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Original Article

COMPARATIVE HYPOGLYCEMIC AND BIOCHEMICAL EFFECTS OF ETIOLATED WHEAT GRASS, TRITICUM AESTIVUM (LINN.) AND LAGERSTROEMIA SPECIOSA (LINN.) PERS. FRUIT IN ALLOXAN INDUCED DIABETIC ALBINO RAT

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ABSTRACT

Objective: Present work aims to explore and compare the hypoglycemic and biochemical activities of *Lagerstroemia speciosa* (Linn.) Pers. fruits squash (FSLS), with the insulin (INS) and fresh etiolated wheat grass juice (FEGJ).

Methods: For this study, rats were divided into five groups (n=6), Group I (NC): Rats kept as normal control. Group II (DC): Injected with alloxan monohydrate (180 mg/kg b.w.) and kept as diabetic control. Group III (DC +INS): These diabetic rats were subcutaneously injected with 1 unit of bovin protamine zinc insulin (INS) half an hour prior to feeding twice a day. Group IV (DC+FEGJ): Instead of INS, this group of rat were orally administrated with fresh etiolated wheat grass juice (FEGJ) at the dose of 30 ml/kg b.w. half an hour prior to feeding twice a day. Group V (DC+FSLS): This group of diabetic rat orally administrated with fruit squash of *L. speciosa* (FSLS) at the dose of 30 ml/kg b.w. half an hour prior to feeding twice a day.

Results: Rats of INS, FEGJ (30ml/kg) and FSLS (30mg/kg) treated groups show significant ($P<0.001$) decrease in blood glucose as well as liver glycogen compared to DC. AST and ALT enzyme significantly ($P<0.001$) lowered in DC+FEGJ while it is not significantly change in DC+ INS and DC+FSLS compared to DC.

Conclusion: Hypoglycemic activity of FSLS is better than FEGJ and it is quite equivalent to the insulin. Biochemical analysis of blood shows significant hepatoprotective activity of FEGJ.

Keywords: FEGJ, FSLS, Bovin protamine zinc insulin, Hypoglycemic, Hepatoprotective.

INTRODUCTION

According to WHO, diabetes is a chronic condition that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin[1]. Hyperglycemia and other related disturbances in the body's metabolism can lead to serious damage to many of the body systems, especially the nerves and blood vessels leading to blindness, amputation and kidney failure[1]. In order to study complications related with diabetes, number of model animals have been suggested in past decades[2]. Use of alloxan, a urea derivative is one of the potent methods to induce diabetes in experimental animals like mice, rat, rabbit and dog [2-3].

Oral hypoglycemic compounds from various medicinal plants as well as dietary supplements provide a useful source for the development of new pharmaceutical leads to existing therapies for diabetes[4]. *Triticum aestivum* (Linn.) is commonly known as bread wheat, belongs to family Poaceae, native to Southwest Asia and the Mediterranean region. Various authors reported anti-cancerous activity[5], anti-thalassemic activity[6], hypoglycemic activity[7], anti oxidant activity[8] and hepatoprotective activity[9] of *T. aestivum. Lagerstroemia speciosa* (Linn.) Pers. belongs to the family Lythraceae. In the past studies, various pharmacological activities of *L. speciosa* was noted by various authors, such as antioxidant[10], anti- inflammatory activity[11] and antimicrobial activity[12].

People with type 1 diabetes requires insulin while type 2 diabetes often require oral drugs, and sometimes insulin to control their blood glucose levels. Although insulin has been designated an essential drug by WHO, it is not yet universally accessible to all those who need it in the majority of developing countries. In some of these countries, people with diabetes die due to lack of timely supply of insulin[1]. Hence beside exploring hypoglycemic activity of *L. speciosa* fruit, present work compares its hypoglycemic efficacy with insulin and fresh etiolated wheat grass juice.

MATERIALS AND METHODS

Plant materials

Lagerstroemia speciosa ripen fruits was collected from the Nagpur university campus.

Etiolated yellow grass, *T. aestivum* was grown in 24 hrs dark on bamboo stick pad (60cm diameter). Pad was layered with three inch thick 3:1 soil compost mixture. Overnight soak grains were sown evenly and sprinkled with sufficient water every day for proper growth. On 14th day yellow grass was harvested just two inch above the surface.

Fresh and concentrated extract of fruit and etiolated wheat grass juice was prepared in laboratory with mortar and pastel at each time of dosing. To fulfill the requirement of fresh etiolated wheat grass other ten pads also processed at one day interval with same procedure.

Experimental animal

Healthy albino rats (9 months old) of both sexes, weighing 150-190 gm were used for the experiment. Animals were free to access drinking water but food was given twice a day at 9:00 am and 9:00 pm only.

