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Original Article

Effect of Diazepam on Thyroid Function Hormone and Associated Histological Markers

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Abstract

Diazepam is widely used to manage anxiety and depression, but its effect on thyroid function remain poorly understood. This study aimed to explore the potential effects of diazepam on thyroid function hormones (T3, T4, and TSH), lipid profile, glucose, cortisol levels and thyroid gland histology in animal models. Twenty-four pre-pubertal rats (average weight 160g ± 20g) were divided into four groups, receiving diazepam at 15 mg/kg, 30 mg/kg, or 60 mg/kg daily for seven days, while a control group received standard feed and water ad libitum. General health, including body weight, food and water intake, and behaviour, was monitored. Animals was euthanized, the thyroid glands were analysed histologically, and serum levels of T3, T4, TSH, cholesterol, triglycerides, HDL, LDL, glucose, and cortisol were measured. No statistical significant differences were found across groups ($p > 0.05$), though trends suggested dose-dependent effects. TSH levels were slightly elevated in the intermediate (at 30 mg/kg) and high-dose groups (at 60 mg/kg), with a minor T4 increase at 30 mg/kg. Triglycerides showed high variability, significantly increasing in the low-dose group (at 15 mg/kg). Histological analysis showed no major thyroid abnormalities. While diazepam does not significantly disrupt thyroid function or metabolism, subtle dose-related effects warrant further clinical investigation.

Keywords: Diazepam, Thyroid Histology, Thyroid Function Hormones, Histological Analysis.

1. Introduction

Diazepam, a widely prescribed benzodiazepine, is known for its anxiolytic, sedative, muscle relaxant, and anticonvulsant properties. It is commonly used for treating anxiety disorders, panic attacks, and certain seizure disorders due to its potent and long-lasting effects on the central nervous system (CNS) (Bachhuber et al., 2016). Diazepam exerts its pharmacological effects by binding to benzodiazepine receptor sites on GABAA receptors, enhancing GABAergic inhibition and leading to sedation, anxiolysis, and anticonvulsant activity (Sharma et al., 2019). Although the effects of diazepam on the CNS are well characterized, its potential influence on thyroid function remains inadequately understood. While limited direct research exists on diazepam's effect on thyroid hormones, benzodiazepines as a class have been suggested to influence the hypothalamic-pituitary-thyroid (HPT) axis. Studies indicate that benzodiazepines may modulate thyroid hormone metabolism, receptor binding, and peripheral conversion of thyroxine (T4) to triiodothyronine (T3) (Ashton, 2002; Haddad & Wieck, 2004; Riss et al., 2008). The HPT axis plays a critical role in regulating metabolism, growth, and neurodevelopment, and its dysregulation may have

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far-reaching physiological and metabolic consequences. Thyroid hormones are crucial for regulating metabolism, growth, and development (Macvanin et al., 2023). However, the effect of diazepam on tissue organs is relatively scarce compared to its well-documented pharmacological effects. Most studies on benzodiazepine-induced toxicity are based on case reports, case series, or retrospective analyses, which often lack the rigor of controlled experimental or clinical trials (Ogah et al., 2024).

Moreover, benzodiazepines have been implicated in altering thyroid peroxidase activity, which is essential for thyroid hormone synthesis (Khadem-Ansari et al., 2014). Some evidence also suggests that diazepam may affect nuclear thyroid hormone receptor density in the brain, indicating potential CNS interactions with thyroid function (Constantinou et al., 2005). Furthermore, benzodiazepines may influence stress-related hormonal changes that indirectly affect thyroid function, given that chronic stress and anxiety disorders have been linked to HPT axis dysregulation (Khadem-Ansari et al., 2014).

Despite these findings, there is a significant gap in recent literature exploring diazepam's direct effects on thyroid function, particularly in human populations. Most available studies are dated and lack contemporary validation, underscoring the need for further investigation into the potential endocrine-disrupting effects of chronic benzodiazepine use. Given diazepam's widespread prescription as an anxiolytic and adjunctive treatment for depression, its potential role in thyroid hormone regulation warrants deeper exploration.

The primary research problem driving this study is the insufficient understanding of the long-term effect of diazepam on thyroid function. Addressing this gap is crucial, considering the integral role of thyroid hormones in maintaining metabolic balance, mood stability, and overall physiological well-being (Baldwin et al., 2014). This study aimed to explore the potential effects of diazepam on thyroid function hormones (T3, T4, and TSH), lipid profile, glucose, cortisol levels and thyroid gland histology in animal models would provide valuable insights into the broader endocrine implications of benzodiazepine use.

