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EVALUATION OF ANTIDIABETIC ACTIVITY OF *XANTHIUM STRUMARIUM* L. (COMPOSITAE) IN ALLOXAN INDUCED DIABETIC MICE

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ABSTRACT

The concerned study reveals the experimental investigation of the biological activity of *Xanthium strumarium* L. (Family: Compositae) used as a traditional anti diabetic agent in past and present culture. The alcoholic extracts of *Xanthium strumarium* was studied for anti-diabetic activity in alloxan induced diabetic mice by oral administration of extracts 400mg/kg body weight for 24 days. The effect was compared with oral dose of 5mg/kg Glibenclamide. Alloxan induced hyperglycemia and glucose fed hyperglycemia mice models were used for the evaluation of anti-diabetic activity. The effect of *Xanthium strumarium* on alloxan induced hyperglycemic activity was shown in dose dependent manner. The alcoholic extract of leaf and fruit of *Xanthium strumarium* significantly decreases the blood glucose of hyperglycemic mice. Phytochemical study showed the presence of glycosides. It is concluded that *Xanthium strumarium* leaf and fruit extract have significant antidiabetic activity, which lowered the fasting blood glucose level in alloxan induced diabetic Mice.

Key Words: *Xanthium strumarium*, Anti-diabetic activity, Alloxan, Glibenclamide.

INTRODUCTION

Diabetes mellitus is the most important non - infective epidemic to hit the globe in the present millennium. By the year 2025, India shall have the maximum number of diabetes in the world making it, the" Diabetes capital of the world" (Hillary K *et al.*, 1998), .Despite the great strides, made in understanding and management of diabetes, the disease and disease related complications are increasing unabated due to multiple defects, in its pathophysiology (Lvorra *et al.*, 1989). Parallel, to this, the holistic approach of herbs has accelerated the global efforts to harness and harvest medicinal plants having multiple beneficial effects (Kameswara Rao *et al.*, 1997). Some of them have been evaluated and active principles isolated; however, the search for novel anti diabetic drugs continues. Diabetes mellitus (DM) is a chronic disease caused by insufficient production of insulin by pancreatic glands and decreases in absorption of glucose by the cells in the human systems and caused increase the concentration of glucose in blood. It is also produced due to the hereditary Characters. Due to increase glucose level in blood causes various deficiencies and hampers the normal Physiological effects of the human system like blood vessels and nerves system etc. It is projected that the Diabetes is the main disease which can increase the deaths retain next coming 25 years in Asian countries and Africans (Ragavan *et al.*, 2006 & Trivedi NA *et al.*, 2004).

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Commonly *Xanthium strumarium* L. (Family: Compositae), it is a Indian system of Medicine, the various plants parts like leaves, roots, bark, roots, fruits etc are used for the treatment of diabetic, mouth ulcers, Malaria, cancer, diarrhoea, fever, inflammation etc. Hence the present investigation was under take to evaluate the anti-diabetic activity of alcoholic extracts of *Xanthium strumarium* L. leaf and fruit in alloxan induced diabetic Mice to confirm the Pharmacological evidence in support of folklore claim.

MATERIALS AND METHODS

Plant Material

Collection of Plant

Xanthium strumarium was collected in the month of December 2010 from Chittoor, Andhra predesh. The plant authentification was done by Madhava chetty, assistant professor, botany department, college of sciences, S.V. University, Tirupati, Andhra Pradesh.

PREPARATION OF ALCOHOLIC EXTRACTS

The plant leaves and fruits were dried in the laboratory at room temperature and powdered in a mixer grinder. To prepare the extract, 50 g of the plant leaf and fruit powder in 250 ml of methyl alcohol was performed by Soxhlet apparatus for 8 h at room temperature for 15 days. The residue was removed by filtration. The filtrate was evaporated to dryness at 40-50°C under reduced pressure in a rotary evaporator. (The yield of ethyl alcohol extract was approximately 10%). The extract was suspended in sodium carboxy methyl cellulose and used for oral administration.

Phytochemical screening

A Preliminary phytochemical screening of alcoholic extract Leaf and fruit of *Xanthium strumarium* was carried by using standard procedures (Khandelwal KR, 2003).

