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COMPARATIVE ANTIHYPERGLYCEMIC ACTIVITY OF AQUEOUS EXTRACTS OF GARLIC (*ALLIUM SATIVUM)* AND GINGER *(ZINGIBER OFFICINALE)* IN ALLOXAN-INDUCED MALE RABBITS

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ABSTRACT

This study was undertaken to investigate the effect of Garlic (*Allium sativum)* and Ginger *(Zingiber officinale)* aqueous extracts on serum glucose, cholesterol, urea levels and body weight in normal and alloxan-induced diabetic male rabbits. Before alloxan monohydrate (120mg/kg) introduction rabbits were left in fasting condition till twelve hours. After seven days of alloxan monohydrate induction diabetes mellitus was confirmed animals with fasting serum glucose level ≥ 250mg/dl and were considered as diabetes. In whole experimental work twelve male rabbits were selected divided in to four groups and each group contains three male rabbits 1st and 2nd group normal and diabetic control 3rd to 4th group containing male rabbits were treated with 1% garlic and ginger aqueous extract to check the comparison between them against serum glucose and their variable effects on serum cholesterol, serum urea and body weight. Blood serum was used to determine all selected biochemical parameters, on every third day. It was observed that 1% Garlic and ginger aqueous extract controlled 51% and 45.26% glucose, 35.29% and 21.95% cholesterol, 46.22% and 21.69% urea level and body weight gradually increased in alloxan induced diabetic male rabbits. It is observed in the present investigation that oral administration of garlic and ginger aqueous extracts are hypoglycemic agents but according to the results garlic is the best hypoglycemic agent, which also controlled cholesterol and urea level more than ginger in alloxan-induced diabetic male rabbits

Key words: Alloxan, Rabbits, garlic. Ginger, glucose, cholesterol body weight

INTRODUCTION

Diabetes Mellitus is the heterogeneous metabolic disorder generated due to fault in carbohydrate, lipid and protein metabolism. The syndrome of diabetes mellitus (DM) has been increasing rapidly in worldwide. It has been estimated that the number of adults affected by diabetes in the world will grow from 135 million in 1995 to 300 million in the year 2025 (Mutalik *et al*., 2003, King *et al*., 1998). The therapy of diabetes has changed markedly in the past 20 - 25 years. By using synthetic hypoglycemic agents usually produces many side effects and also they are too much expensive. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. These facts show that proposing an immediate strategy for diabetes prevention and treatment is a global subject. For a long time, diabetics have been treated with several medicinal plants or their

extracts based on the folklore medicine (Akhtar and Ali., 1984).

A number of investigations of oral antihyperglycemic agents from plants used in traditional medicine have been conducted and many of the plants have been found with good activity (Rind *et al*., 2010, Khushk et al., 2010, Kesari *et al*., 2007). Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypglycemic agents (therapeutic agent) for the treatment of diabetes mellitus (Kim *et al*., 2006).

Garlic (*Allium sativum*) is a member of the Liliaceae family, which is one of the most popular herbs used worldwide to reduce various risk factors associated with several diseases (Thomson *et al*., 2007, Jamison, 2003). Garlic has been found to be effective in lowering serum glucose levels in STZ-induced as well as alloxan-induced diabetic rats and mice. Most of the studies showed that garlic can reduce blood glucose levels in diabetic mice, rats and rabbits. In addition, Liu and co-workers (2005) reported that both garlic oil and diallyltrisulphide improved glycaemic control in STZ-induced diabetic rats. Ingestion of garlic juice resulted in better utilization of glucose in glucose tolerance tests performed in rabbits, while allicin at a dose of 250 mg/kg was 60% as effective as tolbutamide in alloxan induced diabetic rabbits (Mathew and Augusti, 1973).

A few isolated studies about the hypoglycaemic properties of ginger in animals have been reported (Sharma and Shukla, 1977). It is reported that a small but significant blood glucose lowering effect of ginger juice in diabetic and non-diabetic animals was observed (Akhani *et al*., 2004). Some hypoglycemic activity was found in both garlic and ginger as compared to diabetic controls groups.

