30 days treatment

Values are given as mean ± S.D of six individuals

Mean values that do not share same superscript differ significantly from each other at $P < 0.05$

The activities of aminotransferases (AST and ALT) were found to be significantly increased ($p < 0.001$) in the liver of diabetic rats (Fig. 3 and Fig. 4). In the kidney of diabetic animals, the activities of AST and ALT were unaltered (Fig. 5 and Fig. 6). The daily oral administration of CALEt to diabetic rats for 30 days reversed the above changes in a significant ($p < 0.001$) manner. In this study improved marker enzymes in CALEt treated diabetic rats were similar with that of glibenclamide-induced augmentation.

DISCUSSION

Proteins are the building blocks of all cells and tissues. They are the basic components of enzymes, many hormones, antibodies and clotting agents. Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. Proteins play a major role in maintaining the delicate acid-alkaline balance of blood. The tissue proteins levels depend on the balance between their synthesis and catabolism. The present investigation reveals the decreased protein concentration in the liver of diabetic rats. The decreased protein levels may be due to the proteinuria [21] or may be due to increased protein catabolism [22] or may be due to poor functioning of the liver. In this study treatment of diabetic rats with CALEt has restored the protein concentration to the normal levels in a significant manner.

Aspartate aminotransferase and Alanine aminotransferase are members of transaminase family of enzymes. AST and ALT enzymes are highly concentrated in the liver and kidney and are found in the serum in significant amount when the cell membrane becomes leaky and even completely shattered. [23-24] Measurement of enzymatic activities of aminotransferases is of clinical and toxicological significance as changes in their activities is indicative of tissue damage by toxicants or in disease conditions. Rogers *et al*., [25] reported that AAT activity was increased 3 to 4 folds in the liver of STZ induced diabetic rats. Mild elevation of transaminases is frequently found in diabetic patients. [26]

In the current study the activities of aminotransferases (AST and ALT) were significantly increased in the liver of STZ induced diabetic rats while in kidney the activities of AST and ALT levels were not altered. Zafar *et al*., [27] and Subbaiah Rajasekaran *et al*., [28] also reported increased levels of AST and ALT in the liver of STZ induced diabetic rats. Supporting to our findings, Larcan *et al*., [29] has reported that the liver was necrotized in diabetic patients. The increase in ALT activity in diabetes is due to hepatocellular damage and is usually accompanied by an increase in AST activity. [30-31] Although the precise mechanism underlying the link between liver functional markers and incidence of diabetes remains unclear, some possibilities can be considered. One is that increased serum AST and ALT levels reflect a surplus deposit of fat in the liver, a state known as non-alcoholic fatty liver disease (NAFLD) that causes hepatic insulin resistance which is directly toxic to hepatocytes [32, 33]. Our unpublished data, representing higher serum triglycerides and lower HDL cholesterol in the raised ALT group, support such supposition. Another mechanism explaining the relationship between liver markers and diabetes may be related to oxidative stress that contributes to the development and progression of diabetes [34]. The other possible mechanism is that increased protein catabolism accompanying gluconeogenesis and urea formation that are seen in the diabetic state might be responsible for the elevation of the tissue transaminases. [35] The present results are in accordance with the previous studies [35-36] who reported elevated levels of AST and ALT in liver without any significant change in the activities of AST and ALT in kidneys. In the present study, the enhanced AST and ALT activities were significantly reduced after administration of CALEt to the diabetic rats. The reversal of AST and ALT activity in CALEt treated diabetic rats towards near normal level is evidence of the prevention of cellular and tissue damage under diabetic condition.

In view of the above, it is concluded that methanol extract of *Centella asiatica* leaves may possibly be used to alleviate the liver functions impairment associated with diabetic condition. However, further pharmacological and biochemical investigations are needed to find out the active constituent and its mechanism of action to distinguish the bioactive and ameliorative potential of the plant.

REFERENCES

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006; 3(11):e442.
2. World Health Organization. Global Health Estimates: Deaths by Cause, Age, Sex and Country, 2000-2012. Geneva, WHO, 2014.
3. Almdal TP, Vilstrup H. Effect of streptozotocin induced diabetes and diet on nitrogen loss from organs and the capacity of urea synthesis in rats. Diabetologia1987; 30:952-956.
4. Jorda A, Gomez M, Cabo J, Grisolia S. Effect of streptozotocin diabetes on some urea cycle enzymes. Biochem Biophys Res Commun. 1982; 106:37-43.
5. Akanji MA, Ngaha EO. Effect of repeated administration of Berenil on urinary enzyme excretion with corresponding tissue pattern in rats. Pharmacol Toxicol. 1989; 64: 272-279.
6. Adesokan AA, Akanji MA, Aderibigbe A. Serum glucose and lipid levels in alloxan-induced diabetic rats following oral administration of *Aloe barbadensis* miller juice extract. Trop J Health Sci. 2006; 2: 13-15.
7. Pushparaj P, Tan CH, Tan BK. Effects of *Averrho abilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol. 2000; 72: 69-76.
8. Bopanna KN, Kannan J, Godgil S, Balaraman R, Rathod SP. Antidiabetic and antihyperlipidemic effects of neem seed kernel powder on alloxan-diabetic rabbits. Indian J Pharmacol. 1997; 29:162-172.
9. Eskander EF, Jun HW, Ibrahim KA, Abdelal WE. Hypoglycaemic effect of a herbal formulation in alloxan-induced diabetic rats. Egypt J Pharm Sci. 1995; 36:253-270.

