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Research Article

Evaluation of anti-diabetic activity of Lycopene and its synergistic effect with Metformin hydrochloride and Glipizide in Alloxan induced diabetes in rats

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Abstract: The present study was conducted to investigate the anti-diabetic activity of Lycopene and its synergistic effect with Metformin hydrochloride and Glipizide in Alloxan induced diabetes in rats. Lycopene at a dose of 2 and 4 mg/kg were selected for the investigation of antidiabetic activity alone and combination with Glipizide and Metformin . Diabetes was induced by s.c. injection of alloxan monohydrate (110 mg/kg) in healthy male albino wistar rats. Serum glucose, cholesterol, HDL were evaluated for the assessment of antidiabetic activity of Lycopene. Lycopene alone and combination with Glipizide and Metformin decrease the serum glucose, cholesterol and increase the HDL levels. Lycopene alone showed very significant antidiabetic activity on 14th day of the treatment and blood glucose level came to normal on 21st day of treatment. Lycopene in combination with Glipizide and Metformin showed the very significant antidiabetic activity on 14th day of the treatment and blood glucose level came to normal on 21st day of treatment.
Keywords: Lycopene, anti-diabetic activity, Alloxan induced diabetes , Glipizide, serum glucose level

INTRODUCTION

Diabetes mellitus is a chronic major endocrine disorder and growing health problem in most countries and is characterized by hyperglycemia, hyperlipemia, negative nitrogen balance and sometimes ketonemia [1]. Diabetes is one of stress related disorder. Diabetic subjects are shown to have increased oxidative stress and decreased anti oxidant levels. Antioxidants are claimed to work as antistress agents by decreasing oxidative stress [2]. Lycopene, a carotinoid is mostly found in tomatoes and tomato products. It is a powerful anti oxidant with a singlet-oxygen quenching capacity. It is 100 times greater than that of β-carotene and vitamin-E respectively [3].

Glipizide belongs to novel class of oral hypoglycemic drug. Their principle action is a β-cell stimulating insulin secretion and then reducing plasma glucose. Metformin belongs to biguanide class of oral hypoglycemic drug. They lower blood glucose level by increasing glucose uptake and utilization in skeletal muscle. It causes gluconeogenesis and also reduces low density and very low denisity lipo proteins. The combination of Glipizide and Metformin is used to treat the high blood sugar levels [1].

Drugs are used to prevent, diagnose, treat, or to cure many diseases or disorders. However, they must be used safely with precaution to ensure that they are safe and effective. Many drugs owe to interacts with the body in different ways, like with our daily diet or lifestyle, which has significant impact on a drug's ability to show its effects which may be enhanced or decreased. An extensive literature survey from all scientific sources revealed that lycopene has antioxidant and anti-diabetic activity [3, 4]. But the influence of lycopene on diabetic patients who are under the treatment is not clear. Hence, the present study is planned to find out the influence of lycopene alone and also on anti diabetic effect of glipizide and Metformin combination.

The present study was taken to understand the anti-diabetic effect of lycopene alone and synergistic influence of Lycopene with Glipizide and Metformin combination.

MATERIAL AND METHODS

Collection of Lycopene

The drug Lycopene powder was collected as a gift sample from Parry Phytoremedies private limited. Pune.

Chemicals

All the chemicals and reagents used are of analytical grade and procured from approved vendors.

Preparation of alloxan solution

Alloxan monohydrate 110 mg/Kg was dissolved in sterile saline and injected by subcutaneous route immediately within five minutes to avoid degradation.

Lycopene solution

20 mg lycopene powder was weighed and dissolved in 10 ml of distilled water to give 2 mg/ml solution. This solution was administered at a dose of 2 mg/kg and 4 mg/kg body weight using clean and dry oral feeding needle for 21 days.

Glipizide solution

50 mg standard glipizide was weighed and dissolved in 10 ml of distilled water to give 5 mg/ml solution. This solution was administered at a dose of 5 mg/kg body weight using clean and dry oral feeding needle for 21 days.

Metformin Solution

1000 mg standard Metformin was weighed and dissolved in 5 ml of distilled water to give 200 mg/ml solution. This solution was administered at a dose of 300 mg/ kg body weight using clean and dry oral feeding needle for 21 days.