MATERIALS AND METHODS

Plant Material Collection: Garlic (*Allium sativum)* and Ginger *(Zingiber officinale)* were purchased from the market of Jamshoro, identified and authenticated in the Institute of Biotechnology and Genetic Engineering (IBGE). The samples were stored for future references.

Animals used: Healthy and young male rabbits were purchased from Hyderabad city weighing 1-2 kg body weight. Before experimental work rabbits were kept under observation for 15 days in animal house. Animals were offered a balanced diet consisting of green leaves, fodder, pulses and water. All rabbits were kept in wooden cadges during whole experiment.

Preparation of plant extract: Garlic and ginger cut in to small pieces dried in open environment than grinded and converted in to fine powder by commercial blender. Nine gram garlic or ginger were dissolved in 800ml Distilled water and filtered two times by using filter paper through suction pump than made

volume up to 900ml as reported by Akhtar and Ali (1984).

Chemicals: Alloxan mono-hydrate ($C_4H_2N_2O_4.H_2O$) was purchased from Sigma Chemical Company, USA. Glucose oxidase (Human Centronic GmbH-65205, Germany), cholesterol (Human Centronic GmbH-65205 Germany) and urea, (Biomerieux, France) kits were used for performing the respective tests.

Preparation and induction of diabetes mellitus in male rabbits: Alloxan Monohydrate was used to induce diabetes mellitus in normo-glycemic young male rabbits. Animals were allowed in fasting condition for 12 hours and then were injected 120 mg/kg alloxan mono-hydrate through marginal ear vein with freshly prepared in 0.9% normal saline, this dose destroys the β cells of pancreas. 50% mortality rate in alloxan induced male rabbits was observed. Rabbits having blood serum glucose level ≥250mg/dl were considered as diabetes rabbits. After 72 hours of induction of alloxan in healthy rabbits Blood glucose level was determined by the spectrophoto-meter method (Demerdesh *et al.*, 2005). Rabbits were left at fasting condition for 24 hours before the induction of diabetes. Normal rabbits were injected 0.9% normal saline (NaCl).

Administration of extract: Three times in a day 300ml plant extract was administrated to rabbits. Both groups of diabetic control and normal control rabbits were left on normal tap water, administrated same dose with respective group.

Experimental design: All rabbits were divided into three different groups; normal control, diabetic control, treated with aqueous extracts of garlic and ginger aqueous extract. Normal control was injected with 0.9% normal saline; remaining rabbits were injected with alloxan monohydrate, dose 120mg/kg body weight. After one week the rabbits were screened for diabetes and fasting blood glucose ≥ 250mg/dl were selected for the treatment. Rabbits are divided in to different groups.

GROUP – A: 0.9% (NaCl) normal saline, normal diet (Normal Control)

GROUP – B: 120mg/ kg (Alloxan), normal diet (Diabetic control)

GROUP – C: 120mg/ kg (Alloxan), normal diet plus 1% garlic treated (aqueous extracts oral administration)

GROUP – D: 120mg/ kg (Alloxan), normal diet plus 1% ginger treated (aqueous extracts oral administration)

Treatment of rabbits: Diabetic rabbits were treated with aqueous extract (tap water) of garlic and ginger with oral administration of total volume up to 900ml/three rabbits in each group for 15 days.

Collection of blood: For the deter-mination of blood glucose, cholesterol and urea, the blood (1.0 ml) was collected from marginal ear vein of rabbit. After collection of blood, pricked site of the ear was rubbed with cotton wool soaked with 70% ethanol to protect the rabbit against

infection. The serum was removed from the blood; samples were labeled and stored for future analysis at 4 °C.

