BIOKEMISTRI 35(1): 34617 - 34631 (Dec 2023) ***An international journal published by the***

Society for Experimental Biology of Nigeria

Printed in Nigeria

THERAPEUTIC EFFECT OF QUERCETIN ON THE HYPOTHALAMUS OF STZ-NICOTINAMIDE INDUCED DIABETIC RATS

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MS/No BKM/2022/012

ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia and disturbances in sugar, fat, and protein metabolism. The aim of this study was to investigate the therapeutic effect of quercetin on the hypothalamus in adult male Wistar rats with STZ-nicotinamide-induced diabetes. Forty rats were divided into eight groups, each receiving different treatments. The control group received a standard diet, while other groups were administered various doses of quercetin alone, diabetes induction agents alone, or a combination of quercetin and diabetes induction agents. Treatments were administered orally for eight weeks. Results revealed significant differences in body weight among the groups, with diabetic rats showing a decrease compared to the control group. However, rats treated with quercetin or metformin exhibited a significant increase in body weight compared to the diabetic control group. Additionally, diabetes induction significantly decreased plasma insulin, plasma testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone levels, while quercetin treatment led to significant increases in these hormone levels. Histological examination of the hypothalamus showed notable findings. Control group rats displayed normal histomorphological features, while diabetic control rats exhibited swollen and distorted hypothalamic cells with cellular apoptosis. Interestingly, rats treated with quercetin showed a reduction in apoptotic cells, indicating a protective effect on hypothalamic cells. In conclusion, our findings suggest that quercetin possesses potential therapeutic properties in ameliorating the detrimental effects of STZ-nicotinamide-induced diabetes on the hypothalamus in adult male Wistar rats.

KEY WORDS: Quercetin, hypothalamic dysfunction, diabetes, STZ-Nicotinamide

INTRODUCTION

Diabetes, one of the most prevalent and serious metabolic disorders globally, ranks among the top five leading causes of death. It is characterized by persistent hyperglycemia and disturbances in the metabolism of carbohydrates, proteins, and lipids (Ramesh and Pugalendi, 2006; Go *et al.,* 2015). This condition arises from decreased insulin secretion or insensitivity to insulin, coupled with heightened cellular resistance, often accompanied by oxidative stress induction. (Machha *et al.,* 2007) and alterations in glucose and lipid metabolism-regulating enzymes (Ramesh and Pugalendi, 2006). In this manner, controlling of blood glucose level is fundamental for forestalling diabetic entanglements, improving the wellbeing of patients with diabetes (Ceriello, 2005). However, currently available antidiabetic medications are associated with various limitations, including undesirable side effects such as hypoglycemia and increased rates of cell death, as well as high rates of secondary failure.

As of late, accentuation has been set on corresponding, elective medicines for diabetes concentrated on useful nourishments and their bioactive mixes (Tahrani *et al.*, 2010).

Streptozotocin (STZ) is an antibiotic produced by *Streptomyces achromogenes*. It has been widely used for inducing experimental diabetes mellitus in animals, it stimulates the naturally occurring metabolic disorder DM by causing degeneration of pancreatic β cells. The selective β 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 (Elsner *et al.*, 2000).

For many reasons in recent years the popularity of alternative medicine has increased, including herbal products with anti-diabetic activity (Ahmad Khan and Ahmad, 2019). Flavonoids occur commonly, and are widespread, in the plant kingdom. Querectin is one of the most widely distributed flavonoids, present in foods, including vegetables, especially

onions, fruits, tea, and many other dietary sources. Flavonoids, including quercetin, are often known for their antioxidant properties, which contribute to their potential health benefits. An increased consumption of antioxidants in the diet of individuals is strongly recommended (Abd El-Baky, 2011).

Quercetin, a naturally occurring phenolic compound is found abundantly in plants and various natural food sources and has been reported to have numerous beneficial effects including anti-inflammatory, antioxidant, antihypertensive, anticancer, antiviral, neuroprotective, hepatoptotective, anti-diabetic, and calming activities (Yang and Kang, 2018).

MATERIALS AND METHODS

Animals

Forty male Wistar rats with an average weight of 200g were used in this study. They were housed in the animal house of the faculty of basic medical science, college of health sciences, university of Ilorin, at room temperature and maintained under a 12 hours' light 12 hours' dark cycle. They were randomly grouped into 8 groups consisting of five animals per group. The animals were housed in a wire gauzed cage with double cross ventilation. They were fed daily with pelletized grower feed from Ogo-Oluwa livestock and aqua feed enterprises, Kwara state, Ilorin, containing 15% crude protein, 7% fat, 10% crude fibre, 1% calcium, 0.355 phosphorus, 2.55kcal/kg of metabolized energy as indicated in the feed packing. Clean drinking water was provided and proper environmental hygiene was ensured. Streptozotocin was obtained from sigma-aldrich and was stored at temperature -4 degree Celsius.

