Effects of Eugenia Jambolana and Zizyphus Sativa on Blood Glucose Levels of Normal and Alloxan Diabetic Rabbits

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Summary

The alcoholic extracts of the seeds of Eugenia jambolana and leaves of Zizyphus sativa were administered in doses of 150 mg/kg, 200 mg/kg and 250 mg/kg body weight to normal and alloxan-diabetic rabbits. The blood glucose levels were estimated before and 2, 4, 6 and 8 hours after the administration of the extract. The extract of Eugenia jambolana exerted a significant (P < 0.05) hypoglycaemic effect in normal rabbits. The hypoglycaemic effect was not significant (P > 0.01) in alloxan-diabetic rabbits. The extract of Zizyphus sativa did not produce any significant (P > 0.01) hypoglycaemic effect in normal as well as in alloxan-treated diabetic rabbits. The doses used did not show any acute toxicity and behavioural changes. From this study it may be concluded that the extract of Eugenia jambolana acts by initiating the release of insulin from pancreatic beta cells of normal rabbits. Moreover, Zizyphus sativa, which is used as anti-diabetic in folk medicine, did not show any hypoglycaemic effect in normal as well as in diabetic rabbits.

Introduction

A number of indigenous plants are claimed to be useful in the treatment of diabetes mellitus based on folk medicine[17]. Several such plants show hypoglycaemic activity when taken orally, for example, Allium cepa[12], Momordica foetida[11], Coccinia indica[10], Momordica charantia[2], and Cuminum nigrum[1]. Eugenia jambolana and Zizyphus sativa, locally known as Jaman and Unab respectively, have been used by practitioners of Unani sys-

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tem of Medicine for the treatment of diabetes mellitus[8,13].

Eugenia jambolana belongs to the family Myrtaceae. This tree which yields an abundant crop of edible fruit, during the hot weather, is cultivated all over the subcontinent[18]. Its bark is astringent and used in case of dysentery, and the decoction as a tooth gargle. The powdered seeds had the reputation in the recent years of being useful in the treatment of diabetes. The juice of fruits is stomachic and carminative[6].

Zizyphus sativa belongs to the family Rhamnaceae. It is a shrub or small tree which grows in hilly areas of Abbottabad and Bugnotar. Its dried fruit is suppurative, expectorant and is "purifier" of the blood. Its bark is employed to clean wounds. Its leaves are used to treat diabetes mellitus[6].

The present work has been undertaken with the aim to study the effect of the alcoholic extract of the seeds of Eugenia jambolana and leaves of Zizyphus sativa on the blood glucose levels of normal and alloxan-treated diabetic rabbits. Acute toxicity and behavioural changes were also studied to check the safety of these doses.

Material and Methods

Experiments were performed on male, adult rabbits of local strain weighing 0.51-1.72 kg. They were fed on green vegetables and grains and allowed tap-water ad libitum.

Chemicals

Alloxan monohydrate was supplied by B.D.H. Laboratories (Chemical Division), Poole, England, O-toluidine, Glacial acetic acid, Thiourea and Trichloroacetic acid were obtained from E. Merck, Dermstadt, West Germany, Tolbutamide was provided by Hoechst, West Germany.

Preparation of Extract

Alcoholic extracts of the seeds of Eugenia jambolana and leaves of Zizyphus sativa were prepared by the method described by Qayum et al[16]. "The dried, powered plant material was extracted three times with alcohol in a percolator. The alcoholic extract obtained after removal of solvent under vacuum was triturated with pet-ether (40-60°) a sufficient number of times until a fresh addition of pet-ether failed to remove significant colouring material. This pet-ether treated alcoholic extract was charcoaled, filtered and dried under vacuum.

Preparation of Diabetic Rabbits

The method described by Akhtar et al[2] was adopted. A group of rabbits was made diabetic by injecting intravenously, 150 mg/kg body weight of alloxan monohydrate. Eight days after injection, the blood glucose levels of surviving rabbits were estimated. Rabbits with blood glucose levels above 200 mg/100 ml were considered as diabetic.