2. Materials and Methods

2.1. Procurement and care of the animals

Twenty-four (24) Wistar rats (12 male and 12 female rats) weighing 160g±20 were obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State. Animals were kept in the Animal Care Unit of Bingham University, Nasarawa state, Nigeria. All experimental investigations, animals handling and treatment, conform to the guidelines of the National Institute of Health (NH publication 85-23, 1985). The Animal Ethics Committee of Bingham University, Karu, Nasarawa State, approved the study protocol.

2.2. Chemical/Drug of choice

Diazepam (Swiss Pharma Nigeria Ltd/5mg tablet) was purchased from Swiss Pharma Nigeria Ltd, Lagos, Nigeria.

2.3. Experimental Design

This study used twenty-four pre-pubertal Wistar rat strains that are four weeks old, weighing 160g±20 (6 rats per group). The animals were allowed to acclimatize for two weeks and were fed with vital feed chow and water ad libitum. The Wistar rats were then divided randomly into four groups of five mice, each as follows:

a) GROUP 1 (Diazepam Low Dose): Rats were administered 15 mg/kg of Diazepam daily with a vehicle solution for seven (7) days via oral gavage.

b) GROUP 2 (Diazepam High Dose): Rats were administered 60mg/kg of Diazepam daily with a vehicle solution for seven (7) days via oral gavage.
c) GROUP 3 (Diazepam Intermediate Dose): Rats were orally administered Diazepam at 30 mg/kg daily for seven (7) days with vehicle solution via oral gavage.
d) GROUP 4 (Negative Control): Wistar rats were provided standard feed and water for seven (7) days. All administration began and lasted for 7 days (1 week).

General health assessments were conducted on the rats (body weight, food intake, water consumption, behavioural habits, that is agility and motility, fur condition, etc.) and were noted.

2.4. Experimental Measurements

a) Body weight: The body weight of rats was taken from the beginning of the experiment to study the effect of Diazepam treatment on body weight and at the end. The rats were monitored for drug effects and thyroid function alterations using other physical markers that is Coat or Skin changes, behavioural changes, energy levels, and swelling or enlargement of the thyroid gland (goitre).
b) Thyroidal histology: Tissue samples of the thyroid gland were obtained to analyse the cytoarchitectural effect of Diazepam in the thyroid gland.
c) Thyroid function evaluation: Blood samples were obtained for thyroid function assessment post-diazepam administration.

2.5. Ethical Approval

Appropriate ethical approval was obtained in writing from the Bingham University's ethical committee for the use of experimental animals.

2.6. Animal Sacrifice

The animals were restrained in a normal standing position on a firm, flat surface, and the tail's base was grasped firmly with one hand. Performing the procedure on a surface that the animal can grip (such as the wire bar grid of the cage top) may make it easier to gain access to the base of the skull because rodents often stretch themselves forward when held by the tail. A sturdy stick-type pen, a rod-shaped piece of metal, a closed scissor/hemostat or the thumb and first finger of the other hand were placed against the back of the neck at the base of the skull. To produce the dislocation, there was a quick push forward and down with the hand or object restraining the head while pulling backwards with the hand holding the tail base. The feeling of separation of cervical tissues can verify the effectiveness of dislocation. When the spinal cord is severed, a 2-4 mm space will be palpable between the occipital condyles and the first cervical vertebra. Occasionally, however, the dislocation occurs between thoracic vertebrae. Respiratory arrest was confirmed, and verification by palpation of the absence of a heartbeat was confirmed.

3. Laboratory Analyses

Tissues were collected for histological analysis, and biochemical analysis; serum levels of triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose, and cortisol were measured.

3.1. Biochemical Analysis

Blood samples were collected in a plain bottle through *venepuncture*; they were centrifuged at 3000 rpm for 10 minutes at room temperature using a centrifuge machine. The

serum obtained was used to determine the levels of TSH and T4 using an enzyme-linked immunosorbent assay (ELISA) kit. A lipid profile analysis was also carried out on serum samples.

3.2. Histological Analysis

This is the study of the microscopic anatomy of cells and tissues of organisms. Histological analysis was performed by examining the thin section of the thyroid gland under the light (optical) microscope. All Animals were sacrificed on the 29th day by cervical dislocation, and the thyroid gland was dissected and weighed using chemical balance.