CHEMICALS

All the chemicals were of analytical grade. Carboxy methyl cellulose, Alloxan (SD fine, Mumbai), 0.9% normal saline. All other chemicals used were of analytical grade.

ANIMALS

Male & Female Wister mice (25-35g) were housed in group of 8 animals and maintained under standardized condition (12-hours light/dark cycle, 24 ^{0}C) and provided free access to pelleted CHAKKAN diet (Sai Enterprise Pvt. Ltd., Chennai) and purified drinking water ad libitium. All the animals were divided in five groups and treatment schedule is as follows. After treatment, histopathological studies were carried out. All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Venkateswara College of Pharmacy, Chittoor and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute Toxicity Studies

Healthy adult female Swiss albino mice weighing between 25 and 30 g body weight were selected for the acute toxicity study with the extracts of *Xanthium strumarium* doses of 5, 50, 300 and 2000 mg/kg were selected based on the fixed dose (OCED Guideline No. 401) method of CPCSEA (OCED, 1993). Animals were divided into four groups of three animals each and fasted overnight. The doses of 5, 50, 300 and 2000 mg/kg b.wt. were administered to the animals of Groups I, II, III,IV and V, respectively. The extracts were administered orally. The animals were continuously observed for 24 hours to detect changes in autonomic or behavioral responses. Mortality in each group was observed for 7 days.

INDUCTION OF DIABETES

Diabetes was induced in male swiss albino mice by intraperitoneal administration of alloxan monohydrate (150 mg/kg body weight), dissolved in 0.9% normal saline. Since the alloxan induction is done two days to avoid mortality of mice. Alloxan is capable of destruction of pancreatic beta cells and hyperglycemia was confirmed by the elevated blood glucose level, determined at 48 hours. Mice with fasting blood glucose levels >126mg/dl said to be diabetic and those with >200mg/dl used for study.

Treatment protocol

The diabetic mice were randomly divided into five groups (n = 6/groups).

Group 1: Normal group receives standard food pellets and water, *ad libitum* for the period of 24 days.

Group 2: Diabetic group receives standard food pellets and water, *ad libitum* and one single alloxan dose 150 mg/kg i.p. for the period of 24 days.

Group 3: Standard group receives standard food pellets and glibenclamide (5 mg/kg i.p.) for the period of 24 days.

Group 4: Test group 1 (leaf extract) receives standard food pellets and test drug (400 mg/kg oral), for the period of 24 days.

Group 5: Test group 2 (fruit extract) receives standard food pellets and test drug (400 mg/kg oral), for the period

of 24 days.

Mice were fasted overnight and the blood was collect from the retro orbital plexus to determine blood glucose by GOD – POD kit method. The change body weight was observed once a week. After 24 days, body weights were determined and the animals were sacrificed under the influence of anesthetic ether. The blood sample withdrawn from the sacrificed animals was centrifuged at 3000 rpm for 15 min (Kumar 2008 & Basu V *et al.*, 2009).

Analysis of blood sugar levels

Blood samples were collected from the retro orbital plexus after overnight fast animals at 1st, 3rd, 6th, 9th, 12th, 15th, 18th, 21st and 24th day. The blood glucose level in the samples was estimated using god-pod method by UV-Spectrophotometer.

Statistical analysis

All values were expressed as Mean ± SD. The differences between Diabetic control and treatment group were tested for significance using ANOVA followed by Dunnet's test. P<0.05 were considered significant.

RESULTS

Phytochemical tests

Phytochemical screening of the extracts and fractions of *Xanthium strumarium* showed the presence of various chemical constituents. Saponins, proteins, carbohydrates, resins, alkaloids, flavonoids, terpenoids, and steroids are conspicuously present in large amount in the crude extract.

Effect of Alcoholic extracts of leaf and fruit on diabetic mice

The anti-hyperglycemic effect of the alcoholic extract of the leaves and fruits of *Xanthium strumarium* (400mg/kg) and glibenclamide (5 mg/kg) on blood glucose levels of diabetic and non-diabetic rats are shown in Fig 1 and Table.1 The extract exhibited effect in a dose dependent manner. The 400 mg/kg of alcoholic extracts showed a significant ($p < 0.05$) decrease in the blood sugar level while the other doses showed no significant effect ($p>0.05$). The percentage reduction in blood sugar was significant at 24th day after treatment with 400 mg/kg of *Xanthium strumarium*.