Grouping of Rabbits

Normal rabbits were divided into 5 groups (1-5) of six animals each. Group 1 served as control and received 15 ml of 2% gum tragacanth solution. Group 2 received tolbutmide 500 mg/kg body weight. Group 3-5 received extract of Eugenia jambolana dissolved in 15 ml of 2% gum tragacanth solution in doses of 150 mg/kg, 200 mg/kg and 250 mg/kg respectively. The diabetic rabbits were also divided in 5 groups on the same pattern.

Similar to above, 5 groups of 6 rabbits each were made to study the effects of extract of Zizyphus sativa. Group 1 served as control and received 15 ml of 2% gum tragacanth solution. Group 2 received tolbutamide 500 mg/kg body weight. Group 3-5 received extract of Zizyphus sativa dissolved in 15 ml of 2% gum tragacanth solution in doses of 150 mg/kg,, 200 mg/kg and 250 mg/kg respectively. The diabetic rabbits were also divided in 5 groups on the same pattern.

Preparation and Administration of Extract Solutions

The amount of Eugenia jambolana and Zizyphus sativa extract required for each rabbit was calculated on body weight basis and it was dissolved in 10 ml of 2% gum tragacanth solution and final volume made upto 15 ml. The extract was administered to each rabbit by using a stomach tube attached to a standard syringe containing the 15 ml of extract solution. The tube was inserted into the stomach through oesophagus and the plunger was pressed slowly and steadily. Immediate sneezing and coughing indicated injection into the lungs and in such a condition the tube was at once withdrawn and another animal was taken instead. The tolbutamide solution was administered in a similar manner.

Collection of Blood

The procedure for collection of blood was adopted as described by Akhtar et al[1]. The rabbit was held in a wooden rabbit holder and immediately before administration of drug, 0.2 ml of blood for glucose estimation was collected from an ear-vein. Similar blood samples were collected at 2,4,6 and 8 hours after the drug administration. After collection of blood, the pricked site of the ear was rubbed with cotton wool soaked with 70% alcohol to protect the rabbit against infection.

Blood Glucose Estimation

Blood glucose estimation was done by the method of Winckers and Jacobs[19].

Studies on Acute Toxicity of the Extracts

The possible toxic effects of the extracts were studied on rabbits of local strains weighing between 0.54-1.68 kg. The rabbits were divided into 4 groups (1-4) of six animals each. Group 1 served as control and received 15 ml of 2% gum tragacanth solution. Group 2-4 rceived extract of Eugenia jambolana dissolved in 15 ml of 2% gum tragacanth solution in doses of 150 mg/kg, 200 mg/kg and 250 mg/kg respectively. Animals were observed for 8 hours after administration of the extract to check toxic symptoms. They were kept under observation for 7 days.

Similar to above, 4 groups of six animals were made to study the possible toxic effects of Zizyphus sativa.

Results

The effects of different doses of Eugenia jambolana, Zizyphus sativa, tolbutamide and gum tragacanth on blood glucose levels of normal and alloxan-diabetic rabbits are shown in Tables I, II and Figs. 1, 2.

Effects of Eugenia jambolana extract on blood glucose levels in normal rabbits.

The mean percent decreases in blood glucose levels produced by 150 mg/kg of Eugenia jambolana at 2, 4, 6 and 8 hours were 13.92 ± 0.78, 18.38 ± 0.87, 22.48 ± 0.91 and 20.0 ± 0.84 respectively, which are significant ($P < 0.05$). The mean percent decreases in blood glucose levels produce

by 200 mg/kg of Eugenia jambolana at 2, 4, 6 and 8 hours were 19.80 $\pm$ 0.84, 22.66 $\pm$ 0.79, 26.48 $\pm$ 0.91 and 24.53 $\pm$ 0.88, which are significant (P < .05). The mean percent decreases in blood glucose levels produced by 250 mg/kg of Eugenia jambolana at 2, 4, 6 and 8 hours were 23.66 $\pm$ 0.76, 26.30 $\pm$ 0.91, 28.31 $\pm$ 0.94 and 26.92 $\pm$ 0.88 respectively, which are significant (P < 0.05).

