



Original Research Paper

Therapeutic Response of Aqueous Shilajit Extraction, Commercial Shilajit, and Amaryl on Pituitary–Thyroid Axis Hormones in Alloxan -Induced Diabetic Female Rats

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Summary

A traditional healer has been used shilajit as a treatment for diabetes. Since diabetes causes thyroid dysfunction, this experiment designed to compared the ameliorative action of aqueous shilajit extract and commercial shilajit on hormonal and structural of thyroid gland in diabetic female rats. The study done on 48 adult female rats, experimental period was 32 day animals divided into six group each one have eight rats, Group I as a negative Control, Group II administrated aqueous shilajit extract at dose 100 mg/Kg B.W orally one dose daily other four groups induce diabetic by a single intraperitoneal injection of alloxan monohydrate (100 mg/kg body weight) Group III Diabetic Control, Group IV diabetic treated orally with aqueous shilajit extract 100 mg/kg bw. Group V diabetic treated with commercial shilajit 100 mg/kg/bw. Group VI diabetic treated with Amaryl (Glimepiride) 0.1mg/ kg bw., the concentration of thyroid stimulating hormone, TSH, thyroxine T3 and T4 measured in serum and harvested the thyroid gland for histological study. Results; treatment DM with shilajit Extract and commercial shilajit caused increase in T4 and partially restored TSH and some histological change in glands tissue. Amaryl lowest T4 level among all groups. Conclusion: treatment with shilajit extract in diabetic female rats improved thyroid hormone status more than commercial shilajit and Amaryl.

Key words: diabetic, shilajit, thyroid hormones and Amaryl

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, impaired insulin action, or both (Magliano and Boyko, 2021). Among the chemical diabetogenic agents used to induce experimental diabetes, alloxan monohydrate is one of the most commonly employed compounds. Alloxan is selectively transported into pancreatic β -cells through the GLUT2 glucose transporter, where it undergoes redox cycling and generates excessive reactive oxygen species (ROS). These free radicals cause oxidative damage to β -cell DNA, proteins, and membrane lipids, resulting in β -cell necrosis, insulin deficiency, and sustained hyperglycemia (Rohilla and Ali, 2012).

Consequently, the alloxan-induced diabetic model is widely used for investigating diabetes-associated metabolic and endocrine alterations (Biondi et al., 2019). One of the endocrine organs markedly affected by diabetes is the thyroid gland. Chronic hyperglycemia impairs the peripheral conversion of thyroxine (T4) into triiodothyronine (T3) and suppresses the hypothalamic–pituitary–thyroid (HPT) axis,

leading to reduced secretion of thyroid-stimulating hormone (TSH). Conversely, thyroid dysfunction further disturbs glucose metabolism, decreases insulin sensitivity, and aggravates diabetic complications (Penugonda and Vamsi Krishna, 2021). Current therapeutic strategies for diabetes include insulin and oral antihyperglycemic agents such as metformin, glibenclamide, empagliflozin, and aminoguanidine (Avgerinos et al., 2021). Although these drugs effectively control blood glucose levels, prolonged treatment may be associated with adverse effects, and inadequate protection against diabetes-induced oxidative damage in various organs, including the thyroid gland (Salehi et al., 2019). Therefore, increasing attention has been directed toward natural products against diabetic complications.

Shilajit is a naturally occurring dark brown mineral-rich phytocomplex that has been used in Ayurvedic medicine for centuries. It is primarily found in high-altitude mountainous regions, particularly the Himalayas, and is formed through the gradual decomposition of plant materials and microbial metabolites over long geological periods (Agarwal et al., 2007).

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Its chemical composition varies according to geographical origin because of differences in vegetation, microbial activity, mineral composition, and geological conditions (Ghosal et al., 1991; Al-Galbi et al., 2026). The pharmacological properties of shilajit are mainly attributed to its high content of fulvic acid, humic substances (Mishra et al., 2019), dibenzo- α -pyrones, amino acids, minerals, and trace elements. These bioactive constituents exhibit potent antioxidant, anti-inflammatory, immunomodulatory, and mitochondria-protective activities (Stohs, 2014; Alrafas et al., 2023). Shilajit safety in the long term as a dietary supplement was also revealed in animal studies, in which 24 Wistar rats (12 males and 12 females) were given 5000 mg/kg with water once daily for 91 days without any significant toxicity (Velmurugan et al., 2012).