Animals were cared for and used in accordance with the Institutional Animal Ethics Committee (IAEC), P.G.T. Department of Zoology, RTM Nagpur University, Nagpur (Registration no.- 478/01/a/CPCSEA).

Experimental induction of diabetes

Diabetes was induced in 16 hrs fasted albino rats with single 180mg/kg dose of alloxan monohydrate. Alloxan injection was prepared in 0.9 % normal saline. Rats with fasting blood glucose (FBG) more than 220 mg/dl was considered for study.

Hypoglycemic study

For this study rats were divided into five groups (n=6),

GroupI (NC): Kept as normal control.

Group II (DC): Injected with alloxan monohydrate (180 mg/kg b.w.) and kept as diabetic control.

Group III (DC +INS) : These rats were injected with alloxan monohydrate (180 mg/kg b.w.) and from day 2 to 15, half an hour prior to feeding rats were subcutaneously injected with 1 unit of Hypurin® Bovin protamine zinc insulin (manufactured by Wockhardt UK Ltd.) twice a day.

Group IV (DC+FEGJ): This group was injected with alloxan monohydrate (180 mg/kg b.w.) and from day 2 to 15 half an hour prior to feeding, orally administrated with FEGJ (30 ml/kg b.w.) twice a day.

Group V (DC+FSLS): These rats were injected with alloxan monohydrate (180 mg/kg b.w.) and from day 2 to 15 half an hour prior to feeding, orally administrated with FSLS (30 ml/kg b.w.) twice a day.

Estimation of Blood glucose

Insulin treatment was discontinued after last dose and rats were kept fasted for 16 hrs before study. Blood was collected from retro-orbital plexus of each rat to measure blood glucose with Glucose (HK) assay kit (Sigma-Aldrich).

Estimation of liver glycogen

Liver sample was dissected out from 16 hrs fasted rat and digested in hot 30% KOH. Liver glycogen was precipitated with alcohol and the precipitate was dissolved in 10% TCA. The sample was processed for centrifugation to sediment the proteins. After centrifugation, supernatant was precipitated once again with alcohol. After suitable dilution of the sediment with water, estimation of liver glycogen was carried out with anthrone reagent[13].

Estimation of ALT and AST

On 16th day, blood was collected from the retro-orbital puncture from every animal of each group for estimation of Aspartate aminotransferase (AST) and Alanine aminotranferase (ALT) which was carried out by 2, 4-DNPH Reitmann and Frankel Method[14].

Histological study

After 15 days of dosing, animals from each group were sacrificed, pancreas was dissected out and fixed in Baker's formalin-calcium for 4 hrs at 4^{0}C. Paraffin embedded pancreatic tissue was sectioned at 6µ. Modified Gomori's aldehyde fuchsin (AF) staining procedure was used to stain the sections[15].

Statistical analysis

The results were expressed as Mean ± SEM, comparison between groups was done with t-test using online GraphPad Prism t-test calculator.

RESULTS AND DISCUSSION

Effect of insulin, etiolated wheat grass juice and *L. speciosa* fruit squash on blood glucose

Oral administration of 30 ml/kg b. w. dose of both FEGJ and FSLS in diabetic rat significantly decreases (P<0.001) the blood glucose when compared with diabetic control, this proves their hypoglycemic activity. When hypoglycemic activity of DC+FEGJ group compared with DC+INS and DC+FSLS individually, it was found that DC+FEGJ group shows significantly less hypoglycemic activity than DC+INS (P<0.01) and DC+FSLS (P<0.05) groups.

This suggests that hypoglycemic activity of fresh etiolated wheat grass juice is not efficient like insulin and FSLS. When DC+FSLS group was compared with the DC+INS, no significant difference in their hypoglycemic activity was found. The hypoglycemic activity of FSLS may be due its bioactive insulin mimicking components. Corosolic acid and ellagitannins were identified in the water extract of *L. speciosa* leaves after HPLC purification[16]. Ellagitanins such as Lagerstroemin is the activator of glucose transporter in fat cells[16], same group of authors also reported the activation of insulin receptor by Lagerstroemin[17]. This indicates that at dose of 30 ml/kg b.w. hypoglycemic activity of FSLS is nearly equal to the 1U of insulin dose.