Experimental Animals

Male albino wistar rats weighing (150-250 g) were obtained from Indian rabitry enterprises, Bangalore, Karnataka and housed three animals per cage with paddy husk as bedding. Animals were housed at temperature of 25 ± 2 oC, relative humidity of 30-60% and 12:12 h light and dark cycle was followed. The animals had accessed to feed and purified water. The animals got CPCSEA clearance from IAEAC, Acharya & B.M. Reddy College *of* Pharmacy, Registration No. IAEC /P Cology /10/2009-10

Experimentally induced diabetes mellitus

Male albino wistar rats weighing (150-250 g) were fasted for overnight before challenging with single subcutaneous route (s.c) of alloxan monohydrate, freshly prepared and injected within 5 min of preparation to prevent degradation at a dose of 110 mg/kg. After administration of alloxan monohydrate 5% glucose solution was given for 72 h to prevent hypoglycemic shock. Animals had access to feed and water. The development of hyperglycemia in rats was confirmed by fasting serum glucose estimation 72 h post alloxan monohydrate injection where in the animals were fasted again for 14 h before blood collection from retro orbital plexus. The rats with fasting serum glucose level of above 200 mg/dl at 72 h were considered as diabetic and are included in the study. Body weight and blood glucose levels were estimated on initial, 1st, 3rd, 7th, 14th, and 21st day of the treatment. On the 21st day, blood samples were collected from overnight fastedrats by cardiac puncture under mild ether anesthesia for biochemical estimations [5,6].

Experimental study design

Male albino wistar rats were divided into twelve groups each consisting of six animals as follows.

Group 1: Normal control.
Group 2: Diabetic control
Group 3: Glipizide (5 mg/kg)[7]
Group 4: Metformin (300 mg/kg)[8]
Group 5: Lycopene (4 mg/kg)[3]
Group 6: Lycopene (4 mg/kg) + Glipizide (5 mg/kg)
Group 7: Lycopene (4 mg/kg) + Metformin (300 mg/kg)
Group 8: Lycopene (2 mg/kg)
Group 9: Lycopene (2mg/kg) + Glipizide (5 mg/kg)
Group10: Lycopene (2 mg/kg) + Metformin (300 mg/kg)
Group 11: Lycopene (2 mg/kg) + Glipizide (5 mg/kg) + Metformin (300 mg/kg)
Group 12: Lycopene (4mg/kg) + Glipizide (5mg/kg) + Metformin (300mg/kg)

Collection of serum samples

The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anaesthesia on different occasions i.e., day 0, day 1, day 3, day 7, day 14 and day 21. The blood samples were allowed to clot for 30 min at room temperature and then they were centrifuged at 5000 rpm for 20 min. The resulting upper serum layer was collected in properly labelled, clean and dry micro-centrifuge tubes. The blood samples were stored at 2-8 ºC and analyzed within one week. This serum specimen was used for the estimation of different biochemical parameters.

RESULT

Effect of Lycopene and its combination with Glipizide and Metformin on serum glucose levels in diabetic rats

The Effect of Lycopene and its combination with Glipizide and Metformin evaluated for its anti-diabetic activity in alloxan induced diabetic rats. The serum glucose level on treatment with Lycopene and its combination with Glipizide and Metformin on diabetic rats were given in Table-1and plotted in Fig-1.

Both 2, 4 mg/kg were not produced anti-diabetic effect till 7th day but showed very significant ($p<0.001$) anti-diabetic activity on the 14th day of treatment and blood glucose level came to normal on 21st day of treatment. No dose dependent effect was observed. Glipizide (5 mg/kg) showed very significant ($p<0.001$) anti-diabetic activity from 3rd day of the treatment and it brought the blood glucose level to normal level on 14th and 21st day of treatment. Metformin (300 mg/kg) showed the significant ($p<0.05$) anti-diabetic activity from the 3rd day of the treatment and it brought the blood glucose level to normal level on 7th, 14th and 21st day of treatment. Lycopene in combination with Glipizide (5 mg/kg) or Metformin (300 mg/kg) and combination with both Glipizide (5 mg/kg) and Metformin (300 mg/kg) have not showed the anti-diabetic effect till 7th day of the treatment. The combination showed very significant ($p<0.001$) anti-diabetic effect on 14th day and the blood glucose levels brought to normal level on 21st day of the treatment. The inhibition of antidiabetic effect of Metformin and Glipizide was observed when used in combination with Lycopene and the antidiabetic effect was observed only on 14th day of treatment as it observed with Lycopene.