Materials and Methods

Animals maintenance

The study was carried out on 48 female rats (180 ± 20 gm average weight), according to instructions and regulations of animal care and use committee, Faculty of Veterinary Medicine / University of Basrah. From (temperature, humidity and 12- hours light-dark cycle) with in two week acclimatization before experimental. The animals were randomly allocated into different cages, each cage containing 2 animals (Abdulkareem et al., 2025).

Induced experimental diabetes.

Diabetic mellitus (DM) was induced in thirty –two female rats, fasted for 12 h with free access to water, then injected with a single intraperitoneal injection of a freshly prepared solution of alloxan (Al-khazaali and Zeiny, 2025). At a dose of 100 mg/kg, b.w The rats kept for the next 24 h in 5% glucose solution bottles in their cages to prevent hypoglycemia. Three to five and 32 days after injection, serum glucose level were monitored by a glucometer by punctured the tail. Rats with fasting blood glucose levels >200 mg/dL were considered diabetic and were included in the experimental study. (Kelechi et al., 2025).

Preparation of Aqueous Shilajit extraction.

Preparation of shilajit aqueous extract: Shilajit bought from local herbal –medicine store of Basrah governorate. First, shilajit had been washed, air-dried, before being pieces thinly as possible. After the pieces

had dried fully in a food drier for 12 hours, they were crushed into a rather fine powder. Following that, aqueous shilajit extracts were made according to (Perumal et al., 2024). To prepare a stock solution with concentration of 100 mg/mL, approximately 5 grams of shilajit powder was weighed and added to 250 mL beaker containing 100 mL of double-distilled water. Then, the mixture was heated about 60°C for 15 minutes. After cooling it to 25°C (room temperature), the mixture was filtered to inhibit any solid debris through What man No. 1 filter paper, causing in a clear solution. The filtrate was subsequently stored at 4°C for further analysis.

Commercial Shilajit from Himalayan shilajit 2000 mg manufactured by piping Rock Health Products, ny 11779 USA was prepared by dissolved one capsule in distilled water to obtain concentration included (100 mg/kg) according to (Rajpoot, 2025).

Sample collection

Collection of blood sample at the last 32 day of the trial, blood samples were taken from the heart of each animal. For hematological analysis, 4 ml of blood were placed in jell tube anticoagulant-free, centrifuged for fifteen minutes at 3000 rpm, and serum samples-which were used to examine thyroid hormones TSH, T3, and T4 were kept in polyethylene Eppendorf tubes at -20°C (Jasim et al., 2025; Habeeb et al., 2026).

Hormonal assay

Used T3 and TSH enzyme immunoassay test kit catalogs number: 125- 300 Total triiodothyronine product code: 325 300 Thyrotropin: All from monobind inc. Lake forest, CA92630, USA

Histopathological examination

Following the sacrifice of animals, thyroid gland removed and washed with a (0.9%NaCl), for preparation section of gland by staining according to (Luna, 1992; Kadhim et al., 2026). Fixation,

Shilajit has also been reported to preserve cellular integrity and improve organelle function by sustaining cellular energy production (Bhattacharyya et al., 2010). Given the pivotal role of oxidative stress in diabetes-induced thyroid dysfunction and the antioxidant and cytoprotective properties of shilajit, evaluating its effects on thyroid gland function in experimental diabetes is of considerable scientific interest. Therefore The present study aimed to investigate the physiological and structural alterations in the thyroid gland of alloxan-induced diabetic animals and to compare the potential protective effects of aqueous Shilajit extract and commercial Shilajit with those of the conventional antidiabetic drug glimepiride (Amaryl).

dehydration, cleaning, wax infiltration, embedding sample in wax, sectioning, de-waxing and staining (Hematoxylin and Eosin) (ALquraishy et al., 2026).

