Short Communication

EFFECT OF STREPTOZOTOCIN AND POLYHERBAL EXTRACT ON BIOCHEMICAL PARAMETERS IN WISTAR RATS

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ABSTRACT

Gymnema sylvestre, Annona muricata and Ocimum sanctum are well known plants available in India and they are commonly used for the treatment of various diseases including diabetes mellitus. A study was conducted to assess the hypoglycemic effect of a polyherbal extract containing these plants (at two different doses of 300 and 600 mg/kg bwt) compared with metformin as a standard hypoglycemic drug at a dose of 100 mg/kg bwt in streptozotocin-induced diabetes in rats. As a part of the study, the effect of the polyherbal extract on the renal and hepatic functions was tested in rats. The serum parameters both ALT and AST values in rats were significantly ($p < 0.05$) higher in diabetic rats treated with polyherbal extract at 300 mg/kg bwt when compared to Groups I, II and III. BUN values were significantly higher in diabetic and metformin treated groups. Creatinine did not differ significantly among different groups. In conclusion, in streptozotocin induced diabetic rats, the polyherbal extract at 300 and 600 mg/kg bwt had no hepatoprotective action. But the same doses had some renal protective function.

Key words: *Gymnema sylvestre, Annona muricata and Ocimum sanctum,* streptozotocin, metformin, biochemical parameter

Received : 09.12.2023 Revised : 19.02.2024 Accepted : 17.10.2024

INTRODUCTION

Diabetes mellitus (DM) is a complex and chronic metabolic disease of human beings, dogs and cats with elevated blood sugar levels due to lack or deficiency of insulin production, increase in hepatic glucose production or impaired insulin action. It is a common endocrinopathy in dogs (Fall *et al*., 2007).

Streptozotocin (2-deoxy-2-(methyl-3-nitrosoureido)-D-glucopyranose) (STZ) is an antibiotic derived from *Streptomyces achromogenes.* It is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (Ganda *et al.,* 1976; Szkudelski, 2001).

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Ocimum sanctum commonly known as "Tulsi" is a medicinal plant commonly grown in India. Ethanolic extract of leaves showed hypoglycemic effects in normal as well as streptozotocin induced diabetic rats (Sethi *et al.,* 2004). The bark, roots and leaves of *Annona muricata* were reported to be used as an anti-diabetic herb in the Peruvian Amazon (Adeyemi *et al.,* 2010). A member of the milkweed family (Family: Asclepiadaceae) *Gymnema sylvestre,* a woody plant found in tropical forests of India and Africa has been proven to have antidiabetic effect (Mall *et al.,* 2009). As the leaves of Gymnema sylvestre, Annona muricata, and Ocimum sanctum have anti-diabetic potential,to evaluate the efficacy this polyherbal formulation on the biochemical parameters of streptozotocin induced diabetic Wistar rats was undertaken.

MATERIALS AND METHODS

The research work was carriedout in the Department of Veterinary Pathology, Veterinary College and Research Institute, Orathanadu - 614 625, Thanjavur District of Tamil Nadu Veterinary and Animal Sciences University,Tamil Nadu, during the period between March 2023 and November 2023.

Sources of plant extract

Fresh leaves of *Annona muricata* (AM) were collected from a private garden in Thanjavur district. *Gymnema sylvestre* (GS) and *Ocimum sanctum* (OS) leaves were collected from different locations within Thanjavur district of Tamil Nadu. Plants were identified at Sidda Central Research Institute (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India) and accession numbers were G22042327S, O22042328T and A22042329M respectively.

Experimental animal

Forty healthy adult Wistar rats (*Rattusnorvegicus*) of either sex, weighing between 150 – 250 g (7 - 8 weeks old) were used for the experiment. The rats were procured from the Laboratory Animal Science unit of TANUVAS, Madhavaram milk colony, Chennai (40 Nos.) The animal trial works were conducted at Laboratory Animal House, Veterinary College and Research Institute, Tirunelveli (Reg No.: 2161/GO/Re/S/22/ CPCSEA) according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and as per the guidelines set by the Organization for Economic Co-operation and Development (OECD) as per theapproval of the Institutional Animal Ethical Committee, VCRI, Tirunelveli proposal No. 06/SA/IAEC/ VPT, VCRI, TNI/2023.