HISTOPATHOLOGY STUDY

Animals were sacrificed on 24th day of treatment. Pancreas of all group of animals were removed, washed with cold saline and preserved in 10% formalin in buffered form. The results were shown in Fig. 2, 3, 4, 5 & 6 respectively.

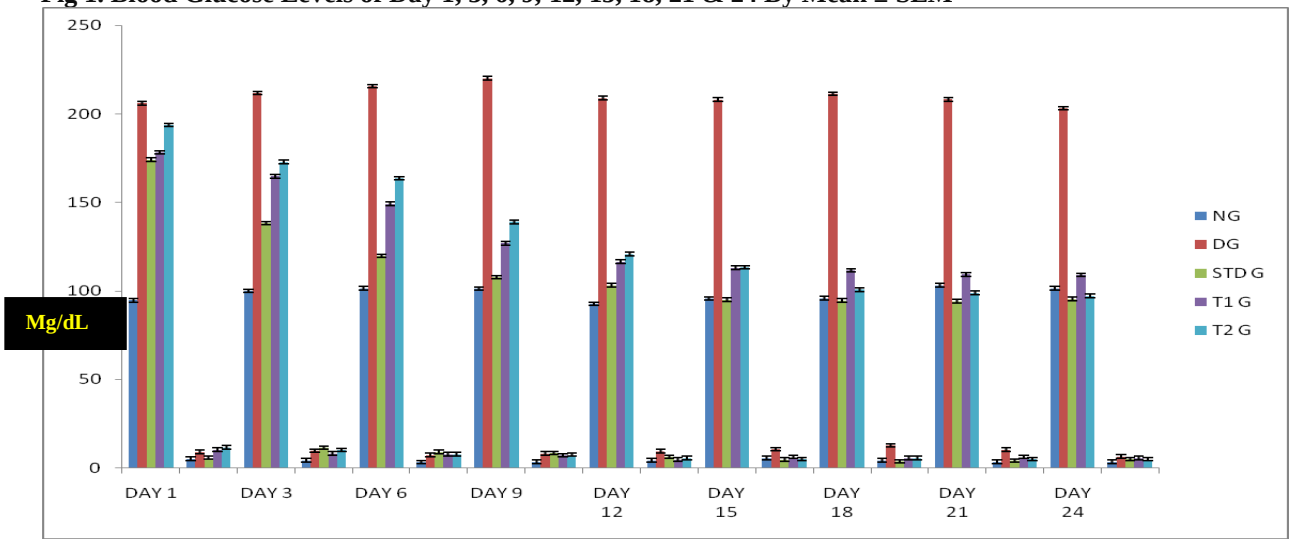
The anti-hyperglycemic effect of the alcoholic extracts of *xanthium strumarium* on the fasting blood sugar levels of diabetic mice is shown in Figures illustrated. Administration of alloxan (150 mg/kg, i.p.) led to elevation of fasting blood glucose levels, which was maintained over a period 24 days. Twenty four days of daily treatment of leaf and fruit alcoholic extracts of *xanthium strumarium* led to a dose-dependent fall in blood sugar levels. Effect seems to reach maximum after 15 days of treatment and remains constant in third week. Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 24 days. Alloxan causes excellent body weight reduction in diabetic group. When compare diabetic group with the normal group, normal group shows significant blood glucose reduction. When compare to diabetic group standard group, test groups shows blood glucose level reduction. When compare to test 1 (leaf) group, test 2 (fruit) group shows significant blood glucose level reduction during experimental period.