EXPERIMENTAL DESIGN

The forty-eight female albino rats were randomly divided into six experimental groups, with eight animals in each group, as follows:

Group I (Control): Eight healthy female albino rats were left untreated and served as the normal control group throughout the experimental period.

Group II Extracted Shilajit 100 mg/kg bw orally once daily

Group III (Diabetic Control) induced diabetic by a single intraperitoneal injection of freshly prepared alloxan monohydrate (100 mg/kg bw).

Group IV diabetic rats treated orally with shilajit extract 100 mg/kg.bw

Group V diabetic rats treated orally with commercial shilajit 100mg /kg/bw.

Group VI diabetic rats treated orally with (Glimepiride) 0.1mg/kg bw.

Statistics analysis

All data of the present study were statistically analyzed by using Microsoft windows excel (version 2019) and SPSS version 26, based on using One Way ANOVA Post-Hoc multiple comparisons were performed using [Tukey HSD /LSD]. The data were presented as means $\pm$ standard deviation (Abduljaleel et al., 2025).

Results

Table (1) appeared the value of serum T3 concentration in the shilajit -treated group exhibited the highest value (2.108 ± 0.179), which was significantly higher than that of the diabetic group (1.665 ± 0.191). Likewise, the groups diabetic rats treated with shilajit extract (1.958 ± 0.201) and commercial shilajit (1.915 ± 0.081) showed increase in T3 concentrations that were not significantly different from the shilajit-treated group. Also, the amaryl-treated group (1.765 ± 0.044) increase in T3 concentration but do not reached to the significant concentration of T3 when comparable with control group and all other group. Serum T4 levels differed significantly among the experimental groups at ($P < 0.05$) level in diabetic group. The highest T4 concentration was observed in the diabetic rats treated with shilajit extract (4.925 ± 0.096), which was significantly greater than all other groups this result may be attributed to the fact Shilajit extract may be have activated or restored the hypothalamic-pituitary-thyroid (HPT) axis, which is affected by diabetes. Diabetic rats, shilajit-treated rats, and diabetic rats treated with commercial shilajit exhibited comparable T4 concentrations (3.958 ± 0.204 , 4.223 ± 0.804 , and 3.935 ± 0.196 , respectively). The control group showed a significantly lower T4 level (3.173 ± 0.155) whereas, the amaryl-treated group had the lowest T4 concentration (2.505 ± 0.124) compared with all groups. Table (1) appeared concentration of serum TSH in these groups at level ($P < 0.05$) there was no significant difference in diabetic group compared with the negative control group. In contrast, treatment of diabetic female rats with the aqueous shilajit extract group and commercial shilajit group significantly increased TSH concentration compared with the diabetic group, the negative control group, and the other experimental groups ($P < 0.05$), indicating that the aqueous shilajit extract produced the highest TSH levels among all treatments. While in group treated diabetic with amaryl did not exhibited differ significantly when compared with the negative control group or the shilajit group ($P > 0.05$).

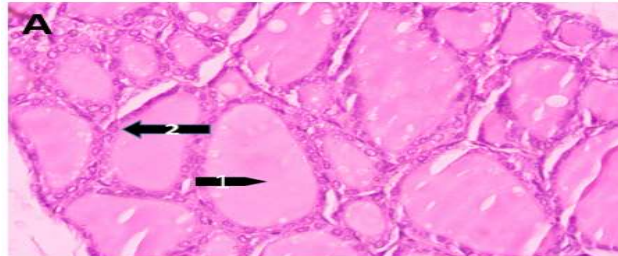
Table 1: The effect of treated with shilajit extract, commercial shilajit and amaryl on pituitary-thyroid hormones axis in diabetic female rats (means $\pm$ SD).