Effect of Zizyphus sativa extract on blood glucose
levels in normal rabbits.

The mean percent decreases in blood glucose levels produced by 150 mg/kg of Zizphus sativa at 2, 4, 6 and 8 hours were 1.98 + 0.65, 2.74. + 0.70, 2.55 $\pm$ 0.61 and 2.0 $\pm$ 0.52 respectively, which are non-significant (P > 0.10). The mean percent decreases in blood glucose levels produced by 200 mg/kg of Zizyphus sativa at 2, 4, 6 and 8 hours were 0.48 $\pm$ 0.21, 1.17 $\pm$ 0.34, 1.85 $\pm$ 0.40 and 1.88 $\pm$ 0.46 respectively, which are non significant (P > 0.10). The mean percent decreases in blood glucose levels produced by 250 mg/kg of Zizyphus sativa at 2, 4, 6 and 8 hours were 1.21 $\pm$ 0.35, 2.63 $\pm$ 0.57, 3.03 $\pm$ 0.67 and 3.00 $\pm$ 0.65 respectively, which are non significant (P > 0.10).

Effect of Tolbutamide on blood glucose
levels in normal rabbits

The mean percent decreases in blood glucose levels produced by 500 mg/kg of tolbutamide at 2, 4, 6 and 8 hours were 13.70 $\pm$ 0.85, 26.68 $\pm$ 1.2, 24.01 $\pm$ 0.96 and 19.2 $\pm$ 0.94 respectively, which are significant (P < 0.05).

Effects of Gum Tragacanth on blood glucose
levels in normal rabbits

The mean percent changes in blood glucose levels produced by 15 ml of 2% gum tragacanth solution at 2, 4, 6 and 8 hours were 1.29 $\pm$ 0.51, 1.53 $\pm$ 0.63, 0.51 $\pm$ 0.09, and 0.50 $\pm$ 0.07 respectively, which are non significant (P > 0.10).

Effect of Eugenia jambolana extract on blood glucose
levels in diabetic rabbits

The mean percent decreases in blood glucose levels produced by 150 mg/kg of Eugenia jambolana at 2, 4, 6 and 8 hours were 0.82 $\pm$ 0.13,

1.75 + 0.32, 2.29 + 0.49 and 3.01 + 0.53 respectively, which are non significant (P > 0.10). The mean percent decreases in blood glucose levels produced by 200 mg/kg of Eugenia jambolana at 2, 4, 6 and 8 hours were 0.60 + 0.13, 1.29 + 0.40, 2.79 + 0.84 and 2.0 + 0.77 respectively, which are non significant (P > 0.10). The mean percent decreases in blood glucose levels produced by 250 mg/kg of Eugenia jambolana at 2, 4, 6 and 8 hours were 0.53 + 0.07, 1.16 + 0.45, 1.82 + 0.61 and 1.25 + 0.53 respectively, which are non significant (P > 0.10).

Effect of Zizyphus sativa extract on blood
gucose levels in diabetic rabbits

The mean percent decreases in blood glucose levels produced by 150 mg/kg of Zizyphus sativa at 2, 4, 6 and 8 hours were 0.56 + 0.09, 0.26 + 0.02, 0.64 + 0.01 and 0.50 + 0.05 respectively, which are non significant (P > 0.10). The mean percent decreases in blood glucose levels produced by 200 mg/kg of Zizyphus sativa at 2, 4, 6 and 8 hours were 0.61 + 0.07, 0.81 + 0.09, 1.04 + 0.84 and 0.90 + 0.63 respectively, which are non significant (P > 0.10).

The mean percent decreases in blood glucose levels produced by 250 mg/kg of Zizyphus sativa at 2, 4, 6 and 8 hours were 0.51 + 0.02, 0.62 + 0.04, 1.98 + 0.72 and 2.25 + 0.82 respectively, which are non significant (P > 0.10).