Table 1. Effect on Alcoholic extract of *Xanthium strumarium* on blood glucose level in Alloxan induced diabetic Mice

Groups	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24
NG	C 94.75± 5.20	C100± 4.32	c101.38± 3.22	c101.25± 3.45	c92.625± 4.46	c95.75± 5.73	c 95.875 ± 4.30	c103.25± 3.35	c101. 38± 3.40
DG	206± 8.98	211.75± 9.73	215.63± 7.27	220.13± 8.15	208.88± 9.44	208± 10.57	211.43 ± 9.77	208.14± 9.44	203.14± 6.53
STD G	a174.13± 5.77	c138.25± 1.41	c119.71± 9.16	c107.71± 8.49	c103.29± 6.35	c95± 4.865	c 94.714 ± 3.76	c94.143± 4.17	c 95.429± 5.00
T1 G	178.25± 10.46	c164.75± 8.22	c149.25± 7.70	c127± 7.18	c116.5± 4.66	c 113.13± 6.22	c 111.63 ± 5.54	c109.38± 6.22	c 109± 5.66
T2 G	193.5± 11.64	b172.5± 10.10	c163.63± 7.89	c139± 7.50	c120.88± 5.55	c 113.38± 4.96	c 100.75± 5.51	c99± 4.957	c97.125± 4.919

a p<0.05, b p<0.01, c p<0.001 compared to normal group, Dunnett comparison test through one way ANOVA test All values are expressed mean ± SEM (n=8)

Fig 1. Blood Glucose Levels of Day 1, 3, 6, 9, 12, 15, 18, 21 & 24 By Mean ± SEM



Histopathology Study

Fig 2. The Normal group- Architecture of the Pancreatic Tissue with Lower Magnification (10x); And Higher Magnification (40x),

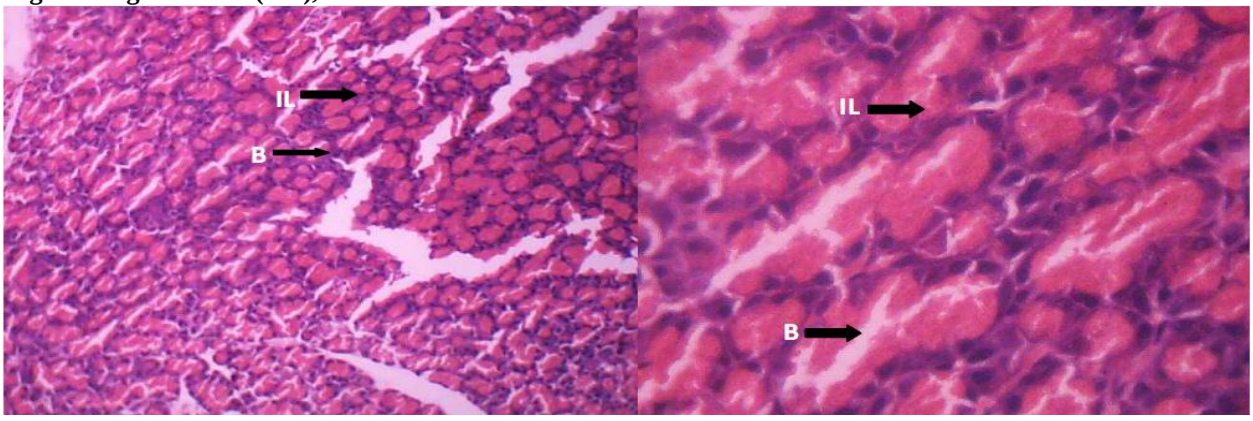


Fig 3. Diseased Group- The Degenerative Changes In Islets Langerhans With Lower Magnification (10x); and Higher Magnification (40x)