Effect of Lycopene and its combination with Glipizide and Metformin on serum Lipid parameters

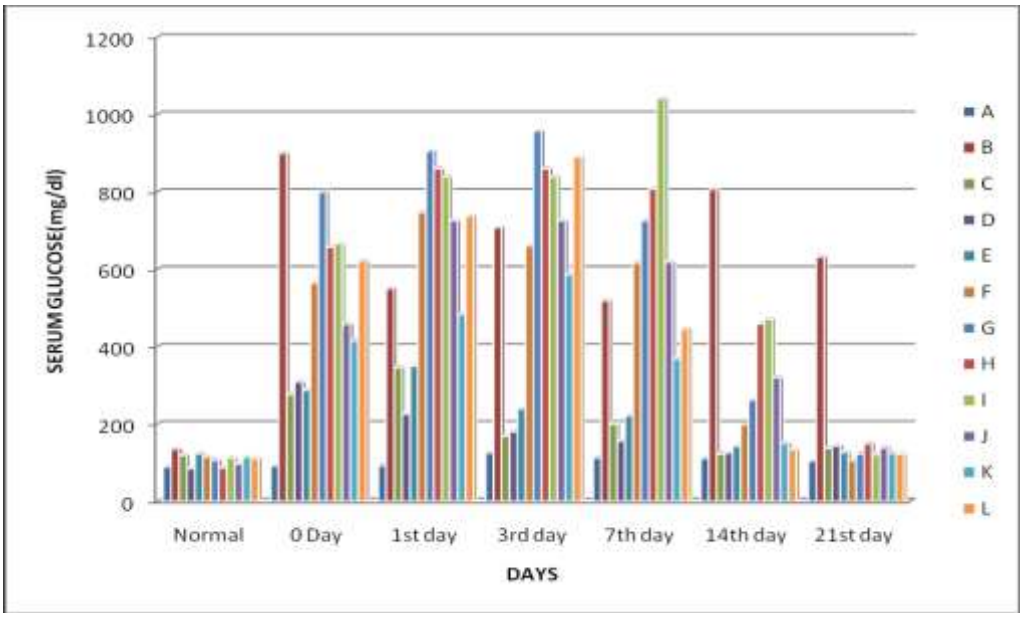
The effect of Lycopene and its combination with Glipizide and Metformin the serum lipid parameters are given in Table-2.

The serum cholesterol was reduced very significantly ($p < 0.01$) to 61.08 and 49.65% after daily treatment of diabetic rats with Lycopene -2, 4 mg/kg in GP-VIII and GP-V respectively. Serum cholesterol level was reduced to 48.36 and 30.01% after daily treatment with Glipizide 5 mg/kg and Metformin 300 mg/kg respectively. With combination treatment serum cholesterol level was reduced to 65.55, 65.28, 53.26, 68.87, 66.76 and 67.40% in GP-VI, GP-VII, GP-IX, GP-X, GP-XI and GP-XII respectively. The increased serum cholesterol level was observed in diabetic rats and which was decreased after treatment with lycopene (2 and 4 mg/kg). The lycopene effect is more than the individual glipizide and metformin treated groups. Even the effect of lycopene was almost similar when used in combination with Glipizide and Metformin. The percentage increase of serum HDL was 136.82 and 256.10% after treatment of alloxan induced diabetic rats by Lycopene -2, 4 mg/kg in GP-VIII and GP-V respectively. Serum HDL level was increased to 238.05 and 61.33% after treatment with Glipizide 5 mg/kg and Metformin 300 mg/kg respectively. With combination treatment serum HDL level was increased to 167.68, 279.10, 162.50, 154.54, 131.64 and 144.12% in GP-VI, GP-VII, GP-IX, GP-X, GP-XI and GP-XII respectively. The reduction in serum HDL cholesterol level was observed in diabetic rats and which was increased by treatment with Lycopene (2 mg/kg) and the effect observed is more than the Metformin treated groups. The Lycopene (4 mg/kg) effect was slightly more than Glipizide. The effect of their combination was almost same and no additional effect was seen.

