

HEPATOPROTECTIVE EFFECT OF PERILLYL ALCOHOL (POH) ON HIGH-FAT DIET-LOW-DOSE STREPTOZOTOCIN-INDUCED TYPE 2 DIABETES IN EXPERIMENTAL RATS

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ABSTRACT

Objective: The aim of the present study was to investigate the hepatoprotectivity effect of perillyl alcohol in high-fat diet (HFD)-low-dose streptozotocin (STZ)-induced diabetic rats.

Methods: Diabetes was induced in male albino Wistar rats by 4 weeks diet manipulation followed by a low single intraperitoneal injection of STZ (35 mg/kg body weight). Perillyl alcohol was administered orally (50 mg/kg body weight and 100 mg/kg body weight) for 30 days. Glibenclamide was used as a standard drug and given orally 6 mg/kg body weight. At the end of treatment period, levels of alkaline phosphatase (ALP), aspartate transaminase (AST) alanine transaminase (ALT), and bilirubin and serum protein were measured in serum of experimental rats. Histopathological studies of liver were also done with microscope.

Results: The levels of ALP, AST, ALT, and bilirubin were increased significantly in HFD-STZ-induced diabetic rats. Perillyl alcohol-treated diabetic rats showed marked restoration in the activity of ALP, AST, ALT, and bilirubin when compared with diabetic control rats. Perillyl alcohol and glibenclamide drug-treated rats showed reversible tissue regeneration with prominent hepatocytes.

Conclusion: The present results clearly indicate that the perillyl alcohol has a potent efficacy for hepatoprotective effect in HFD- low-dose STZ-induced diabetic rats.

Keywords: High-fat diet, Streptozotocin, Perillyl alcohol, Glibenclamide, Bilirubin.

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INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia, means high blood glucose levels disturbances in carbohydrate, protein and fat metabolism, and is a widespread metabolic disease almost found in all countries [1]. DM the metabolic disorder is caused by hereditary (or) acquired shortage in insulin secretion and by decreased sensitivity of the organs to secrete insulin [2]. The number of adults with diabetes in the world is predictable to increase from 135 million in 1995 to 300 million in the year of 2025 [3]. Elevated levels of glucose in blood cause oxidative stress as a result of increased creation of mitochondrial reactive oxygen species (ROS), non-enzymatic glycation of proteins, and glucose autoxidation [4]. Glycosylation of hemoglobin and lipid peroxidation are important parameters that help in the progression of complications of diabetes in patients [5]. Liver helps to store the body excess glucose as glycogen by this process liver plays an important role in glucose homeostasis [6]. When oxidative stress increases in liver, the liver cells are affected so the important thing is to protect the liver to maintain the normal metabolic activity. When diabetes is not treated, it will lead to so many problems such as eye problem, neuropathy, kidney disease, aging, liver problems, and other complications [7]. Synthetic antidiabetic drugs have so many side effects [8], and many traditional plants decrease glucose levels and have no side effect at all [9]. Therefore, recently proper hypoglycemic agents have been focused in traditional medicine because some natural products in traditional medicine may be better treatments than currently used drugs [10]. Perillyl alcohol (POH) (Fig. 1) is a naturally occurring monocyclic monoterpene that can be purified from various plants such as peppermint, spearmint, cherries, and celery [11]. It has been shown by the researchers that POH possesses considerable antitumor, anticancer, anti-inflammatory, and antifungal activity [12,13].

In view of the above medicinal properties, the present study was designed to investigate the hepatoprotective activity of perillyl alcohol in high-fat diet (HFD)-STZ-induced diabetic rats.

METHODS

Animals

Healthy male albino Wistar rats (160–180 g) were obtained from Biogen Laboratory Animal Facility, Bengaluru, India, and maintained at a constant temperature (25±1°C) on a 12 h light/12 h dark cycle with standard pellet diet (National Institute for Nutrition, Hyderabad, India) and water was provided *ad libitum*. The study was approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College (Reg. no. 160/PO/ReBi/S/1999/CPCSEA, Proposal no. 1192), Annamalai University.

Chemicals

Perillyl alcohol (POH) and streptozotocin (STZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and other chemicals were obtained from E. Merck, Himedia (Mumbai India). All of the chemicals and reagents used in these experiments were analytical grade.