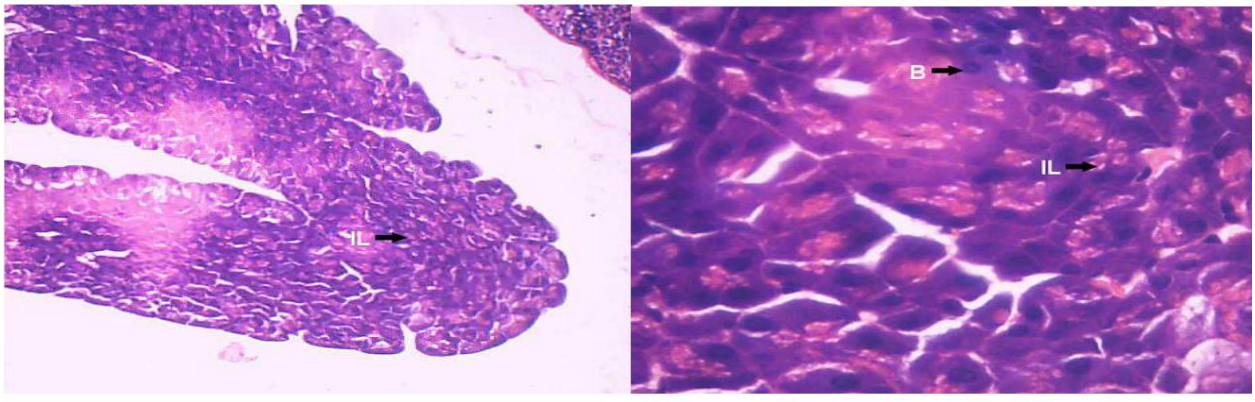


Fig 4. Standard Group- the Normal Architecture of the Pancreatic Tissue with Lower Magnification (10x); and Higher Magnification (40x), but when compare to normal group standard is having somewhat lesser architecture of pancreatic tissue

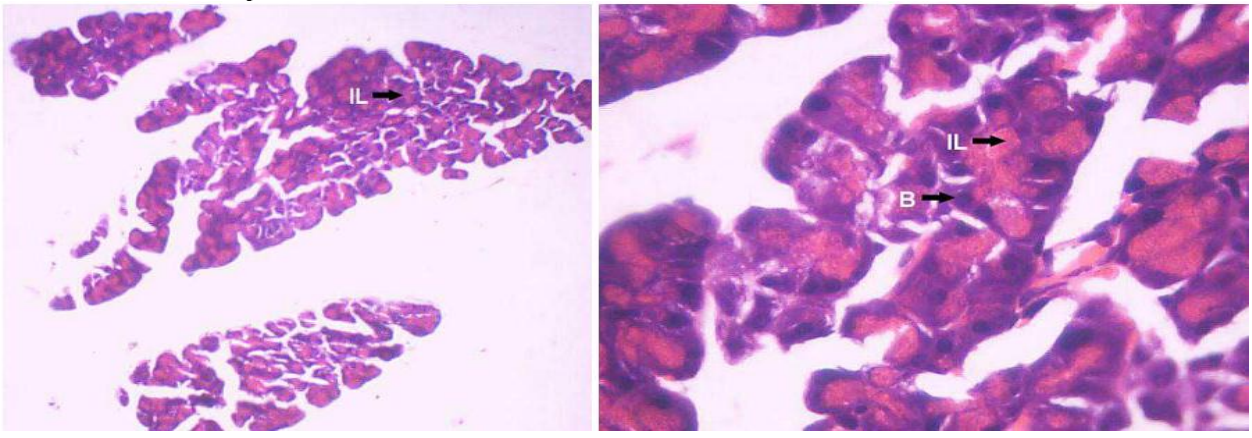


Fig 5. Test 1 Group- The regenerative changes in the Pancreatic Tissue with Lower Magnification (10x); and Higher Magnification (40x)