10. James JT, Dubery IA. Pentacyclic triterpenoids from the medicinal herb, *Centella asiatica* (L.) Urban. Molecules 2009; 14(10): 3922-3941.
11. Goh SH. Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases, Pelanduk Publications, Malaysia, 1995, 162.
12. Sasikala S, Lakshmi Narasaiah S, Dhananjaya Naidu M. Antidiabetic activity of *Centella asiatica* on streptozotocin induced diabetic male albino rats. World J Pharm Sci. 2015; 3(8):1701-1705.
13. Sharma VK. Streptozotocin: An experimental tool in diabetes and Alzheimer's disease. Int J of Pharma research & Development 2010; 2(1):9.
14. Singh V, Singh M, Shukla S, Singh S, Mansoori MH, Kori ML. Antidiabetic activity of *Flacourita indica* Merr in streptozotocin induced diabetic rats. Global Journal of Pharmacology 2011; 5(3):147-152.
15. Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Antidiabetic and anti-hyperlipidemic potential of *Abelmoschus esculentus* Moench. In streptozotocin-induced diabetic rats. J Pharm BioallSci. 2011; 3:397-402.
16. Djomeni PDD, Tedong L, Asongalem EA, Dimo T, Sokeng SD, Pierre K. Hypoglycaemic and antidiabetic effect of root extracts of *Cebia pentandra* in normal and diabetic rats. Afr J Trad Compl Altern Med. 2006; 3: 129–136.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 1951; 193(1):265-275.
18. Reitman S, Frankel S. Calorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. American Journal of Clinical Pathology 1957; 28: 56–63.
19. Bergmayer HU, Bruns E. Methods of enzymatic analysis, 1965, Academic Press, New York.
20. Steel RGD, Torrie JR. Principles and procedures of statistics with special reference to the biological sciences. McGraw, New York, 1960, 481.
21. Mauer SM, Steffes MW, Brown DM. The kidney in diabetes. Am J Med 1981;70:63-6
22. Almdal JP, Vilstrup H. Strict insulin therapy normalizes organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. Diabetologia 1998; 31: 114–118.
23. Cotran R, Kumar V, Robins S. Robin's Pathological Basis of Disease. 4th Edn. W.B Saunders Co. Harcourt. 1989: 212-217.
24. Ngaha EO. Renal effects of potassium dichromate in the rat: composition of urinary excretion with corresponding tissue pattern. Gen Pharmacol. 1981; 12: 291-358.
25. Rogers KS, Higgins ES, Kline ES. Experimental diabetes causes mitochondrial loss and cytoplasmic enrichment of pyridoxal phosphate and aspartate aminotransferase activity. Biochem Med Metab Biol. 1986; 36(1): 91-97.
26. Elizabeth H, Harris MD. Elevated liver function tests in type 2 diabetes. Clinical Diabetes 2005; 23:3.
27. Zafar M, Naeem-Ul-Hassan naqvi S, Ahmed M, Kaimkhani ZA. Altered liver morphology and enzymes in streptozotocin induced diabetic rats. Int J Morphol. 2009; 27(3): 719-725.
28. Rajasekaran S, Ravi K, Sivagnanam K, Subramanian S. Beneficial effects of *Aloe vera* leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clinical and Experimental Pharmacology and Physiology 2006; 33(3):232-237.
29. Larcan A, Lambert H, Laprevote-Heully MC, Delorme N. Light and electron microscopic study of hepatic lesions in the course of hyperlactatemia in diabetic patients. Diabetes Metabolism 1979; (5)103-112.
30. Mohan Rao GM, Morghom LO, Kabar MN, Benmohamud BM,Ashibani K. Serum glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) levels in diabetes mellitus. Indian J Med Sci. (1989); 5: 118-122.
31. Sekar N, William S, Balasubramaniyam N, Kamarajan P, Govindasamy S. Optimization of sodium orthovanadate to treat streptozotocin indued diabetic rats. J. Biosci. 1990; 15:67-75.
32. Marchesini G, Forlani G. NASH: from liver diseases to metabolic disorders and back to clinical hepatology. Hepatology. 2002; 35:497-499.
33. Harris EH. Elevated liver functions test in type 2 diabetes. Clinical Diabetes 2005; 23(3):115-119.
34. Ceriello A. Oxidative stress and glycemic regulation. Metabolism. 2000; 49: 27-29.
35. Kaleem M, Medha P, Ahmed QU, Asif M, Bano B. Beneficial effects of *Annona squamosa* extract in streptozotocin-induced diabetic rats. Singapore Med J. 2008; 49 (10): 800–804.
36. Mandade R. Protective effects of *Carthamus tinctorius* on streptozotocin-induced diabetic complications in Rats and the possible morphological changes in the liver and kidney. International Journal of Science Innovations and Discoveries 2012; 2(5):502-510.

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