Effect of Tolbutamide on blood glucose
levels in diabetic rabbits

The mean percent decreases in blood glucose levels produced by 500 mg/kg of Tolbutamide at 2, 4, 6 and 8 hours were 0.36 + 0.05, 0.53 + 0.08, 0.60 + 0.10 and 0.57 + 0.09 respectively which are non significant (P > 0.10).

Effect of gum tragacanth on blood glucose
levels in diabetic rabbits

The mean percent changes in blood glucose levels by 15 ml of 2% gum tragacanth solution at 2, 4, 6 and 8 hours were 0.39 + 0.03, 1.04 + 0.24, 0.13 + 0.02 and 0.35 + 0.04 respectively, which are non-significant (P > 0.10).

Acute Toxicity Study

The rabbits receiving 150 mg/kg, 200 mg/kg and 250 mg/kg body weight of the extracts of Eugenia jambolana and Zizyphus sativa did not show any visible signs of toxicity e.g. excitement, restlessness, respiratory distress, convulsions or coma. Moreover, they remained alive for upto 7 days.

Discussion

The observations show that the gum tragacanth solution used as vehicle in these experiments did not produce any significant change on blood glucose levels of normal as well as on that of alloxan induced diabetic rabbits. This finding is in accordance with the observations of Marquis et al[11] and Akhtar and Ali[1].

The present study revealed that the alcoholic extract of Eugenia jambolana produced a significant hypoglycaemic efffect when administered orally to normal rabbits. The observations revealed that Eugenia jambolana extract in dose of 250 mg/kg produced a maximum decrease in blood glucose levels of normal rabbits. The observations also indicate that hypoglycaemic effect in normal rabbits develops slowly and is most pronounced about 6 hours after the administration.

The extract, however, did not show any significant effect on blood glucose levels of alloxan treated rabbits. For comparison, the effect of the standard hypoglycaemic drug tolbutamide– (500 mg/kg) was observed on the blood glucose levels of normal and alloxan treated diabetic rabbits. Tolbutamide produced significant hypoglycaemic effect in normal rabbits but not in alloxan-treated diabetic rabbits. This finding is in accordance with the observation of Augusti and Benaim[7] and Akhtar et al[5].

Sulphonulureas including tolbutamide, have been reported to produce hypoglycaemia by stimulating pancreatic beta cells to release more insulin into the blood stream, thus increasing glycogen deposition in the liver, causing a reduction of glycogen levels, and having an extrapancreatic effect to possibly increase the number of insulin receptors[1,9].

In view of the similarity between the effects of tolbutamide and Eugenia jambolana, it may be likely that the hypoglycaemic effect of Eugenia jambolana also be mediated through the release of insulin from the pancreatic beta cells. A similar mechanism has been proposed to explain the hypoglycaemic effect in normal rabbits of other indigenous plants such as Tecoma stans[14]. Momordica foetida[11], Euphorbia prostrata and Fumaria parviflora[3], Bergenia lingulata[4], Taraxacum officinale[5] and Eriobotria japonica[15].

The observations show that the extract of the leaves of Zizyphus sativa did not produce any significant hypoglycaemic effect in normal or in alloxan

treated diabetic rabbits. This indicates that extract of *Zizyphus sativa* is devoid of antidiabetic activity but is traditionally used only on empirical grounds in the folk medicine.

Acknowledgements

References

Akhtar, M.S. and Ali, M.R. (1985): Study of hypoglycaemic activity of Cuminum nigrum seeds in normal and alloxan diabetic rabbits. Planta med. 51:81-85.

Akhtar, M.S., Akhtar M.A. and Yaqub, M. (1981): Effect of Momordica charantia on blood glucose levels of normal and alloxan diabetic rabbits. Planta med., 42:205-12.

Akhtar, M.S., Khan, Q.M. and Khaliq, T. (1984): Effect of Euphorbia prostrata and Fumaria parviflora in normoglycaemic and alloxan treated hyperglycaemic rabbits. Planta med., 66:138-142.