Preparation of Streptozocin

Immediately before administration, STZ was dissolved in freshly prepared iced-cold 0.1M citrate buffer with pH of 4.5 and was covered with foil paper (Deeds *et al.*, 2011)

Preparation of 0.1M citrate buffer

To prepare 0.1M citrate buffer, approximately 50ml of distilled water was added to a 100ml volumetric flask placed on a magnetic stirrer. Next, 1.4705g of sodium citrate was added and dissolved using the magnetic stirrer. Citric acid was added gradually to adjust the pH of the solution to 4.5.

Preparation of Nicotinamide

Nicotinamide that was gotten from sigma-aldrich was dissolved in normal saline

Groupings and Doses of drugs administered to Rats of Control and Experimental Group.

In this study, forty male Wistar rats, weighing approximately 200g each on average, were utilized. The rats were divided into eight groups, with five rats per group. One (A)group served as the control, receiving standard treatment. The Diabetes-only (B)group received a single dose of 230mg Nicotinamide and 65mg STZ. The Quercetin-only (C) group received a daily dose of 20mg/kg Quercetin, while the Quercetin-only (D) group received a daily dose of 50mg/kg Quercetin. The Quercetin + diabetes (E) group received the same diabetes induction treatment as the Diabetes-only group along with a daily dose of 20mg/kg Quercetin. Similarly, the Quercetin + diabetes (F) group received the diabetes induction treatment along with a daily dose of 50mg/kg Quercetin. The Methformin + diabetes (G) group received the diabetes induction treatment along with a daily dose of 20mg/kg Methformin, while the Methformin + diabetes (H) group received the same treatment along with a daily dose of 20mg/kg Methformin. The respective doses were administered orally to the rats. The experiment spanned a duration of 8 weeks.

Sacrifice Of Animals, Collection of Blood and Tissue Sample

The blood glucose level of the diabetic rats both treated (Group E, F and H) and untreated (Group B) was taken using a glucometer to determine their blood glucose level. The animals were sacrificed using the cervical dislocation (euthanasia method), which is the act of inducing human pain in an animal by a method that induce rapid loss of consciousness and death within of pain, discomfort or distress. It is done by applying pressure on the neck and dislocating the spinal cord from the brain. The aim is quickly separate the spinal cord from the

brain so as to provide the animal with a fast and painless death.

Histological Study

The hypothalamic tissues were fixed in 10% formalin. They were dehydrated with varying percentage of ethanol. Sections were cleared with xylene and embedded in molten wax. The sections were cut, stained with haematoxylin and eosin and microscopically analyzed.

Table 1: Groupings and Doses of drugs administered to Rats of Control and Experimental Group.

Group Of Rats	Doses of Drugs Administered	Days of Exposure
Control (A)	1ml of distilled water	57
Diabetes only (B)	230mg of NC and 65mg of STZ	1
Quercetin only (C)	20mg/kg/day	57
Quercetin only (D)	50mg/kg/day	57
Quecertin + diabetes (E)	20mg/kg/day	57
Quecerin + diabetes(F)	50mg/kg/day	57
Metformin only (G)	50mg/kg/day	57
Metformin + diabetes(H)	50mg/kg/day	57