HFD-fed STZ-induced diabetes

The rats were divided into two dietary regimens by feeding either normal or HFD for the initial period of 4 weeks [14]. The composition (Table 1) and preparation of HFD (58% fat, 25% protein, and 17% carbohydrate, as a percentage of total kcal) have been described earlier [15]. After

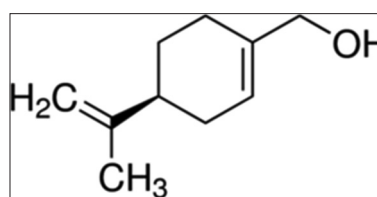


Fig. 1: Structure of perillyl alcohol (C₁₀H₁₆O)

4 weeks of dietary manipulation, the groups of rats fed with HFD were injected intraperitoneally with a low dose of STZ (35 mg/kg b.w.) dissolved in 0.1M cold citrate buffer, pH 4.5). 3 days after STZ injection, the rats were screened for blood glucose level. Rats having fasting blood glucose >250 mg/dl that exhibited random hyperglycemia and glycosuria were selected for the experiment. The rats were allowed to continue to feed on the respective diets until the end of the experiments.

Experimental design

A total number of 30 rats were divided into 5 groups of six animals each (6 normal rats and 24 diabetic rats). Saline was used as vehicle. Perillyl alcohol and glibenclamide were dissolved in saline and administered orally once in a day for 30 days.

- Group 1 – Normal rats treated with vehicle alone
- Group 2 – HFD-fed STZ-induced experimental diabetic rats (35 mg/kg b.w.)
- Group 3 – HFD-fed STZ-induced diabetic rats orally treated with perillyl alcohol (50 mg/kg b.w./day for 30 days)
- Group 4 – HFD-fed STZ-induced diabetic rats orally treated with perillyl alcohol (100 mg/kg b.w./day for 30 days)
- Group 5 – HFD-fed STZ-induced diabetic rats orally treated with glibenclamide (6 mg/kg b.w./day for 30 days).

During the experimental period, body weight, food and water consumption, and physical examinations were determined at regular intervals. At the end of the treatment period, the rats were fasted overnight and sacrificed by cervical decapitation. The blood was collected with and without anticoagulants for plasma and serum separation, respectively.

Histopathological studies

The liver tissue was fixed in 10% formalin for 48 h. It was then followed by dehydration by passing through a series of graded alcohol, beginning with 50% alcohol and progressing in graded step to 100% (absolute) alcohol and was finally embedded in paraffin. Sections of the liver (5–6 µm thick) were developed using semi-automated rotator microtome, stained with hematoxylin and eosin dye, and observed microscopically [16].

Biochemical analysis

Alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were assayed in serum using commercial kits obtained from Sigma Diagnostics (I) Pvt., Ltd., Baroda, India.

Table 1: Composition of high-fat diet

Ingredients	Diet (g/100 g)
Powdered NPD	36.5
Lard	31
Casein	25
Cholesterol	1
Sodium cholate	0.5
Vitamin mineral mix	6
dl-methionine	0.3
Yeast powder	0.1
Sodium chloride	0.1

*The composition of NPD: 4.1% fat, 22.2% protein, and 12.1% carbohydrates, as a percentage of total kilocal. NPD: Normal pellet diet

Table 2: Effect of perillyl alcohol on hepatoprotective markers Serum Bilirubin, ALT, AST, ALP and liver weight in diabetic animals treated with perillyl alcohol

Groups	Liver weight (g)	Serum bilirubin (mg/dl)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control	6.79±0.52	0.64±0.15	75.44±5.36	49.08±3.46	21.09±2.23
Diabetic control (HFD-STZ)	4.85±0.71 ^{a,*}	1.14±0.08 ^{a,*}	130.32±8.39 ^{a,*}	78.23±3.98 ^{a,*}	31.75±3.11 ^{a,*}
D+POH (50 mg/kg b.w.)	6.00±0.35 ^{b,*}	0.88±0.23 ^b	90.85±3.94 ^{b,*}	60.08±3.46 ^{b,*}	25.13±2.34 ^{b,*}
D+POH (100 mg/kg b.w.)	6.23±0.31 ^{b,*}	0.77±0.20 ^{b,*}	81.32±5.34 ^{b,*}	55.08±3.63 ^{b,*}	24.05±2.12 ^{b,*}
D+GC (6 mg/kg b.w.)	6.66±0.69 ^{b,*}	0.73±0.10 ^{b,*}	80.07±5.62 ^{b,*}	52.08±6.14 ^{b,*}	22.88±2.34 ^{b,*}