Akhtar, M.S. and Ali, M.R. (1984): Study of anti-diabetic effect of a compound medicinal plant prescription in normal and diabetic rabbits, JPMA, 34:239-244.

Akhtar, M.S., Khan, Q.M. and Khaliq T. (1985): Effect of Portulaca oleracae and Taraxacum officinale in normoglycaemic and alloxan treated hyperglycaemic rabbits. JPMA, 35:207210.

Anonymous (1984): "A note on the plants of medicinal value found in Pakistan". Printing Corporation of Pakistan Press, Islamabad: 22, 55.

Augusti, K.T. and Benaim, M.E. (1975): Effect of essential oil of Onion (APDS) on blood glucose, free futty acids and insulin levels of normal subjects. Clin Chim. Acta, 60: 121.

Chopra, R.N., I. Chopra, Handa, K.L. and Kapur, L.D. (1985): Indigenous drugs of India, U.N. Dhur and sons Ltd., Calcutta: 686.

Karam, J.H. (1982): Pancreatic harmones and antidiabetic drugs, in Basic and Clinical Pharmacology by Bertram G. Katzung, Maruzen Asian ed., Huntsmen Offset Printing Pte. Ltd., Singapore, 458-468.

Khan, A.K.A., Akhtar, M.S. and Mahtab, H. (1980): Treatment of diabetes mellitus with Coccinia indica. BMJ. 280:1044.

Marquis, V.O., Adanlawo, T.A. and Olaniyi, A.A. (1977): The effect of foetidin from Momordica foetida on blood glucose levels of albina rats. Planta med., 31:367-74.

Mathew, P.T. and Augusti, K.T. .(1975): Hypoglycaemic effects of onion, Allium cepa Linn on diabetes mellitus. A preliminary report. Ind J Physical and Pharmacol; 19:213-217.

Nadkarni, K.M. (1954): Indian Materia Medica 3rd ed. Popular Book Depot, Bombay; 51:81-85.

Nash, J.B., Albers, C.C., Howard, J.K. and Fly, S.H. Jr. (1950): Lack of antidiabetogenic and antidiabetic effects of Tecoma stans in alloxandiabetes. Tex. Rep; Biol., med. 8:350.

Noreen, W., Wadood, A., Hidayat, H.K. and Wahid S.A.W. (1988): Effect of Eriobotria japonica on blood glucose levels of normal and alloxan diabetic rabbits, Planta med, 26:1-6.

Qayum, A., Ahmed, N. Ahmed, K.D., Khattak, S.G. (1982): Pharmacological screening of medicinal plants. JPMA, 32:103-105.

Said, M. (1970): "Hamdard Pharmacopoeia of estern medicine" II ed. Hamdard National Foundation, Karachi; 53-54.

Said, M. (1972): Pharmacographia indica. 1st ed. Hamdard National Foundation, Karachi; 170-71.

Winckers and Jacobs; (1980): Blood glucose and its determination, in practical Clinical Biochemistry by Varley, H., 5th ed. Heinemann, London, 396-397.