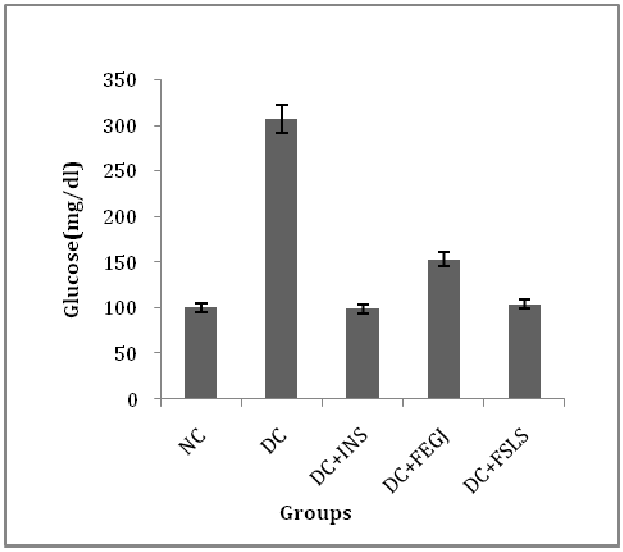

Fig. 1: Effects of INS, FEGJ and FSLS on blood glucose level of rats. Values are expressed as Mean ± SEM. P<0.001

Effect of insulin, etiolated wheat grass juice and L. speciosa fruit squash on biochemical parameters

When NC group was compared with DC, it revealed significant (P<0.05) decrease in insulin level in DC. Insulin level in DC+INS and DC+FEGJ increase significantly (P<0.05) compared to DC while there is no significant difference between insulin of DC and DC+FSLS. Insulin treatment to diabetic rats may prevent the background consequences such as ketoacidosis and provide healthier environment for augmentation of insulin producing β cell mass[18]. Though action of FEGJ on increased insulin level is not certainly known, this may results due to stimulatory effect of FEGJ on regeneration of insulin producing β cells. There is no improvement in the insulin level in DC+FSLS group suggesting that FSLS may not affect the regeneration of alloxan damaged β cell mass.

AST is the marker of the heart, liver, skeletal muscle, red cells, and kidney damage whereas ALT is elevated than normal mainly in the liver and kidney damage[19-20]. In the present study, elevated level of AST and ALT enzymes set as hepatic damage indicators. Significantly elevated levels of AST (P<0.001) and ALT (P<0.001) enzymes in DC group compared to NC proves hepatotoxic effect of alloxan. In DC+FEGJ group, significant reversal of AST (P<0.001) and ALT (P<0.05) values towards normal indicates hepatoprotective activity of FEGJ. In DC+INS and DC+FSLS no significant difference observed in the levels of AST and ALT enzyme in comparison with DC predicting no role of INS and FSLS in hepatoprotection.

The liver glycogen concentration was found very significantly higher (P<0.001) in DC than NC like other authors[21-23]. This may be due to the increase rate of glycogen synthase enzyme in alloxan treated groups, which in turn account for accumulation of glycogen in diabetic rat liver[24]. INS, FEGJ and FSLS treated groups show significantly lowered (P<0.001) glycogen concentration toward normal. This reversal of glycogen concentration to normal indicates preventive effect of INS, FEGJ and FSLS on alloxan induced hike in the level of glycogen synthase. FEGJ possesses significant (*P<0.05) effect on lowering of glycogen concentration than FSLS (fig-2).

Table 1: Effect of INS, FEGJ and FSLS on biochemical parameters

Groups	Parameters		
	Insulin (µU/ml)	AST(IU/L)	ALT(IU/L)
NC	5.63±0.38	94.43±0.66	57.88±1.14
DC	3.83±0.19[c]	124.46±4.5[d]	91.04±2.71[d]
DC+INS	5.30±0.29[a]	127.68±4.41[ns]	92.31±2.32[ns]
DC+FEGJ	5.05±8.32[a]	82.36±1.30[b]	41.26±0.96[b]
DC+FSLS	3.70±0.17[ns]	123.30±2.13[ns]	89.19±0.70[ns]

All values are expressed as Mean ± SEM (n=6), paired t-test was performed to compared between group. [c]P<0.05, when DC compared with NC. [d]P<0.001, when DC compared with NC. [a]P< 0.05 when treated groups compared with the diabetic control group. [b]P <0.001 when treated groups compare with the diabetic control. [ns]P statistically not significant when compared with the diabetic control.