	NO.	T3 ng/dL	T4 μ g/dL	TSH mIU/L
Control -ve	8	1.733 ± 0.119 bc	3.173 ± 0.155 c	0.00850 ± 0.00058 b
Shilajit control positive	8	2.108 ± 0.179 a	4.223 ± 0.804 b	0.00450 ± 0.00058 c
Diabetic	8	1.665 ± 0.191 c	3.958 ± 0.204 b	0.00625 ± 0.00126 bc
<i>D.M+extraction shilajit</i>	8	1.958 ± 0.201 ab	4.925 ± 0.096 a	0.01725 ± 0.00126 a
<i>D.M+commercial shilajit</i>	8	1.915 ± 0.081 ab	3.935 ± 0.196 b	0.01500 ± 0.00577 a
DM+Amaryl	8	1.765 ± 0.044 bc	2.505 ± 0.124 d	0.00650 ± 0.00191 bc

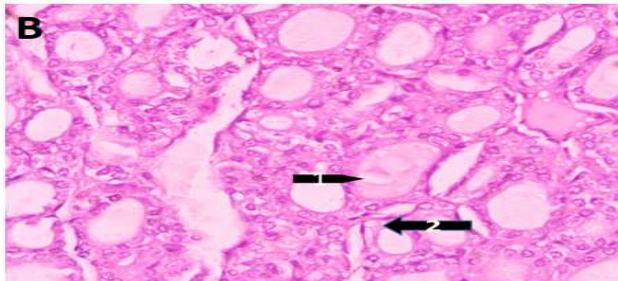
Values ex small letters pressed in mean significant differences at p. value less than 0.05, TSH at ($P > 0.001$).

Histopathology Features of thyroid Tissue

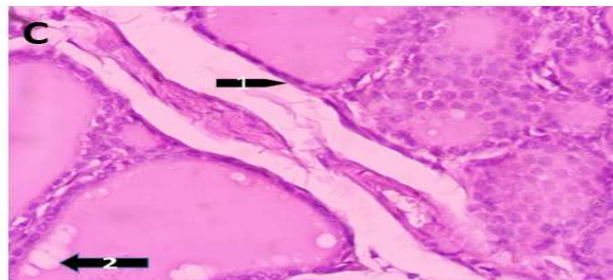
The histopathology of thyroid female rat shown in figure(A) microscopic investigation of thyroid sections of control group showed normal appearance of follicles, normal thyroid stroma and follicular cells (cuboidal to low columnar) with colloid full the follicles. Also, in figures (B) for shilajit group show atrophy of follicles with moderate of colloid, likewise in the figures (C) that induced diabetic female rats with alloxan 100 mg /kg bw showed pathological changes as flattened the follicular epithelial cells, vacuolation in the colloid and fibrin between follicles. Additionally, showed the epithelial cell of follicles arrows 1 with different size of follicles full with colloid in treated with aqueous shilajit D). Also, a section of thyroid showed with excessive vacuolated in the treated group with commercial shilajit in figure E. Whereas, in group treatment diabetic with amaryl showed a colloid full the follicles in figure (F) with flattened epithelial cells of the and the follicles surrounded with fluids.



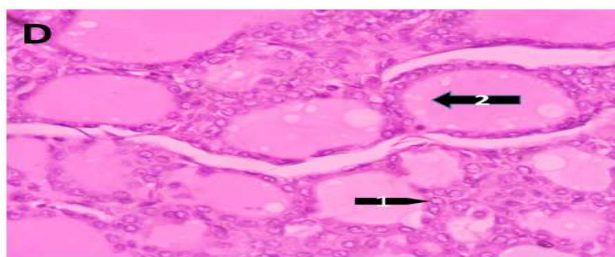
Figure(A) Section of thyroid gland of female rat as a control group ,showed colloid full the follicles arrows1, epithelial cell of follicles arrows 2. (H&E stain X40)



Figure(B) Section of thyroid gland of female rat as a shilajit control positive group showed atrophy of follicles with moderate of colloid (H&E stain X40)



Figures (C) section of thyroid of diabetic female rat show flattened of the follicular epithelial cells arrows 1 , vacuolation in the colloid arrows 2 and fibrin between follicles. (H&E stain X40)