TABLE I

EFFECT OF EUGENIA JAMBOLANA, ZIZYPHUS SATIVA AND TOLBUTAMIDE ON BLOOD GLUCOSE LEVELS OF NORMAL RABBITS

Blood Glucose in mg/100 ml

Time Interval (hours)	Control	Tolbutamide (500 mg/kg)	Eugenia 150 mg/kg	jambolana 200 mg/kg	250 mg/kg	150 mg/kg	Zizyphus sativa 200 mg/kg	250mg/kg
0	92.76 ± 2.80 (6)	102.4 ± 1.50 (6)	98.52 ± 2.61 (6)	100.45 ± 2.74 (6)	102.74 ± 3.52 (6)	105.7 ± 3.4 (6)	102.5 ± 2.4 (6)	98.7 ± 2.0 (6)
2	91.56 ± 3.06 (6)	88.36 ± 0.80 (6)	84.80 ± 2.45 (6)	80.56 ± 2.51 (6)	78.43 ± 2.62 (6)	103.06 ± 2.2 (6)	102.0 ± 2.1 (6)	97.5 ± 2.6 (6)
4	94.18 ± 2.7 (6)	74.87 ± 0.77 (6)	80.41 ± 2.30 (6)	77.68 ± 2.63 (6)	75.71 ± 2.80 (6)	102.8 ± 2.7 (6)	101.3 ± 2.2 (6)	96.1 ± 3.0 (6)
6	92.28 ± 2.7 (6)	77.81 ± 0.72 (6)	76.39 ± 3.1 (6)	74.15 ± 2.4 (6)	73.65 ± 2.31 (6)	103.0 ± 1.99 (6)	100.6 ± 3.1 (6)	95.7 ± 2.2 (6)
8	92.29 ± 2.5 (6)	82.73 ± 1.8 (6)	78.81 ± 2.7 (6)	75.80 ± 2.51 (6)	75.08 ± 2.72 (6)	103.58 ± 2.3 (6)	100.57 ± 2.63 (6)	95.73 ± 2.54 (6)

Figures in parenthesis indicate number of animals. Each value represents the mean $\pm$ standard error.

TABLE II

EFFECT OF EUGENIA JAMBOLANA, ZIZYPHUS SATIVA AND TOLBUTAMIDE ON BLOOD GLUCOSE LEVELS OF ALLOXAN DIABETIC RABBIT

Blood Glucose in mg/100ml

Time Interval (hours)	Control	Tolbutamide (500 mg/kg)	Eugenia - 150 mg/kg	Jambolana 200 mg/kg	250 mg/kg	Zizyphus sativa 150 mg/kg	200 mg/kg	250 mg/kg
0	384.0 ± 4.8 (6)	374.48 ± 2.55 (6)	387.5 ± 5.3 (6)	378.7 ± 3.8 (6)	395.3 ± 4.1 (6)	372.2 ± 6.3 (6)	390.7 ± 3.2 (6)	408.2 ± 5.1 (6)
2	382.5 ± 3.6 (6)	373.11 ± 2.70 (2)	384.3 ± 3.7 (2)	376.4 ± 4.2 (6)	393.2 ± 5.1 (6)	370.1 ± 5.8 (6)	388.3 ± 3.8 (6)	406.1 ± 4.8 (6)
4	380.1 ± 5.2 (6)	372.46 ± 2.72 (6)	380.7 ± 4.5 (6)	373.8 ± 2.9 (6)	390.7 ± 4.8 (6)	371.2 ± 4.9 (6)	387.5 ± 4.2 (6)	405.7 ± 5.4 (6)
6	383.5 ± 4.0 (6)	372.23 ± 2.74 (6)	378.6 ± 3.4 (6)	368.1 ± 3.7 (6)	388.1 ± 4.3 (6)	369.8 ± 4.3 (6)	386.6 ± 4.3 (6)	400.1 ± 4.7 (6)
8	382.65 ± 3.5 (6)	372.34 ± 2.67 (6)	$375.83 + 3.41$ (6)	371.12 ± 3.62 (6)	390.35 ± 3.94 (6)	370.33 ± 3.69 (6)	378.18 ± 3.6 (6)	399.01 ± 4.2 (6)