All values are expressed as mean±SD for six rats in each group. One-way ANOVA followed by Tukey's multiple comparison tests. Statistical significance was compared within the groups as follows: ^aControl rats and ^bDiabetic control rats. Values are statistically significant at *p<0.05. ALP: Alkaline phosphatase, AST: Aspartate transaminase, ALT: Alanine transaminase, SD: Standard deviation, HFD: High-fat diet, STZ: Streptozotocin

Total serum protein was estimated using the method of Lowry *et al.*, 1951 [17]. Serum bilirubin was estimated by the method of Malloy and Evelyn 1937 [18].

Statistical analysis

All values are expressed as mean ± standard deviation for six rats in each group. One-way ANOVA followed by Tukey's multiple comparison tests using IBM SPSS version 22. Statistical significance was compared within the groups as follows: ^aControl rats and ^bdiabetic control rats. Values are statistically significant at *p<0.05.

RESULTS

Effect of perillyl alcohol on AST, ALP, ALT, and serum bilirubin

In the present study, the hepatoprotective markers in serum of HFD-low-dose STZ-induced diabetic rats ALT, AST, ALP (Table 2 and Fig. 2), and serum bilirubin (Table 2 and Fig. 3) level were increased in diabetic group when compared with normal group and treated with perillyl alcohol 50 and 100 mg/kg body weight significantly decrease these markers.

Effect of perillyl alcohol on liver weight

Liver weight was consistently decreased in diabetic group (Fig. 4) and when treated with perillyl alcohol, 50 and 100 mg/kg b.w., liver weight increased when compared with diabetic group (Table 2).

Effect of perillyl alcohol on total serum protein

Fig. 5 depicts the changes in serum total protein in control and diabetic rats, serum protein significantly decrease in diabetic control group (p<0.05), and when the rats were treated with perillyl alcohol, 50 and 100 mg/kg b.w., the serum total protein significantly increased near to the normal when compared with diabetic control group.

Histological observations

The liver of treated diabetic rats with POH 50 and 100 mg/kg body weight showed significant results. Normal control rats show ideal hepatocytes in liver tissue (Fig. 6a), and diabetic control rats show tissue damage in liver hepatocytes and major blood sinusoids and internal blood cysts (Fig. 6b). Perillyl alcohol and glibenclamide drug-treated rats showed reversible tissue regeneration with prominent hepatocytes (Fig. 6c-e).

DISCUSSION

Presently available drug regimens in the market for the management of DM have certain side effects so there is a need to find safer and more effective antidiabetic drugs with no or very less side effects especially from plant origin [19]. For the successful therapeutic strategies, we need to have interaction between the oral hypoglycemic drugs and the phytochemicals in terms of bioavailability, metabolism, and steadiness [20]. The present study was meant to determine the efficiency of perillyl alcohol on hepatoprotective activity of normal and HFD-STZ-induced diabetic rats. HFD is now a day used by researchers to induce the insulin resistance which is the main cause of type 2 diabetes HFD also induces obesity it in turn increases oxidative stress [21]. To induce the experimental diabetes, STZ is commonly used it causes the selective β-cell cytotoxicity through the release of nitric oxide. This results in speedy decrease in pancreatic islet pyridine nucleotide concentration