3.2.1. Grossing

The tissues were observed and cut into small pieces not more than 4mm thick into pre-labelled cassettes. These were further immersed in 10% formal saline for 24 hours to fix.

3.2.2. Tissue Processing

This is done automatically using an automatic tissue processor (Leica TP 1020). The tissues were allowed to pass through various reagents, including stations 1 and 2 containing 10% formal saline, station 3 to station 7, and alcohol (70%, 80%, 90%, 95%, absolute 1, and absolute two) for dehydration. The tissues continued to pass through station 8 and station 9, containing two changes of xylene for clearing, and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 hours; tissues stayed in each station for 1 hour as described (Gamde & Obeagu, 2023).

3.3. Statistical Analysis

The data obtained was then analysed using One Way Analysis of Variance (ANOVA), a statistical package SPSS version 25 was used, and the results obtained were presented as Mean ± Standard error of the mean and a post hoc test of Turkey was used to determine the significance level. P values ($p<0.05$) were also considered statistically significant.

4. Results and Discussion

4.1. Thyroid function hormones

Table 1 presents the effect of diazepam on thyroid function hormones, specifically triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) in four groups: low-dose, high-dose, intermediate-dose, and control. For T3, the mean values for all groups are quite similar, ranging from 0.765 to 0.815 mg/dL. The overall mean T3 level across groups is 0.79 mg/dL, with a p-value of 0.9705, indicating no statistically significant difference in T3 levels between the groups. This suggests that diazepam, regardless of dosage, had little to no effect on T3 levels in this study.

For T4, the mean values range from 4.535 mg/dL in the low-dose group to 5.12 mg/dL in the intermediate-dose group. The control group had a mean T4 level of 4.752 mg/dL. The overall mean T4 level was 4.78 mg/dL, with a standard deviation of 0.25. The p-value of 0.4818 indicates that there was no statistically significant difference in T4 levels between the groups, suggesting that diazepam did not significantly alter T4 levels across the different doses. Similarly, TSH levels showed some variability, ranging from 0.18 mg/dL in the control group to 0.365 mg/dL in the intermediate-dose group, but the p-value of 0.144 shows no significant impact of diazepam on TSH levels either. This overall lack of significance implies that diazepam administration does not appear to affect thyroid function hormones in a meaningful way within the tested doses (Figure 1).

Table 1. Effect of Diazepam on Thyroid Hormones

	Group 1	Group 2	Group 3	Control Group	Mean ± S.D.	*p*-value
T3 (mg/dL)	0.8	0.765	0.78	0.815	0.79 ± 0.002	0.9705
T4 (mg/dL)	4.535	4.7	5.12	4.752	4.78 ± 0.25	0.4818
TSH (mg/dL)	0.2	0.31	0.365	0.18	0.26 ± 0.09	0.144

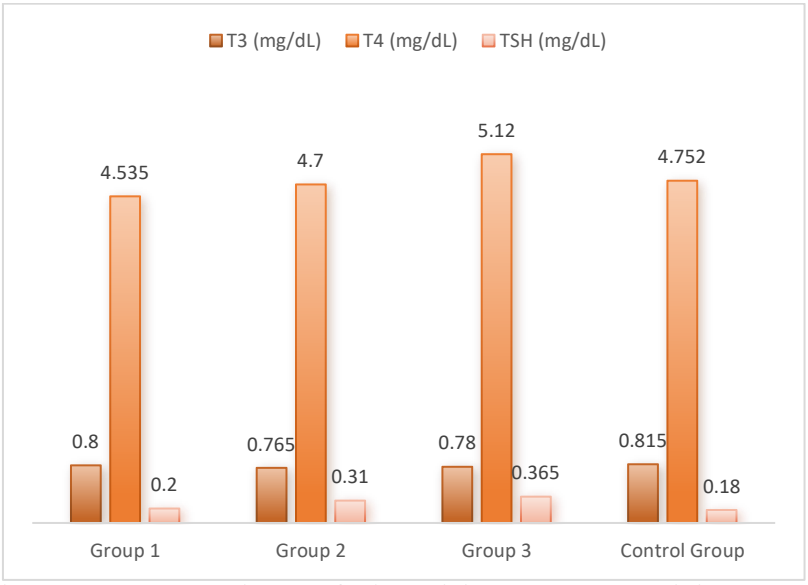


Figure 1. Comparison of Thyroid Hormones with control

4.2. Effect of Diazepam on Lipid Profile

Table 2 presents the effect of diazepam on lipid profile, glucose, and cortisol levels across four groups: low-dose, high-dose, intermediate-dose, and control. For cholesterol, the mean values across the groups range from 76.5 mg/dL in the high-dose and control groups to 84 mg/dL in the intermediate-dose group. The overall mean cholesterol level is 79.13 mg/dL, with a standard deviation of 3.54, and a p-value of 0.887. This high p-value suggests that there is no statistically significant difference in cholesterol levels between the different diazepam doses and the control, indicating that diazepam does not affect cholesterol levels. For triglycerides, there is greater variability, with levels ranging from 53.5 mg/dL in the control group to 234.5 mg/dL in the low-dose group.