Preparation of hydroalcoholic plant extract

The hydroalcoholic extract of individual herb (leaves) was prepared by soaking the respective herbal powder in 70% ethanol for 48 hrs in a Soxhlet extraction apparatus in the Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Orathanadu. The extract was evaporated under room temperature to get a blackishgreen semisolid paste and was stored at 4^{0}C until further use. The individual plant extracts were mixed at the ratio of 1:1:1.

Anti - diabetic study

Group I	Control
Group II	Diabetic control (STZ- 60 mg/kg bwt)
Group III	Diabetic rats treated with metformin (100 mg/kg bwt)
Group IV	Diabetic rats treated with polyherbal extract. (300 mg/kg bwt)
Group V	Diabetic rats treated with polyherbal extract (600 mg/ kg bwt)

Wistar rats were subjected to seven days of acclimatization at the laboratory animal house. Wistar rats of either sex (20 female and 20 male) was divided into five groups of eight rats (4 males and 4 females) each as indicated below.

Experimental induction of diabetes

The rats were fasted for 16 hrs STZ was prepared in 0.1 M citrate buffer having a pH of 4.5. Group I rats served as control (8 rats). The remaining 32 rats were injected with freshly prepared streptozotocin at a dose rate of 60 mg/ kg bwt intraperitoneally once. Diabetes was established in all the STZ treated rats over a period of 2 days. Blood samples were collected before the administration of streptozotocin and two days after streptozotocin administration. Immediately after STZ injection, the rats were given 5% w/v of glucose solution instead of drinking water for 2 days to prevent hypoglycemic mortality (Petchi *et al.,* 2014). Diabetic state was confirmed by measuring fasting blood glucose level after 48h of induction using Q-Line biochemical kit with fully automatic biochemistry analyzer. Rats with fasting blood glucose levels above 200 mg/dL were considered diabetic and were used for the experimental study. Among them four male and four female were used as diabetic control (Group II).

Anti-diabetic treatment

The metformin tablets (250 mg) were powdered and suspended in distilled water (5 ml) to make a concentration of 50 mg/ ml. The solution was prepared every day and administrated orally at the dose rate of 100 mg/kg bwt to rats (Group III) once daily from 3^{rd} to 35^{th} day. Polyherbal extract (3g in 10 ml of Tween 20) was administered orally through rat gavaging needle at 300 and 600 mg/ kg bwt to Group III and IV respectively daily during morning hours (h) from 3^{rd} to 35^{th} day.

Biochemical parameters

On the blood samples collected in sodium fluoride- oxalate tubes on 0^{th}, 3^{rd} and 35^{th} day of experiment, glucosewas estimated by fully automated analyser method. The sera collected on 35^{th} day of experiment were subjected for biochemical estimation of serum levels of alanine aminotransferase, aspartate amino transferase, blood urea nitrogen and creatinine. All the biochemical estimations were done using Q-line biochemical kits by fully automated biochemistry analyser SELECTRA Pro X from LABX medical system.

Statistical analysis

Statistical analysis was performed using the statistical software SPSS version 20

for Windows. Mean values and standard error were calculated, and all values were expressed as mean (±SE). The statistical value of $p < 0.05$ was considered as statistically significant. The data were analysed by analysis of variance (ANOVA) for all biochemical parameters.

RESULTS

Plasma glucose

On the third day of streptozotocin injection, all the rats developed diabetes with plasma glucose value above 200 mg/dL.

Alanine aminotransferase

The mean (±SE) ALT (IU/L) values of rats at the end of experiment (35thday) were 60.00±02.12, 69.00±3.00,89.66±15.70, 130.50±38.50 and 136.50±19.50 respectively for GroupI, II, III, IV and V and presented in Table 1. There was a significant ($p<0.05$) increase in ALT values in rats in both Group IV and V when compared to Group I, II and III. But there was no significant ($p<0.05$) difference noticed in both Group II and III, when compared to Group I.

Aspartate aminotransferase

The mean (±SE) AST (IU/L) values of rats at the end of experiment (35 day) were 118.78±05.28, 127.55±12.25, 136.10±10.59, 198.35±14.45 and 146.22±30.68 respectively for Group I, II, III, IV and V and presented in Table 1. Significant ($p < 0.05$) increase in AST values of rats were noticed in Group IV when compared toGroup I, II and III. But there was no significant ($p\leq0.05$) difference in Group II, III and V when compared to Group I. By comparing the Group III and V, therewas no significant ($p < 0.05$) difference. When comparing the Group IV and V, there was no significant ($p < 0.05$) difference in AST values in rats.