RESULTS

Photomicrograph of the hypothalamus of Rats in Control group A

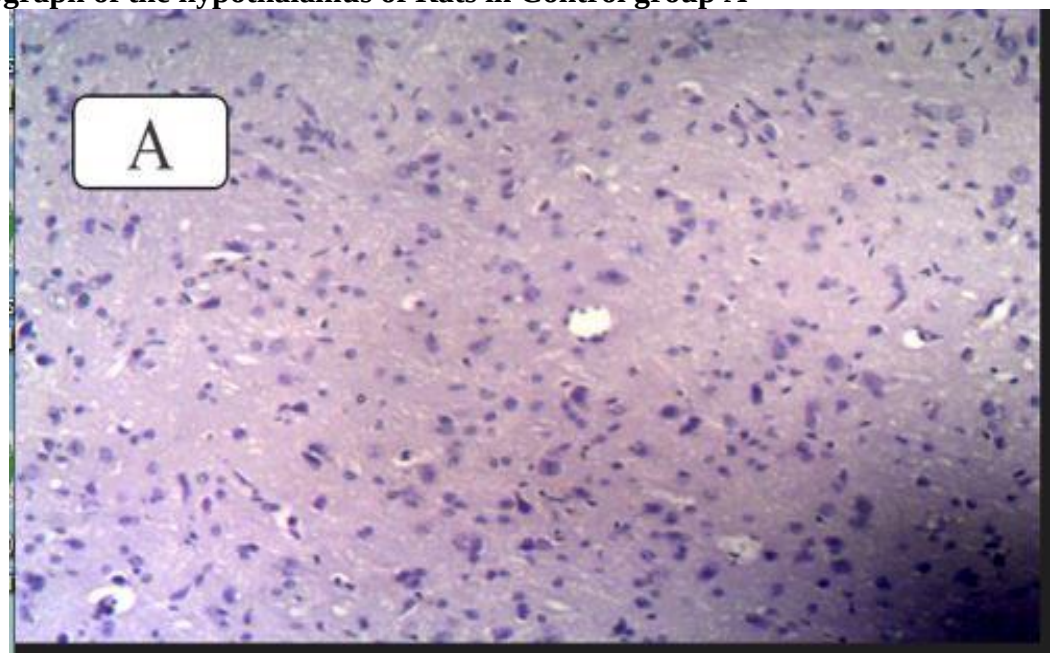


Plate A: - Photomicrograph of plate A (Control) administered with distilled water show a general histomorphological presentation, cellular delineation, histoachitectural assortment and no feature of significant inflammation or degenerative changes on the hypothalamus

Photomicrograph of the hypothalamus of Rats in group B

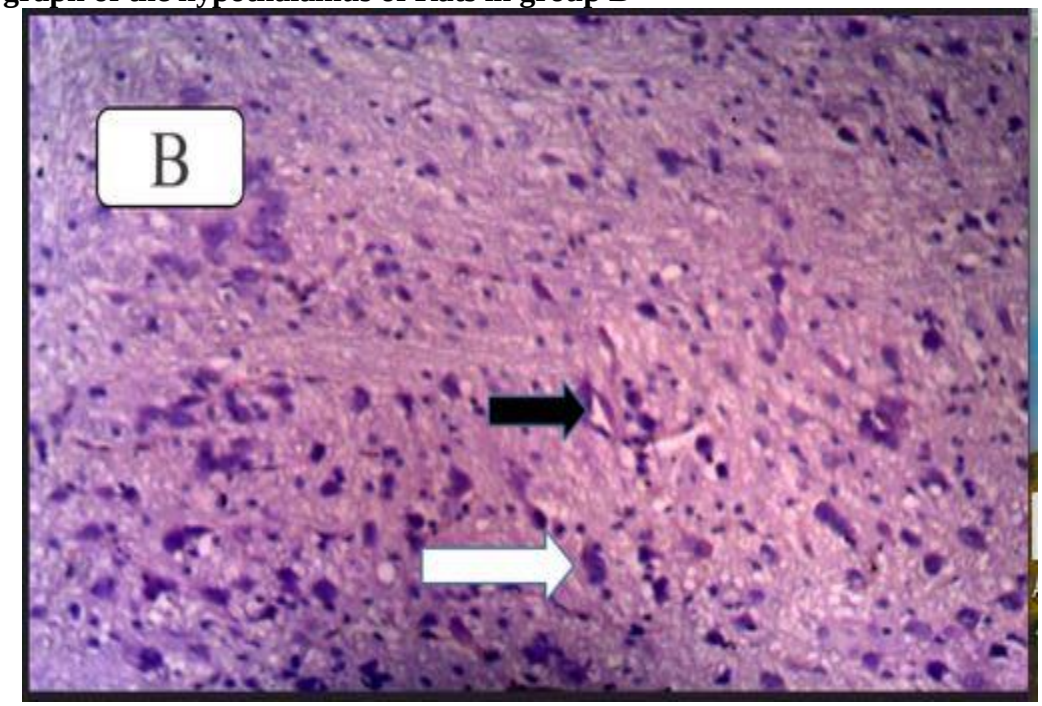


Plate B: Photomicrograph of plate B (Diabetic control) administered with a single dose of STZ-Nicotinamide shows swollen, distorted hypothalamic cells and cellular apoptosis. White long arrow indicates dark cells that are associated with cellular apoptosis caused by induced diabetes. Short black arrow shows free fatty acid associated with diabetes.