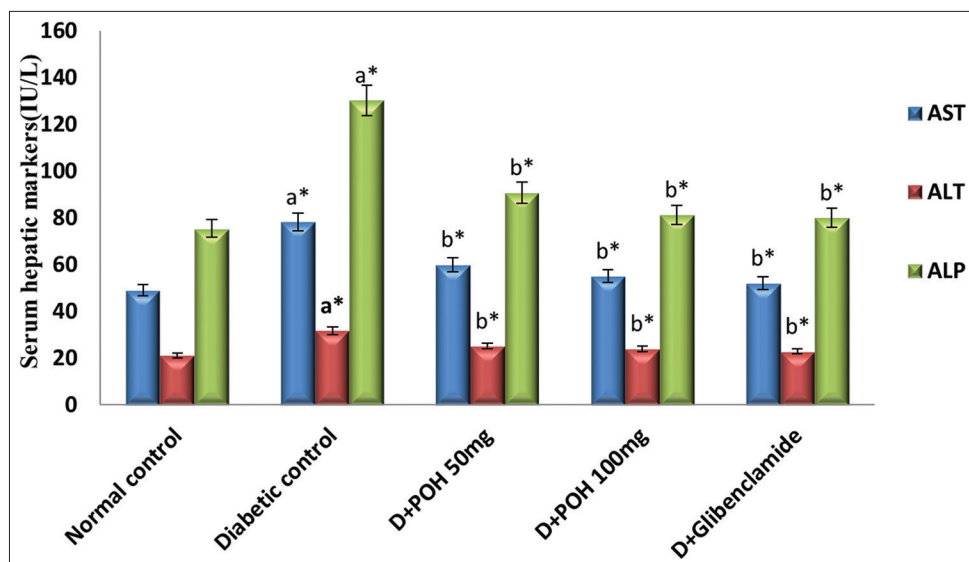


Fig. 2: Effect of perillyl alcohol on hepatic markers on high-fat diet-low-dose streptozotocin-induced diabetic rats. One-way ANOVA followed by Tukey’s multiple comparison tests statistical significance was compared within the groups as follows: ^aControl rats and ^bdiabetic control rats. Values are statistically significant at *p<0.05

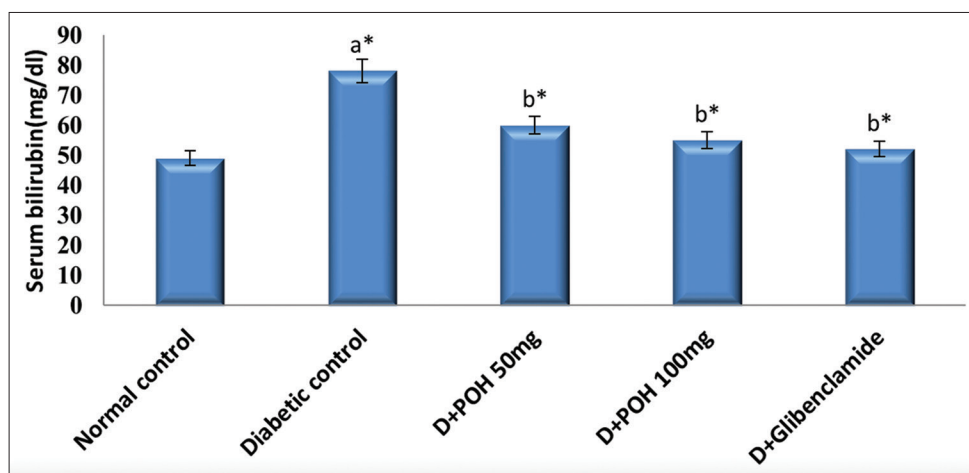


Fig. 3: Effect of perillyl alcohol on bilirubin on high-fat diet-low-dose streptozotocin-induced diabetic rats. One-way ANOVA followed by Tukey’s multiple comparison tests statistical significance was compared within the groups as follows: ^aControl rats and ^bdiabetic control rats. Values are statistically significant at *p<0.05