The overall mean is 144 mg/dL, with a high standard deviation of 74.11, but the p-value of 0.161 shows no statistically significant differences between the groups. Similarly, for high-density lipoprotein (HDL), low-density lipoprotein (LDL), and glucose, the p-values (0.589, 0.265, and 0.333, respectively) indicate no significant differences across the groups, meaning that diazepam had no considerable effect on these parameters. The mean HDL and LDL levels across all groups were 62.63 mg/dL and 20.38 mg/dL, respectively, while the mean glucose level was 5.71 mmol/L (Figure 2).

Table 2. Effect of Diazepam on Lipid Profile

	Group 1	Group 2	Group 3	Control Group	Mean ± S.D.	*p*-value
Cholesterol (mg/dL)	79.5	76.5	84	76.5	79.13 ± 3.54	0.887
Triglycerides(mg/dL)	234.5	137	151	53.5	144 ± 74.11	0.161
HDL (mg/dL)	57.5	63.5	68	61.5	62.63 ± 4.367	0.589
LDL (mg/dL)	21.5	22.5	17.5	20	20.38 ± 2.17	0.265

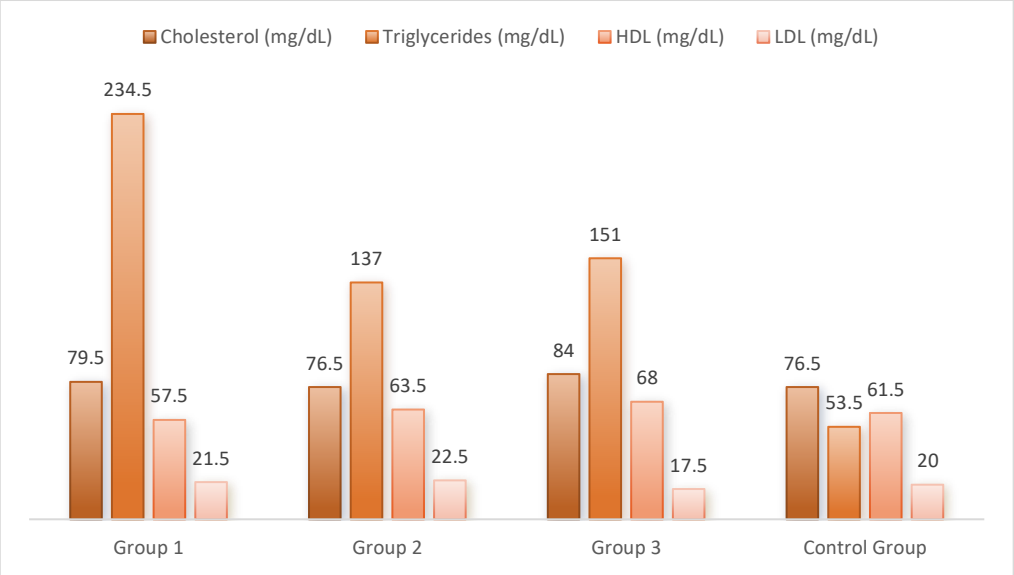


Figure 2. Comparison of Lipid Profile with control

4.3. Effect of Diazepam on Glucose and Cortisol Level

Table 3 presents effect of Diazepam on Glucose and Cortisol Level. Cortisol levels varied from 114.5 nm/dL in the intermediate-dose group to 139 nm/dL in the high-dose group, with an overall mean of 129.13 nm/dL. Despite this variation, the p-value of 0.190 suggests no statistically significant difference in cortisol levels across the groups. These findings imply that diazepam administration does not significantly impact lipid profiles, glucose levels, or cortisol levels across the tested doses.