Table-1: Effect of Lycopene and its combination with Glipizide and Metformin on serum glucose levels in diabetic rats

Group	Treatment	Fasting serum glucose level (mg/dl)						
		Normal	0 day	1st day	3rd day	7th day	14th day	21st day
I	Normal control	93.07 ± 2.34	90.69 ± 7.21	92.98 ± 2.38	125.37 ± 7.6	112.93 ± 5.38	110.9 ± 6.39	104.49 ± 4.52
II	Diabetic control	131.67 ± 4.49**a	885.52 ± 68.02**a	550.49 ± 85.37**a	707.93 ± 203.21**a	519.07 ± 75.89**a	806.59 ± 110.86**a	632.34 ± 103.81**a
III	Glipizide 5mg/kg	119.48 ± 3.53	276.14 ± 24.59**b	346.16 ± 26.36	168.97 ± 8.125**b	199.89 ± 12.57	122.76 ± 10.8**b	137.7 ± 11.88**b
IV	Metformin 300mg/kg	84.50 ± 12.37	308.50 ± 16.56	225.03 ± 26.60**b	180.34 ± 55.20*b	156.44 ± 25.71**b	125.33 ± 20.66**b	143.40 ± 18.04**b
V	Lycopene 4 mg/kg	123.45 ± 4.8	287 ± 45.54**b	350.2 ± 49.07	239.55 ± 10.1	222.17 ± 15.68	142.66 ± 7.97**b	126.44 ± 5.53**b
VI	Lyco 4 mg/kg + Glip5mg/kg	114.52 ± 7.61	565.02 ± 111.3	747.77 ± 197.29	660.78 ± 85.84	617.51 ± 109.62	198.41 ± 28.64**b	106.7 ± 11.27**b
VII	Lyco 4 mg/kg + Met 300mg/kg	106.52 ± 4.61	800.31 ± 147.13	905.55 ± 150.63	957.19 ± 162.96	726.05 ± 160.87	262.16 ± 44.12**b	123.3 ± 18.06**b
VIII	Lycopene 2 mg/kg	86.88 ± 17.67**b	657.80 ± 100.99	690 ± 87.24	860.05 ± 136.62	808.25 ± 111.48	458.89 ± 50.96**b	149.24 ± 16.5**b
IX	Lyco 2 mg/kg + Glip 5mg/kg	104.57 ± 12.31	666.19 ± 74.81	696.51 ± 79.8	839.39 ± 73.27	1041.9 ± 94.21**b	471.53 ± 51.36**b	120.37 ± 14.65**b
X	Lyco 2 mg/kg + Met 300mg/kg	93.53 ± 6.44*b	458.90 ± 73.48**b	584.31 ± 88.27	725.97 ± 68.09	618.85 ± 112.66	320.23 ± 49.92**b	138.82 ± 11.36**b
XI	Lyco 2 mg/kg + Glip 5mg/kg+ Met 300mg/kg	114.86 ± 9.70	421.05 ± 29.74**b	483.5 ± 48.71	586.87 ± 98.152	368.81 ± 41.47	150.64 ± 16.07**b	124.96 ± 6.46**b
XII	Lyco4mg/g +Glip 5mg/kg+ Met 300mg/kg	110.90 ± 12.13	623.02 ± 136.28	738.24 ± 171.29**b	890.36 ± 63.69	446.26 ± 102.49	133.97 ± 17.61**b	122.35 ± 10.29**b

Values are expressed as Mean ± S.E.M; n=6, *p < 0.05. **p < 0.01 and 'a' indicates when the values are compared with normal group, 'b' indicates when values are compared with diabetic control.



A – Normal control , B – Diabetic control, C – Glip 5 mg/kg, D – Met 300 mg/kg, E – Lyco 4 mg/kg, F – Lyco 4 mg/kg + Glip 5 mg/kg, G – Lyco 4 mg/kg + Met 300 mg/kg , H – Lyco 2 mg/kg , I – Lyco 2 mg/kg + Glip 5 mg/kg , J – Lyco 2 mg/kg + Met 300 mg/kg , K – Lyco 2 mg/kg + Glip 5 mg/kg + Met 300mg/kg, L – Lyco 4 mg/kg + Glip 5 mg/kg + Met 300mg/kg

Fig-1: Effect of Lycopene and its combination with Glipizide and Metformin on serum glucose levels in diabetic rats

Table-2: Effect of Lycopene and its combination with Glipizide and Metformin on serum Lipid parameters