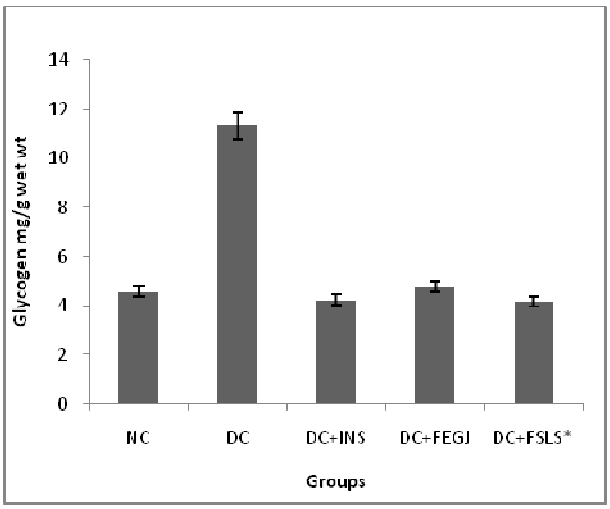

Fig. 2: Effects of INS, FEGJ and FSLS on fasted liver glycogen level of rats.

Values are graph as Mean ± SEM (n=6), P<0.001 when DC+INS, DC+FEGJ and DC+FSLS was compared with DC; *P<0.05 when DC+FEGJ and DC+FSLS was compared.

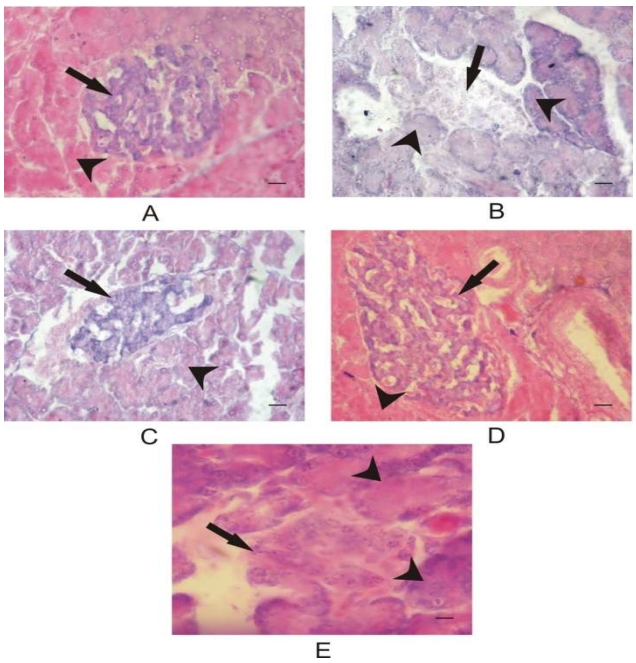

Fig. 3: AF stained photomicrograph of pancreas of NC, DC, DC+INS, DC+FEGJ and DC+FSLS

A. Represents the pancreatic section of NC showing evenly distributed endocrine cells in islets of Langerhans (arrow) surrounded by acini (arrow head).
B. Pancreatic section of DC showing decrease number of endocrine cells in islets of Langerhans (arrow) surrounded by acini (arrow head).
C. Represents the pancreatic section of DC+INS showing invigorating endocrine cells in islets of Langerhans (arrow) surrounded by acini (arrow head).

D. Represents the pancreatic section DC+FEGJ showing replenished endocrine cells in islets of Langerhans (arrow) surrounded by acini (arrow head).

E. Represents the pancreatic section of DC+FSLS showing no augmentation in endocrine cell mass in islets of Langerhans (arrow) surrounded by acini (arrow head).

In all photomicrograph bar =100µ.

Effect of insulin, etiolated wheat grass juice and *L. speciosa* fruit squash on histology of pancreas

Histological study was carried out in order to predict the way of plant extract action. Some authors have shown that plant extract have capability to reverse the chemical induced damage to pancreas [25-28]. Whereas some other reported the insulin mimicking activity of plant extracts [26]. Fig-3B represents the considerable damage to the pancreatic islets due to alloxan. Fig-3C indicates the post insulin treated improved pancreatic islets by regenerating the β cells in diabetic rat as reported by others[18]. Fig-3D shows the evenly distributed endocrine cells (arrow) indicating the regeneration stimulating activity of FEGJ compared to DC. FSLS does not efficiently reverse the alloxan induced pancreatic damage like INS and FEGJ because of persistence of lacuna created by alloxan damage in FSLS treated rats (arrow, Fig-3 E).

CONCLUSION

The hypoglycemic activity of FSLS reported in present work is better than FEGJ and is quite equivalent to the insulin whereas biochemical analysis of blood shows FEGJ have significant hepatoprotective activity while FSLS and INS does not playing role in hepatoprotection. On the basis of biochemical and histological data, it is concluded that, fresh etiolated wheat grass juice possesses hypoglycemic activity due to its stimulating effect on regeneration of alloxan induced damage to pancreas while hypoglycemic activity of *L. speciosa* fruit squash may be due to its insulin mimicking bioactive compound. Hence, we encourage the further studies regarding use of fruits of *L. speciosa* as insulin alternative.

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