Table 3. Effect of Diazepam on Glucose and Cortisol Level

	Group 1	Group 2	Group 3	Control Group	Mean ± S.D.	*p*-value
Glucose (mmol/L)	5.55	6.1	6.45	4.75	5.71 ± 0.74	0.333
Cortisol (nm/dL)	131	139	114.5	132	129.13 ± 10.38	0.190

4.4. Histological Effect

4.4.1. Group 1. Low Dose (15mg/kg body weight)

Diazepam was administered at a dosage of 15mg/kg body weight for 7 days via oral gavage. The thyroid cytoarchitecture remains intact, with no observable distortion or pathological alterations. Thyroid follicles appear normal, with well-defined follicular cells and colloid. The findings suggest no significant morphological impact of diazepam on thyroid tissue under these experimental conditions, as displayed in the images below. Plate 1: Thyroid section of Wistar rat administered 15mg/kg diazepam showed normal thyroid follicles containing homogenous colloid and surrounded by cuboidal follicular cells. (H&E. X Mag. 400).

4.4.2. Group 2. High Dose (60mg/kg body weight)

Histological examination of thyroid tissue from animals treated with 60 mg/kg diazepam shows clear signs of follicular degeneration (black arrow), where the normal structure of the follicles has broken down. Surrounding these degenerated follicles, the cuboidal cells appear distorted (red arrow), indicating some level of cellular disorganization. This suggests that high doses of diazepam may have a significant effect on the thyroid's structure. The tissue was stained using Hematoxylin and Eosin (H&E), and the image was captured at 400x magnification. Plate 2: Thyroid section of Wistar rat administered 60mg/kg

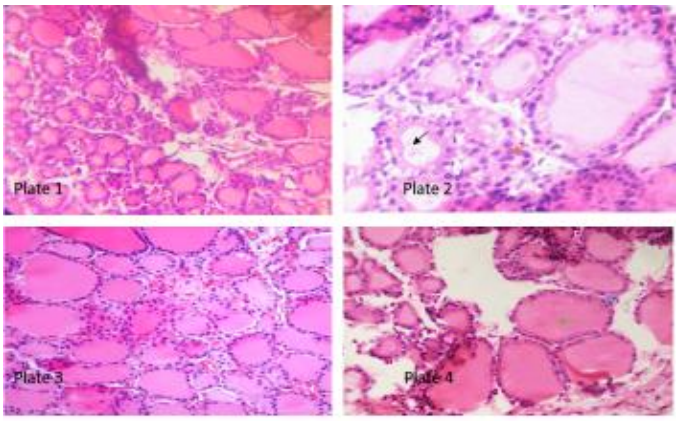
diazepam showed follicular degeneration (black arrow) with surrounding distorted cuboidal follicular cells (red arrow). (H&E. X Mag. 400).

4.4.3. Group 3. Intermediate Dose (30mg/kg Body Weight)

Histological analysis of thyroid tissue following administration of diazepam. Diazepam was administered at a dosage of 30mg/kg body weight for 7 days via oral gavage. The thyroid cytoarchitecture remains intact, with no observable distortion or pathological alterations. Thyroid follicles appear normal, with well-defined follicular cells and colloid. The findings suggest no significant morphological impact of diazepam on thyroid tissue under these experimental conditions, as displayed in the images below. Plate 3: Thyroid section of Wistar administered 30mg/kg diazepam showed normal thyroid follicles containing homogenous colloid and surrounded by normal cuboidal follicular cells. (H&E. X Mag. 400).

4.4.4. Group 4. Control Group

Histological analysis of thyroid tissue from the control group, which received only standard feed and water, with no drug treatment. The cytoarchitecture of the thyroid gland is well preserved, with normal thyroid follicles containing homogenous colloid and surrounded by cuboidal follicular cells. No signs of cellular atypia, hyperplasia, or structural alterations are observed. This image serves as the baseline for comparison with the diazepam-treated group, demonstrating the normal histological characteristics of untreated thyroid tissue. Plate 4: Thyroid section of normal control showed a well-preserved thyroid gland with normal thyroid follicles (green arrow) containing homogenous colloid surrounded by cuboidal follicular cells (blue arrow). No signs of cellular atypia, hyperplasia, or structural alterations are observed (H&E. X Mag. 400).



Thyroid section plates 1&3 showed normal thyroid follicles containing homogenous colloid and surrounded by cuboidal follicular cells while section plate 2 showed follicular degeneration as compared to normal control plate 4. (H&E. X Mag. 400).