Groups	Treatment	Lipid Profiles (mg/dl)			
		Serum Total Cholesterol	% Changed	HDL	% Changed
I	Normal control	82.73 ± 4.81	-	49.88 ± 2.94	-
II	Diabetic control	126.96 ± 7.39**a	↑53.46	17.95 ± 1.88**a	↓64.01
III	Glipizide 5mg/kg	65.56 ± 7.19**b	↓48.36	60.68 ± 9.64**b	↑238.05
IV	Metformin 300mg/kg	88.85 ± 3.31**b	↓30.01	28.96 ± 3.25	↑61.33
V	Lycopene 4 mg/kg	63.92 ± 12.04**b	↓49.65	63.92 ± 12.03**b	↑256.10
VI	Lyco 4 mg/kg + Glip5mg/kg	43.73 ± 8.33**b	↓65.55	48.05 ± 4.3*b	↑167.68
VII	Lyco 4 mg/kg + Met 300mg/kg	44.07 ± 6.3**b	↓65.28	68.05 ± 7.27**b	↑279.10
VIII	Lycopene 2 mg/kg	49.41 ± 2.44**b	↓61.08	42.51 ± 5.78	↑136.82
IX	Lyco 2 mg/kg + Glip 5mg/kg	59.34 ± 4.85**b	↓53.26	47.12 ± 5.96*b	↑162.50
X	Lyco 2 mg/kg + Met 300mg/kg	39.52 ± 8.84**b	↓68.87	45.69 ± 5.48*b	↑154.54
XI	Lyco 2 mg/kg + Glip 5mg/kg+ Met 300mg/kg	42.19 ± 2.89**b	↓66.76	41.58 ± 4.65	↑131.64
XII	Lyco 4 mg/kg + Glip 5mg/kg+ Met 300mg/kg	41.38 ± 4.67**b	↓67.40	43.82 ± 3.7*b	↑144.12

Values are expressed as Mean ± S.E.M; n=6, *p < 0.05. **p < 0.01 and 'a' indicates when the values are compared with normal group, 'b' indicates when values are compared with diabetic control.

DISCUSSION

Management of diabetes is still a challenge to the allopathic medicinal systems. Though, various types of oral anti-hyperglycaemic agents are available in addition to insulin for the treatment of diabetes mellitus but these synthetic agents are also having more side effects[9].

Lycopene has been under considerable investigation for its anti-oxidant benefits in treating various chronic human diseases like cancer, cardiovascular diseases, osteoporosis and diabetes. Hence the present study was under taken to investigate the effect of Lycopene alone and in combination with Glipizide and Metformin on diabetic rats. The literature survey revealed that chronic Lycopene treatment significantly improved the blood glucose levels and body weight of diabetic rats [3].

Alloxan is widely used to induce the experimental diabetes in animals. It acts on the β cells of the pancreas. The cytotoxic action of this is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid; establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells [10].

Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids [11]. Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, sinceinsulin inhibits the hormone sensitive lipase. On the other hand, glucagons, catecholamine and other hormones enhance lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. Our results also conformed the previous report that lycopene has a hypochloestrolemic activity [12, 13].

CONCLUSION

The lycopene has anti-diabetic action in alloxan induced diabetic rats. No dose dependent effect was observed.

Lycopene 2, 4 mg/kg as not produced anti-diabetic effect till 7th day but showed very significant ($p<0.001$) anti-diabetic activity on the 14th day of treatment and blood glucose level came to normal on 21st day of treatment.

Lycopene in combination with Glipizide (5 mg/kg) or Metformin (300 mg/kg) and combination with both Glipizide (5 mg/kg) and Metformin (300 mg/kg) have not showed the anti-diabetic effect till 7th day of the treatment. The combination showed very significant ($p<0.001$) anti-diabetic effect on 14th day and the blood glucose levels brought to normal level on 21st day of the treatment.

The inhibition of antidiabetic effect of Metformin and Glipizide was observed when used in combination with Lycopene and the antidiabetic effect was observed only on 14th day of treatment as it observed with Lycopene. This inhibitory effect is due to pharmacodynamic or pharmacokinetic interaction yet to be revealed.

Lycopene both 2, 4 mg/kg alone and combination with glipizide and metformin decrease the serum cholesterol levels. It indicates that the Lycopene 2 & 4 mg/kg useful in the treatment of diabetes as it has hypolipidemic effect since the diabetes always associated with the hyperlipidemia.

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