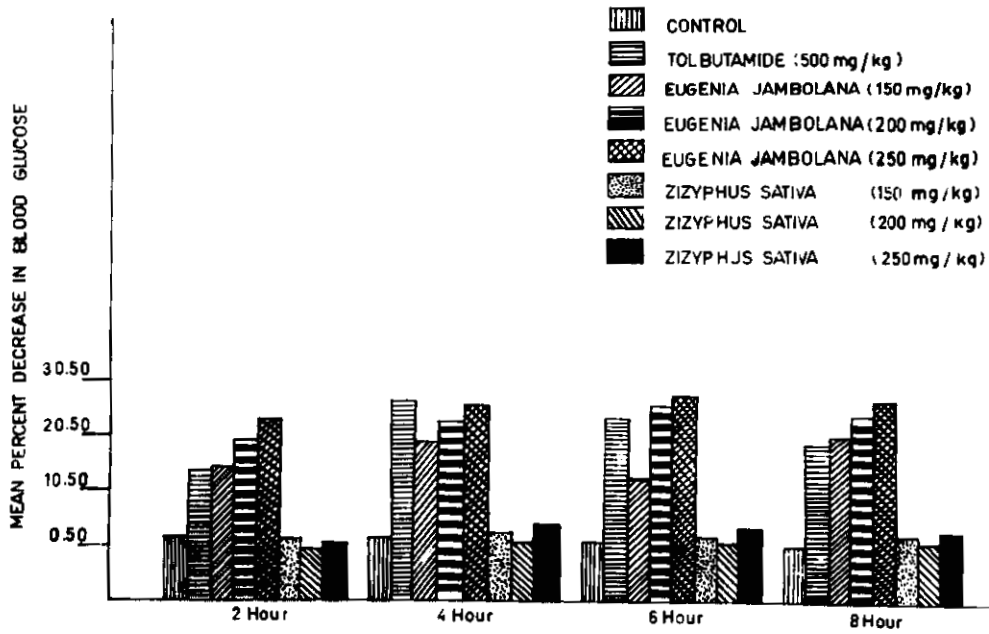Figures in parenthesis indicate number of animals. Each value represents the mean $\pm$ standard error.

Fig: I: Effect of Eugenia jambolana, Zizyphus Sativa and Tolbutamide on blood glucose levels of normal rabbits.
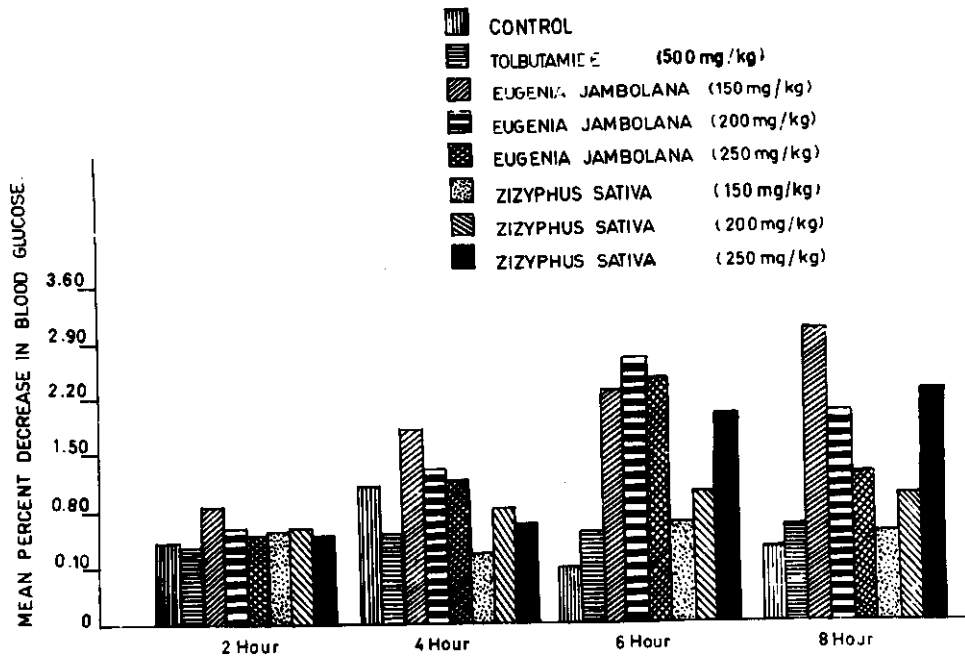
Fig: II: Effect of Eugenia jambolana, Zizyphus sativa and Tolbutamide on blood glucose levels of alloxan-diabetic rabbits.