Photomicrograph of the hypothalamus of Rats in group C

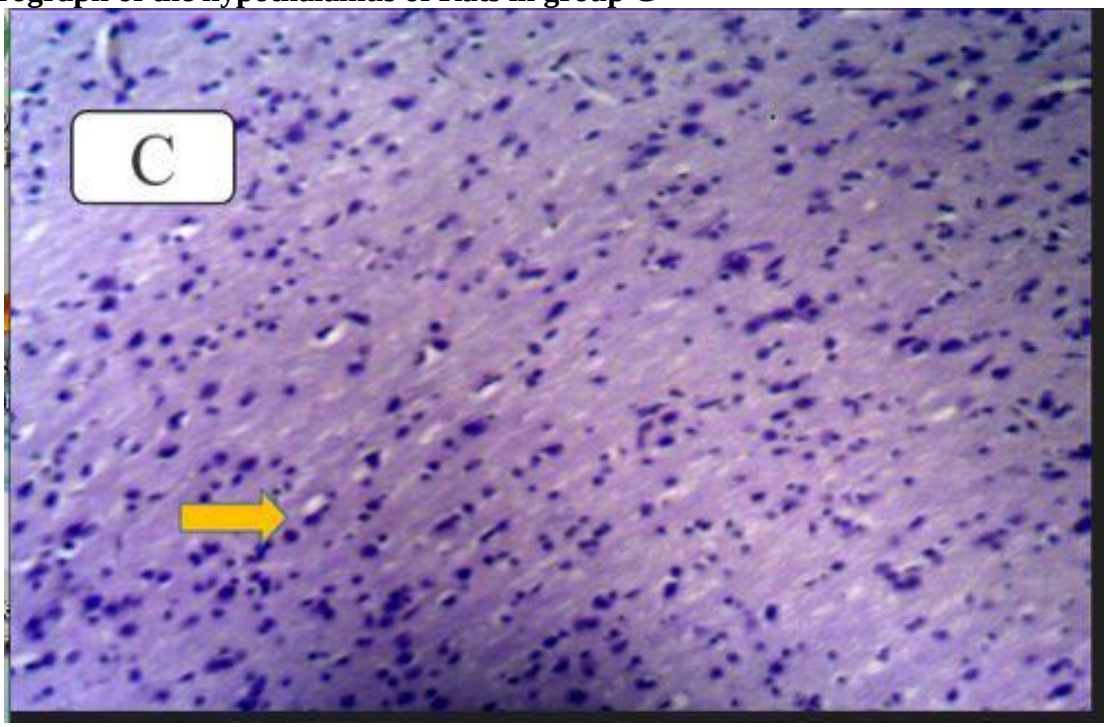


Plate C: Photomicrograph of plate C (Quercetin only low dose administered with 20mg/kg/day) shows cellular delineation, histoachitectural assortment and no feature of significant inflammation or degenerative changes on the hypothalamic cells. The yellow arrow indicates remnant of free fatty acids.