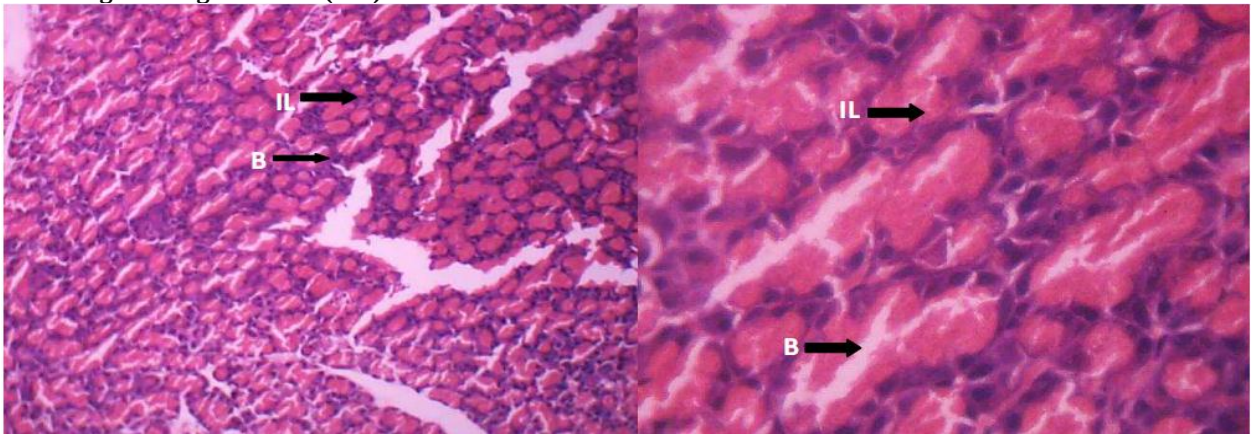
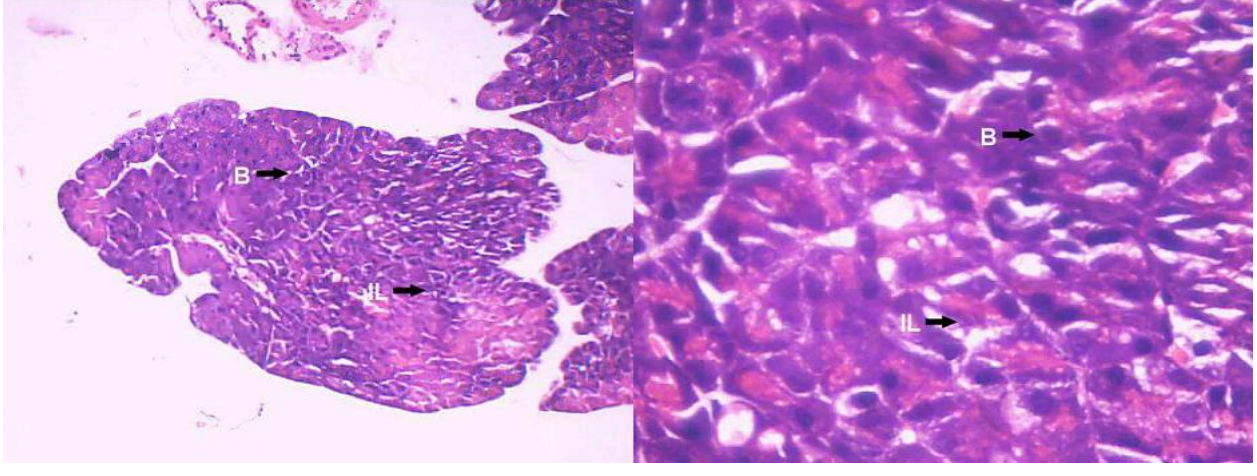


Fig 6. Test 2 Group the regenerative changes in the Pancreatic Tissue with Lower Magnification (10x); and Higher Magnification (40x)



DISCUSSION

The diabetes mellitus is a metabolic disorder associated with derangement of insulin secretion and resistance to the action of insulin. Hyperglycemia is occurred in diabetic state is a condition in which an excessive amount of glucose circulates in the blood plasma. In light of the results, our study indicates that *xanthium strumarium* leaf and fruit extracts have good hypoglycemic activity. Alcoholic extracts of *xanthium strumarium* exhibited significant hypoglycemic activities in alloxan induced hyperglycemic mice.

This present study discussed about the hypoglycemic activity of the T1 and T2 drugs on alloxan induced diabetic mice. Alloxan injection caused diabetes mellitus, which may be due to destruction of β cells of the islet of langerhans of the pancreas. Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and reduced utilization of glucose by the tissues are the fundamental basis of hyperglycemia in diabetes mellitus. Administration of T1 and T2 drugs to glucose loaded normal mice fasted for 18 h, resulted in decrease in blood sugar level after 60 min. The decline in blood sugar level reached its maximum at 2 hrs. In our study the difference observed between the blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group as compared with other group of animals at the end of experimental period. Our investigations indicate the efficiency of the T1 and T2 drugs in the maintenance of blood glucose levels in alloxan induced diabetic mice. Administration of T1 and T2 drugs to diabetic mice showed a significant decrease in the levels of blood glucose. The possible mechanism by which T1 and T2 drugs brings about its hypoglycemic action in diabetic mice may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

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