Biochemical and Biological Parameters: Blood serum glucose, Cholesterol and urea level were estimated from the serum by using standards kits, for the determination of glucose, samples were prepared by taking 20µl of serum in small glass bottle added 1200 µl of reagent present in kit mixed samples and incubated it for 10 minutes at 25°C and absorbance was read at 500 nm of the standard and samples against reagent blanks within 60 minutes, for examination of serum cholesterol level, Samples were prepared by taking 10µl of serum in small glass bottle homogenized 1000 µl of reagent present in kit mixed samples and incubated for 10minutes at 25°C and absorbance was read at 500 nm of the standard and samples against reagent blanks within 60 minutes (Lopes *et al.,* 1977, McGowan *et al.,* 1983) and serum urea level was determined by taking one ml of working solution and 10µl of sample was taken in test tube against standard and dH_2O (blank), left for 5 minutes at 25°C, finally absorbance was taken using spectrophotometer at 580nm. Body weight was determined by weight machine on every third day.

Statistical Analysis: Data were calculated as

$$\text{Mean value} = \frac{\text{Final Value} - \text{Initial value}}{\text{Initial value}} \times 100$$

The data were expressed as mean ± standard devaition (Kondeti *et al.,* 2011)

RESULTS AND DISCUSSION

In this study, the effect of 1% of garlic and ginger aqueous extracts on the serum glucose levels of diabetic male rabbits is shown in Table-1, which indicated that initial mean ± SD values of normal and diabetic control male rabbits is (123.50± 0.46mg/dl) and (314.93±1.50mg/dl), no any effective change was observed till the final reading. The starting mean ± standard division values of serum glucose of garlic treated male rabbits were (316.35 ± 8.02mg/dl) which gradually decreased till the 15th day (152.29± 4.19mg/dl) decreased blood sugar level 51% compared to their respective controls. The initial value of ginger treated male rabbits was (330.25 ±4.63mg/dl) decreased up to (180.75±2.62mg/dl) after 15 days of treatment. The results of present study have showed great antidiabetic affects when compared to Luthra and Tayal (1962) results, which indicates that garlic and petroleum ether extracts contain such substances that can bring down blood sugar level to some extent. Dietary therapy is generally considered to be the first step in the treatment of diabetic patients. Garlic (*Allium sativum*) is used in the traditional medicine of many countries for the treatment of different diseases, including diabetes (Swanston *et al.,* 1991). Some of the sulfur containing compounds such as allicin, ajoene, S-allylcysteine, S-methyl

cysteine, diallyl disulfide and sulfoxides may be responsible for antiathero-sclerotic activity of garlic (Campbell *et al*., 2001, Orekhov *et al*., 1997). Few studies about the hypo-glycemic properties of ginger in animals have been reported with variable results (Sharma and Shukla., 1977). Ginger juice exhibits hypogly-caemic activity in both normal and streptozotocin (STZ)-induced diabetic rats (Mascolo *et al.,* 1989). The results of present study clearly indicate that an aqueous extract of raw ginger effectively lowers serum glucose, cholesterol and triacylglycerol levels in diabetic rabbits. However, it should be noted that serum glucose levels in ginger treated diabetic rabbits did not reach normal levels at the dosage used in the present study and same results were reported by Akhani et al., (2004).

Table-1: Effect of 1 % aqueous extracts of garlic and ginger on serum glucose level on alloxan-induced diabetic male rabbits compared with normal and diabetic controls.

Test Sample		Mean blood glucose concentration ± SD (mg/dl) (n=3)					
		1st day	3rd day	6th day	9th day	12th day	15th day
Normal control	Group - A	123.50 ± 0.80	119.6 ± 0.42	122.37 ±1.40	115.70 ±1.04	125.27 ±0.75	130.06 ±1.62
Diabetic control	Group - B	314.93 ± 2.60	332.3± 0.92	331.25 ± 2.38	295.85 ± 1.24	299.90 ±2.47	300.33 ±2.42
Garlic treated	Group - C	316.35 ±8.02	249.55 ±1.48 21.11%	220.26 ±1.23 30.37%	203.12 ±4.73 35.79%	170.75 ±4.58 46.02%	152.29 ±4.19 51%
Ginger treated	Group - D	330.25 ±4.63	289.71 ±8.94 12.27%	260.70 ±8.06 21.05%	220.21 ±6.36 33.32%	192.23 ±4.43 41.79%	180.75 ±2.62 45.26%