Figure 3. Histological Analysis of Thyroid Tissue in the Control Group

4.5. Discussion

The effect of diazepam, a commonly used benzodiazepine, on various physiological and biochemical parameters are well-documented (Ogah et al., 2024; Soyka, 2017), but its influence on endocrine and metabolic markers remains a subject of ongoing research. This study aimed to explore the potential effects of diazepam on thyroid function hormones (T3, T4, and TSH), lipid profile, glucose, cortisol levels and thyroid gland histology. The present study showed that oral administration of diazepam at 60 mg/kg does not significantly affect thyroid function hormones (T3, T4, and TSH). The observed insignificant differences among experimental groups could be attributed to factors such as developmental stages of the

animals, dosage effect, and environmental conditions. The absence of statistically significant changes in T3 (p = 0.9705) and T4 (p = 0.4818) levels across the groups is in line with findings from previous studies (Brunton et al., 2018; Mazzaferri & Skillman, 1969; Sharma et al., 2019). However, previous studies by Boyadjieva & Ovtcharov (1987) and Humbert (1994) have reported that long-term benzodiazepines are associated with stress-related endocrine changes where the effects on the hormone is dose-dependent. Thyroid hormones are largely regulated by the hypothalamic-pituitary-thyroid axis and diazepam's sedative effects on the central nervous system may not directly interfere with this axis. Hence, further study on molecular level is needed to elucidate the relationship between diazepam and thyroid function.

Similarly, in the present, diazepam administration does not significantly affect the lipid profile, glucose, or cortisol levels. Cholesterol levels, although slightly elevated in the high-dose group, do not differ statistically significant across groups (p = 0.887). The present study is inconsistent with previous studies like Copland & Balfour (1987), which reported that diazepam administration in rats caused a decrease in total serum lipids and cholesterol, with a lower dose increasing HDL2 lipoprotein fraction. However, studies like Copland & Balfour (1987) and Tremblay et al. (2003) aligns with our study, showing that Diazepam administration, whether at low or high doses does not significantly influence the lipid profile, glucose, or cortisol levels in the studied rat groups.

Similarly, no significant effects were observed in glucose (p = 0.333) and cortisol levels (p = 0.190), aligning with research indicating that diazepam, despite its central nervous system activity, does not substantially alter glucose metabolism or stress hormone levels under short-term treatment (Tremblay et al., 2003). The slightly elevated glucose in the treated groups could be a stress response to diazepam administration, but it did not reach statistical significance, suggesting that diazepam does not cause clinically relevant metabolic disturbances in the parameters measured.

Despite the valuable insights provided by this study, certain limitations should be considered. First, the sample size may limit the generalizability of the findings, and a larger cohort could provide more robust statistical power. Second, while efforts were made to minimize stress in the animals, the process of handling and drug administration could have introduced stress-related hormonal changes, potentially affecting the endocrine markers measured. Third, the duration of diazepam exposure was relatively short, and chronic administration studies may reveal different effects, particularly in long-term hormonal regulation and histological changes in the thyroid gland. Overall, the results support the conclusion that diazepam has a limited effect on these metabolic markers in this experimental model.

5. Conclusion

The findings from this study indicate that diazepam, administered 60 mg/kg does not significantly affect thyroid function hormones (T3, T4, and TSH) or key metabolic markers such as cholesterol, triglycerides, HDL, LDL, glucose, and cortisol in rats. Despite some minor variations in hormone and metabolic levels across groups, none of the differences were statistically significant. Overall, these results suggest that acute or short-term diazepam administration does not induce significant alterations in the endocrine and metabolic parameters assessed in this study. While there may be subtle effects, particularly with long-term or chronic use, the current findings imply that under the given experimental conditions, diazepam does not provoke meaningful disturbances in thyroid function, lipid metabolism, or stress-related hormonal responses.

Future research should extend the duration of diazepam administration to assess its long-term effects on thyroid function and metabolism. Intending researchers should consider additional biomarkers to gain a more comprehensive understanding of its endocrine effects. Additionally, exploring the molecular mechanisms underlying these effects and correlating biochemical changes with behavioural outcomes will provide a more comprehensive understanding of diazepam's impact on thyroid function.

5.1. Ethics approval and consent to participate
Not applicable

5.2. Consent for publication
Not applicable

5.3. Availability of data and material
Data are available from the corresponding author upon request

5.4. Competing interests
The authors declare no conflict of interest

5.5. Funding
The study did not receive any additional funding.

5.6. Author's contributions
OSM and SMG conceptualized, performed, and write the original draft, edited the manuscript as well as performed the critical literature search and laboratory analysis with BW and HK. All authors read and approved the final manuscript.

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