Figures (D) section of thyroid treated diabetic with aqueous shilajit extract showed the epithelial cell of follicles arrows 1 with different size of follicles full with colloid arrows 2 . (H&E stain X40)

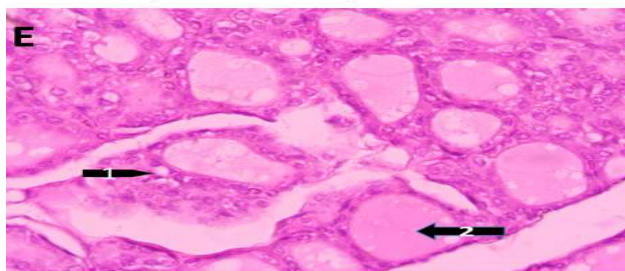


Figure (E) section of thyroid gland of diabetic female rat treated with commercial shilajit showed with excessive vacuolated in the follicle cells arrows 1 , colloid full the follicles arrows 2 .(H&E stain X40)

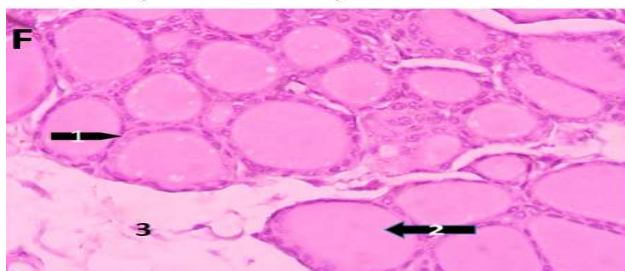


Figure (F) section of thyroid gland of diabetic female rat treated with amaryl showed flatted epithelial cells of the follicles arrows 1 colloid full the follicles arrows 2, surrounded it with fluids 3. (H&E stain X40)

Discussion

The results demonstrated that diabetes significantly disturbed thyroid hormone homeostasis, as evidenced by altered serum T3, T4, and TSH concentrations, whereas treatment with aqueous shilajit, commercial shilajit, and, to a lesser extent, amaryl improved these hormonal alterations. Among the treatment groups, the aqueous shilajit extract produced the greatest improvement in thyroid hormones and thyroid histological architecture. Diabetes mellitus is well recognized as a disorder that affects multiple endocrine organs, including the thyroid gland. Chronic hyperglycemia promotes oxidative stress, inflammation, and metabolic disturbances that impair the normal function of the hypothalamic–pituitary–thyroid (HPT) axis. Experimental and clinical studies have shown that diabetes alters thyroid hormone synthesis, secretion, transport, and peripheral metabolism, resulting in

abnormal circulating concentrations of T3, T4, and TSH (Chaker et al., 2016; Chaker et al., 2017; Brenta. 2021; Mehran et al., 2025; Habeeb et al., 2026). Furthermore, thyroid hormones are major regulators of carbohydrate metabolism because they influence insulin sensitivity through GLUT4-mediated glucose uptake in skeletal muscle and adipose tissue. Consequently, disturbances in thyroid function may further aggravate diabetic metabolic abnormalities (Chen et al., 2019). Consistent with this relationship, (Rong et al., 2021) reported a J-shaped association between circulating TSH and freeT4 concentrations and the risk of diabetes, indicating the close interaction between thyroid dysfunction and glucose metabolism. Among all treatments, aqueous shilajit exerted the most pronounced improvement in thyroid hormone concentrations. Serum T3 levels increased significantly compared with untreated diabetic rats,

suggesting enhanced thyroid hormone synthesis and/or improved peripheral conversion of T4 to T3. This observation agrees with (Ramya et al., 2023) who reported that shilajit possesses antioxidant properties capable of protecting endocrine tissues against oxidative injury. Fulvic acid and dibenzo- α -pyrones, the principal bioactive constituents of Shilajit, have been shown to improve mitochondrial energy production, reduce reactive oxygen species, and preserve cellular integrity (Carrasco-Gallardo et al., 2012). Similarly, aqueous shilajit produced the highest serum T4 concentration among all experimental groups. This finding suggests improved thyroid secretory activity and restoration of thyroid follicular function following attenuation of oxidative stress. Previous reports have described Shilajit as an adaptogenic substance capable of enhancing endocrine homeostasis and improving thyroid gland function under conditions of physiological stress (Meena et al., 2010). Therefore, the restoration of T4 observed in the present study may reflect improved thyroid cellular metabolism and functional recovery.