Blood urea nitrogen

The mean (±SE) blood urea nitrogen (mg/dL) values of rats at the end of experiment (35thday) were 37.85±01.80, 72.50±02.50, 71.66±10.26, 48.00±11.00 and 56.00±04.00 respectively for Group I, II, III, IV and V, and presented in Table 1. Statistically significant ($p < 0.05$) increases in BUN value of rats were noticed in Group II and III when compared to group Iand IV. But there was no significant ($p < 0.05$) difference in Group IV and V and when compared to Group I. By comparing Group III and V, there was no significant difference in BUN value.

Creatinine

The mean (±SE) creatinine (mg/dL) values of rats at the end of experiment (35thday) were 00.68±0.10, 00.70±0.09, 01.01±0.20, 00.71±0.17 and 00.70±0.11 respectively for Group I, II, III, IV and V and presented in Table 1. There was no significant ($p < 0.05$) difference in creatinine values among the different Groups.

DISCUSSION

The induction of diabetes by STZ, a nitrosourea derivative, has been attributed to the selective destruction of insulin producing beta cells by induction of necrosis. The selective beta-cell toxicity of STZ is related to the glucose moiety in its chemical structure, which enables STZ to enter the

cell via the low affinity glucose transporter GLUT2 in the plasma membrane. *Gymnema sylvestre* (Asclepiadaceae), *Annona muricate* (Annonaceae) *Ocimum sanctum* (Lamiaceace) are known plants available in India and they are commonly used for the treatment of various diseases including diabetes mellitus.

Serum biochemistry of both ALT and AST values in rats were significantly ($p< 0.05$) higher in group IV when compared to Groups I, II and III and there were statistically significant ($p < 0.05$) increases in BUN values of rats noticed in Groups II and III when compared to Group I and IV. However, there was no significant ($p < 0.05$) difference in creatinine values among the different Groups II, III, IV and V when compared to Group I. A similar finding was observed by (Zafar *et al.,* 2009) who reported that damage vasodilation kidneys was due to the increased production of Kallikrein and prostaglandin E2 which caused hyperfiltration and vasodilation and increase in diabetes.The increase in aminotransferases levels may be due to the cellular damage in the liver caused by STZ-induced diabetes. Their report concluded that the streptozotocin through its direct alkylating action can cause cellular necrosis and selective destruction of the beta cells producing hyperglycaemia at a dose of 45 mg/kg body weight.STZ has been reported to induce both plasma membrane and organellar membrane damage especially that of RER and mitochondria in hepatocytes because of which the enzymes are leaked into circulation, a factor well supported by the microscopic evidence of hepatic damage (Rajesh and Latha, 2004).

The high serum ALT and AST values in Group IV and V might be due to poor hepatoprotective mechanism attributed to the individual herbs in the present formulation. Mulkawar *et al.* (2018) also reported that *Gymnema sylvestre* caused only a slight reduction in AST in diabetic rats.

In conclusion, in streptozotocin induced diabetic rats, the polyherbal extract at 300 and 600 mg/kg bwt had no hepatoprotective action. But the same doses had some renal protective function.

Table 1. Mean (±SE) ALT, AST, BUN and Creatinine values of rats at the end of the experiment

Group	ALT (IU/L)	AST (IU/L)	BUN (mg/dL)	Creatinine (mg/dL)
I	60.00±02.12^{a}	118.78±05.28^{a}	37.85±01.80^{a}	0.68±0.10^{a}
II	69.00±03.00^{a}	127.55±12.25^{a}	72.50±02.50^{b}	0.70±0.09^{a}
III	89.66±15.70^{a}	136.10±10.59^{a}	71.66±10.26^{b}	1.01±0.20^{a}
IV	130.50±38.50^{b}	198.35±14.45^{b}	48.00±11.00^{a}	0.71±0.17^{a}
V	136.50±19.50^{b}	146.22±30.68^{ab}	56.00±04.00^{ab}	0.70±0.11^{a}

^{ab}Mean (±SE) superscripted with different letters in a column were significantly different ($p< 0.05$)

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