According to the data shown in Table-2**,** the 1% garlic aqueous extract possessed significant hypolipidemic affect during fifteen days of treatment however ginger also controlled 21% serum cholesterol level gradually as mentioned in results. It is shown that the mean Cholesterol level± SE in garlic treated male rabbits was noted (131.3±8.30), and which decreased up to 35% as compared to their controls (84.96±2.09). Diabetic controls (140.41 ±4.72) and in normal control group serum cholesterol level remains same but significantly increased in diabetic control was observed after fifteen days of interval. Ginger has efficacy as antihyperlipidaemic agent was evaluated with the help of biochemical parameters and results were compared with gemfibrozil which is used as a standard reference drug for comparing the data, as its hypolipidaemic activity is well established (Todd and Ward., 1980). In the present study, we have investigated the antihyper-lipidaemic effect of ginger aqueous extract in ginger treated rabbits. It is evident from the results that feeding ginger extract till 15 days resulted in

less marked hyperlipidaemia. It may be suggested that ginger stimulated the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body (Srinivasan and Sambaiah., 1991). Garlic (*Allium sativum*) is clearly one of the most popular herbal remedies worldwide today. Animal studies suggest that garlic has potential antilipidemic, antihypertensive, antiglycemic, antithrombotic, and antiatherogenic properties (Bordia *et al.,* 1975). The administration of garlic extract significantly decreased serum triglycerides and cholesterol in diabetic rats. In continence with the present data, other workers have reported that administration of fresh garlic or etheric garlic extracts improved lipid profile including reduction of serum cholesterol levels (Knipschild and Terpiet 1989).

Table-2: Effect of 1 % aqueous extracts of garlic and ginger on serum cholesterol level on alloxan induced diabetic male rabbits compared with normal and diabetic controls.

Test Sample	Mean blood cholesterol concentration ± SD (mg/dl) (n=3)					
	1st day	3rd day	6th day	9th day	12th day	15th day.
Normal control	72.18 ±4.00	71.96 ±1.09	76.1 ±1.57	90.15 ±2.92	87.2 ±3.69	79.12 ±1.60
Diabetic control	140.4 ±8.18	147.2 ±0.25	152.2 ±0.99	145.5 ±2.38	160.3 ±5.61	165.2 ±3.77
Garlic treated	131.3 ±8.30	128.9 ±4.67 3.1%	117.8 ±3.37 10.28%	107.3 ±4.48 18.27%	93.3 ±1.56 28.94%	84.96 ±2.09 35.29%
Ginger treated	135.5 ±5.57	136.1 ±1.32 0.44%	127.7 ±3.84 5.75%	119.2 ±6.17 12.02%	112.14 ±5.78 17.30%	105.75 ±5.12 21.95%

Table-3 shows that introduction of 120mg/kg of alloxan introduction increased the urea level in diabetic male rabbits. Mean normal value of serum urea in rabbits is 42-80mg/dl as reported by Jones (1975). Normal control male rabbits contained serum urea level (61.04±1.15mg/dl) and diabetic control contained serum urea level is 122.28±2.63mg/dl and diabetic treated rabbits contained 120.18 ± 5.64mg/dl respectively. Diabetic rabbits having initial high serum urea level as compared to normal values because of alloxan induction urea level also increased. Garlic aqueous extract 1% controlled serum urea level 46.22% (68.00± 1.90) in diabetic rabbits after fifteen days. Initial serum urea level of ginger treated male rabbits was noted (115.25±6.93) which decreased up to (90.25±2.23), 21% serum urea level was controlled after fifteen days of treatment. Alloxan treatment increase the serum enzymes levels such as cholesterol, LDL, creatinine, urea and alkaline phosphatase and decrease the HDL level, but glibenclamide (Muhammad *et al*., 2010). Diabetes causes disturbance in renal function so that the blood urea level is

elevated. Extract treated group exhibited reduction in serum urea level (As indicated in results by using 1% garlic aqueous extract significantly decreased serum urea level as compared to ginger.