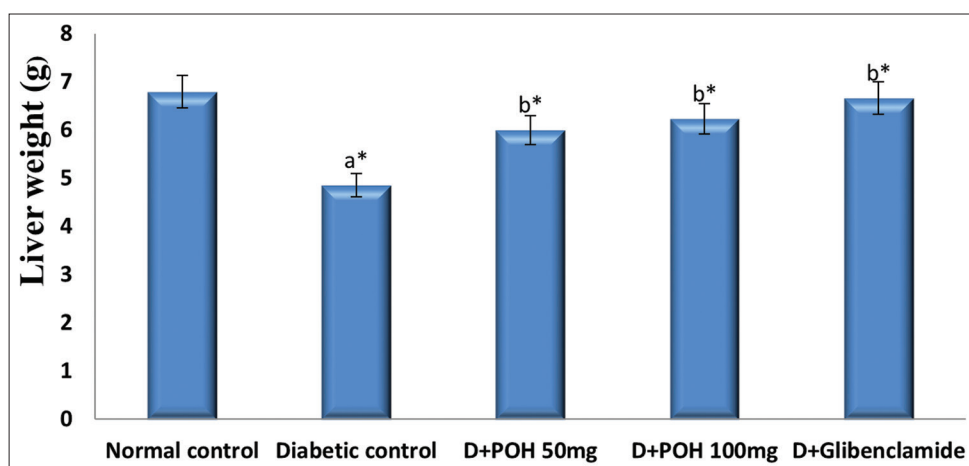


Fig. 4: Effect of perillyl alcohol on liver weight of high-fat diet-low-dose streptozotocin-induced diabetic rats. One-way ANOVA followed by Tukey’s multiple comparison tests statistical significance was compared within the groups as follows: ^aControl rats and ^bdiabetic control rats. Values are statistically significant at *p<0.05

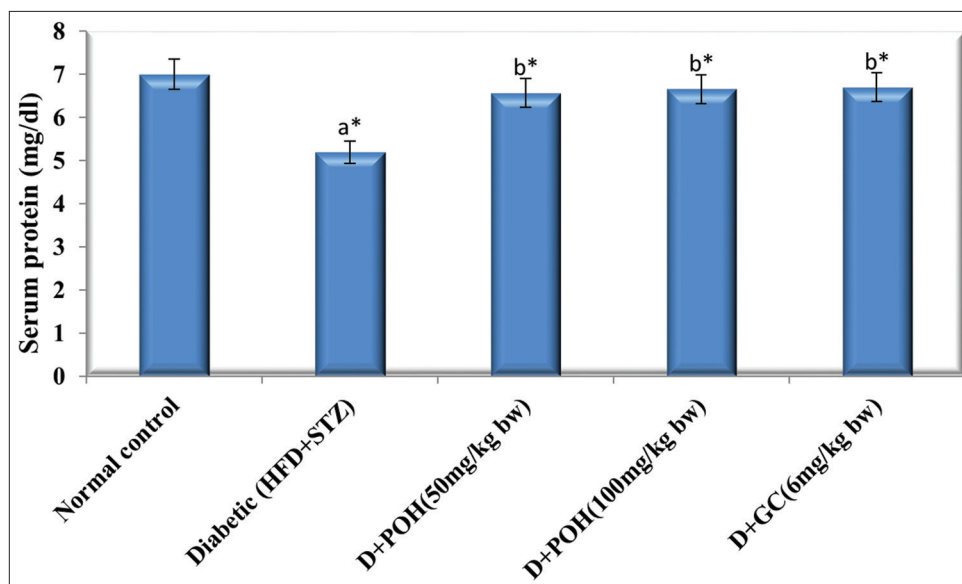


Fig. 5: Effect of perillyl alcohol on serum total protein on high-fat diet- low-dose streptozotocin-induced diabetic rats. One-way ANOVA followed by Tukey's multiple comparison tests statistical significance was compared within the groups as follows: ^aControl rats and ^bdiabetic control rats. Values are statistically significant at * $p < 0.05$