Shilajit naturally contains selenium, zinc, copper, iron, magnesium, and other trace elements required for thyroid hormone biosynthesis and metabolism. Selenium is an essential component of iodothyronine deiodinases responsible for conversion of T4 into biologically active T3, whereas zinc and iron participate in thyroid hormone synthesis and regulation of pituitary TSH secretion. Consequently, these micronutrients may have contributed to the improved thyroid hormone profile observed in the aqueous shilajit-treated animals (Bhattacharya et al., 1995, Carrasco-Gallardo et al., 2012). Both aqueous and commercial shilajit increased serum TSH concentrations compared with untreated diabetic rats this finding may indicate recovery of pituitary responsiveness and partial restoration of hypothalamic–pituitary–thyroid axis regulation. Reduction of oxidative damage by Shilajit may restore physiological endocrine feedback between the hypothalamus, pituitary gland, and thyroid gland (Brenta 2011; Chaker et al., 2017). However, interpretation of TSH changes should be made cautiously because additional assessment of hypothalamic TRH and pituitary function would be necessary to confirm this mechanism.

Commercial Shilajit also improved serum thyroid hormone concentrations, although the response was consistently less pronounced than that produced by the aqueous extract. Differences in biological activity between commercial and freshly prepared aqueous shilajit may result from variations in geographical origin, purification procedures, processing methods, storage conditions, and concentrations of fulvic acid and bioactive minerals. Such variability has been widely reported and may influence the pharmacological efficacy of commercial shilajit products (Schepetkin et al., 2003).

Glimepiride is one of the second-generation sulfonylureas, a group of insulin secretagogues [Nakamura et al., 2014]. Glimepiride stimulates the release of insulin when it binds to specific cisternae of beta cells leading to closure of KATP and hence the depolarization of membrane which leads to release of insulin [Kalra 2015].

In contrast, amaryl (glimepiride) produced only modest improvement in thyroid hormone concentrations. Although glimepiride effectively lowers blood glucose by stimulating pancreatic insulin secretion and improving insulin sensitivity, it has no established direct effect on thyroid hormone synthesis or regulation of the HPT axis. Therefore, the slight improvement observed in thyroid hormone concentrations is most likely attributable to improved glycemic control and the consequent reduction in oxidative stress Wissam et al., 2023 rather than direct stimulation of thyroid function.

The histopathological findings strongly supported the biochemical results. Thyroid glands from diabetic control rats exhibited marked degenerative changes, including follicular epithelial flattening, colloid depletion, disruption of follicular architecture, and interstitial fibrin deposition, confirming severe thyroid injury induced by alloxan-mediated diabetes. Similar histological alterations have previously been associated with oxidative damage and impaired thyroid hormone synthesis in experimental diabetes. In contrast, thyroid tissues from rats treated with aqueous Shilajit demonstrated near-normal follicular organization, (Ghosal 1990). abundant colloid, and preservation of epithelial integrity, whereas commercial shilajit and amaryl produced partial restoration of thyroid morphology. The close agreement between histopathological observations and hormonal findings indicates that improvement of thyroid structure was accompanied by recovery of endocrine function.

Study Limitation:

The study have many limitations such as small sample size $n=8$ per group, Evaluated endocrine and biochemical profile at single time (Day32), Finally the study were depended on the female rats.

Conclusion

In conclusion, the present findings indicate that aqueous Shilajit exerts a stronger protective and therapeutic effect on the pituitary–thyroid axis than commercial Shilajit and Amaryl in alloxan-induced diabetic female rats, as evidenced by improvements in both the functional activity and histological structure of the thyroid gland.

Conflict of Interest:

Competing Interest The authors declare that there is no conflict of interest

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