Table-3: Effect of 1 % aqueous extracts of garlic and ginger on serum urea level on alloxan-induced diabetic male rabbits compared with normal and diabetic controls.

Test Sample	Mean blood urea concentration ± SD (mg/dl) (n=3)					
	1st day	3rd day	6th day	9th day	12th day	15th day.
Normal control	61.4 ±1.98	60.6 ±1.76	63.2 ±2.22	60.12 ±3.64	68.16 ±2.15	65.12 ±2.28
Diabetic control	122.3 ±4.55	119.5 ±3.20	113.9 ±1.58	111.5 ±3.66	129.9 ±12.73	120 ±6.26
Garlic treated	120.18 ±5.64	113.21± 4.89 5.75%	94.98 ±6.35 20.96%	80.76 ±5.11 32.80%	70.72 ±2.59 41.15%	64.63 ±2.00 46.22%
Ginger treated	115.25 ±6.93	106.33± 4.16 7.73%	105.89 ±1.93 8.18%	97.28 ±4.46 15.59%	93.0 ±6.75 19.30%	90.25 ±2.23 21.69%

Table-4 shows the effect of Garlic and ginger aqueous extract oral administration on the body weight of alloxan induced diabetic male rabbits increased compared with their respective controls. Initial body weight of normal and diabetic control male rabbits was 1545.89± 14.05gm and 1258.3±8.46gm, gradually changed on 15th day body weight of normal control was 1555± 10.58gm and diabetic control 1150± 5.50gm. The initial values of body weight of garlic and ginger treated rabbits were 1360±12.91gm and 1100 ±11.80gm which gradually increased up to 1403.2±11.18gm and 1159 ± 11.15gm respectively.

Table-4: Effect of 1 % aqueous extracts of garlic and ginger on body weight of Alloxan induced diabetic male rabbits compared with normal and diabetic controls

Test Sample	Mean body weight ± SD (grams) (n=3)					
	1st day	3rd day	6th day	9th day	12th day	15th day.
Normal control	1545.89 ± 14.05	1737.5± 3.54	1706.6± 26.65	1549.8 ± 11.38	1569.9 ±14.97	1555 ± 10.58
Diabetic control	1258.3 ± 8.46	1362.2± 2.50	1299.97 ±10.1	1200 ± 5.14	1180 ± 7.33	1150 ±5.50
Garlic treated	1360 ± 12.91	1388.6± 11.87	1408.46 ±7.05	1442.3 ± 10.55	1365 ± 10.74	1403.2 ±11.18
Ginger treated	1100 ± 11.80	1190 ± 9.90	1174 ± 5.32	1123 ± 8.10	1168.7 ±13.5	1159 ± 11.15

Data from the present study showed that aqueous extracts of garlic and ginger have hypoglycemic and hypolipidemic effects, but while hypoglycaemic effect was dose dependent, the hypolipidaemic effect tended to be

abolished at higher concentration. Garlic has many medicinal uses and is fame to prevent of heart disease and cancer. Garlic and Ginger controls high cholesterol, atherosclerosis, and high blood pressure (Sugawara and Suzuki 1997). People who suffer with diabetes can greatly benefit from the garlic plant regulate the level of blood sugar in the human body. Present study suggests that Garlic and Ginger may have beneficial effects in diabetes that hold the hope of a new generation of antidiabetogenic drugs. However, comprepensive chemical and pharmacological research is required to find out the exact mechanism of garlic and ginger for its antidiabetogenic effect and to identify the active constituent responsible for to control diabetes.

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