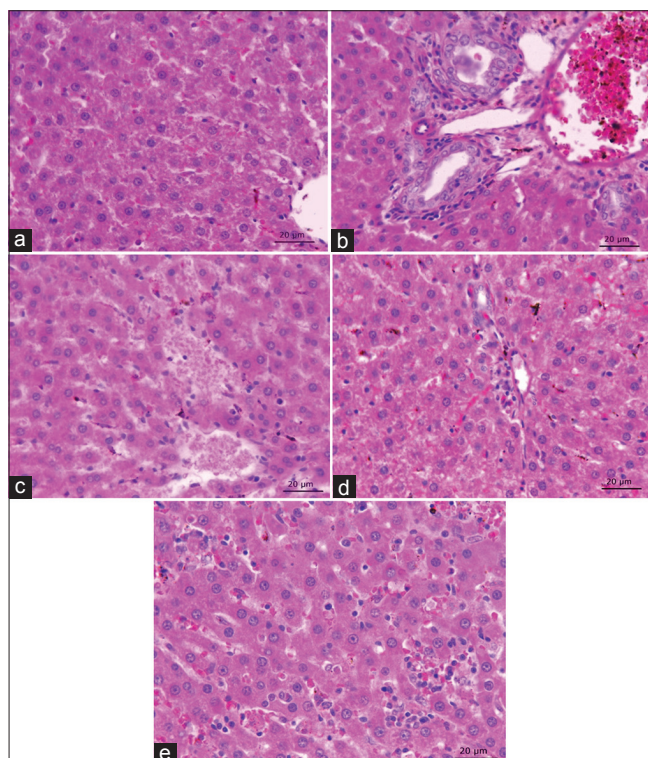


Fig. 6: (a-e) Histological observation of liver of normal and high-fat diet (HFD)-low-dose streptozotocin (STZ)-induced diabetic rats. Histopathological observations of perillyl alcohol and glibenclamide-treated liver in HFD-STZ-induced diabetic rats after 30 days of treatment (H&E staining, $\times 40$). (a) Normal control - normal histological structure of hepatic, hepatic cords, hepatic sinusoids, and central vein. (b) Diabetic control - mild sinusoids damaged hepatocytes were observed. (c) Diabetic + POH 50 mg/kg b.w., (d) diabetic + POH 100 mg/kg b.w.), and (e) diabetic + glibenclamide 6 mg/kg b.w. - showed reversible tissue regeneration and prominent hepatocytes

and following β -cell necrosis. The act of STZ on mitochondria generates SOD anion, which leads to diabetic complications [22-24]. In the

present investigation, HFD/STZ is used to induce diabetic condition in male Wistar rats. Standard antidiabetic drug glibenclamide is used to compare the antihyperglycemic activity in experimental rats. The glibenclamide is a standard antidiabetic drug, used to compare the antihyperglycemic property in experimental rats [25]. In the present study, HFD-STZ-administered rats showed increased levels of plasma glucose and decreased insulin levels. The oral administration of perillyl alcohol to diabetic rats showed the levels of plasma glucose reduced and insulin levels increase toward near normalcy when results were compared with diabetic untreated group. Perillyl alcohol by its ability reverses the hepatoprotective markers to near normal level. The normal ability of liver to synthesis glycogen is impaired in diabetes; there is the problem with the enzymes. Synthase phosphatase not activates glycogen synthase to glycogenesis [26]. Necrosis cause decreased in the cell mass that is, why the liver weight is decreased in diabetes [27]. The diabetic rats treated with perillyl alcohol at the dose of 50 and 100 mg/kg body weight maintained the liver weight near to normal levels. From our investigation, it is well clear that POH prevents the glycation of proteins in the liver and serum. AST, ALT, and ALP are reliable markers of liver function [28,29]. An increase in the activities of AST, ALT, and ALP in serum might be mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream which gives an indication of the hepatotoxic effect of STZ [30]. Treatment of the diabetic rats with perillyl alcohol reduced the activity of these enzymes in serum compared to the diabetic untreated group and consequently alleviated liver damage caused by HFD-low-dose STZ-induced diabetes. This study also supports the possibility of regeneration of tissues even the damage is severe as we found in the liver of experimental rats [31]. Significant reductions in the activities of these enzymes increase in liver weight regeneration of liver tissue damage in POH-treated diabetic rats indicated the hepatoprotective role in preventing diabetic complications.

CONCLUSION

Perillyl alcohol restored the altered serum enzymes (AST, ALT, and ALP) and bilirubin. And also, histopathological studies of tissues confirmed the recovery of tissue damage. The action of perillyl alcohol was comparable to the antidiabetic drug glibenclamide. Results of this experimental study indicated that perillyl alcohol possessed hepatoprotective activities. Further pharmacological and biochemical investigations are underway to explain the mechanism of the antidiabetic effect of perillyl alcohol.

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AUTHORS' CONTRIBUTIONS

Towseef Hassan and Insha Naseer, Ph.D. Research Scholars performed the experimental work, prepared the data, and drafted the write-up. C. Elanchezhian, Associate Professor, Department of Zoology guided the research work and edited the write-up.

CONFLICTS OF INTEREST

There is no conflict of interest.

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