Photomicrograph of the hypothalamus of Rats in group D

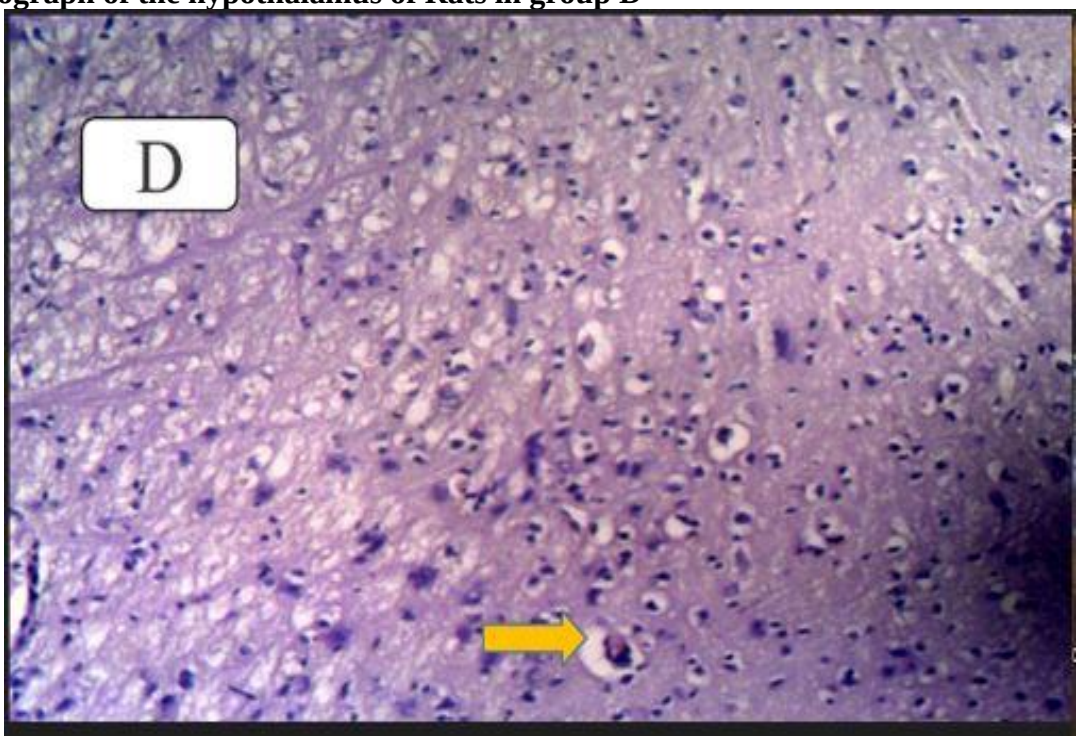


Plate D: Photomicrograph of plate D (Quercetin only high dose administered with 50mg/kg/day) shows the yellow arrow indicates remnant of free fatty acids.

Photomicrograph of the hypothalamus of Rats in group E

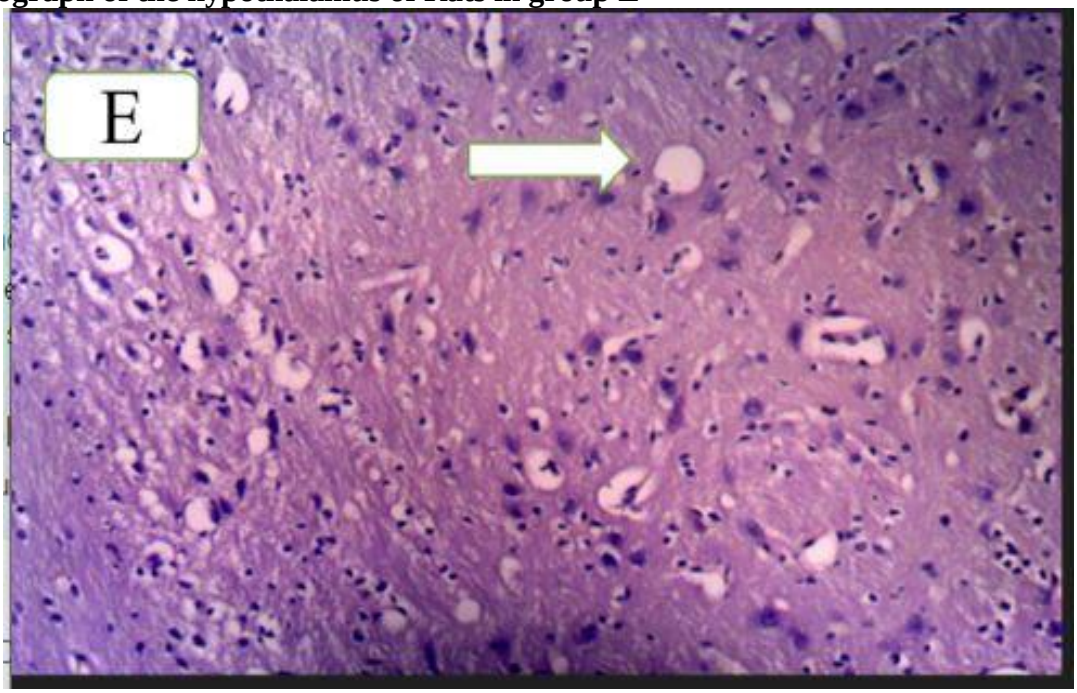


Plate E: Photomicrograph of group (E) Diabetic treated with Quercetin low dose (20mg/kg/day) shows a reduced number of apoptotic cells from the administration of Diabetes (STZ-Nicotinamide) which led to swollen, distorted hypothalamic cells and cellular apoptosis. The destructive effect of Diabetes (STZ-Nicotinamide) was likely to be reversed by Quercetin to a larger extent. White long arrow indicates dark cells that are associated with cellular apoptosis caused by induced diabetes.

Photomicrograph of the hypothalamus of Rats in group F

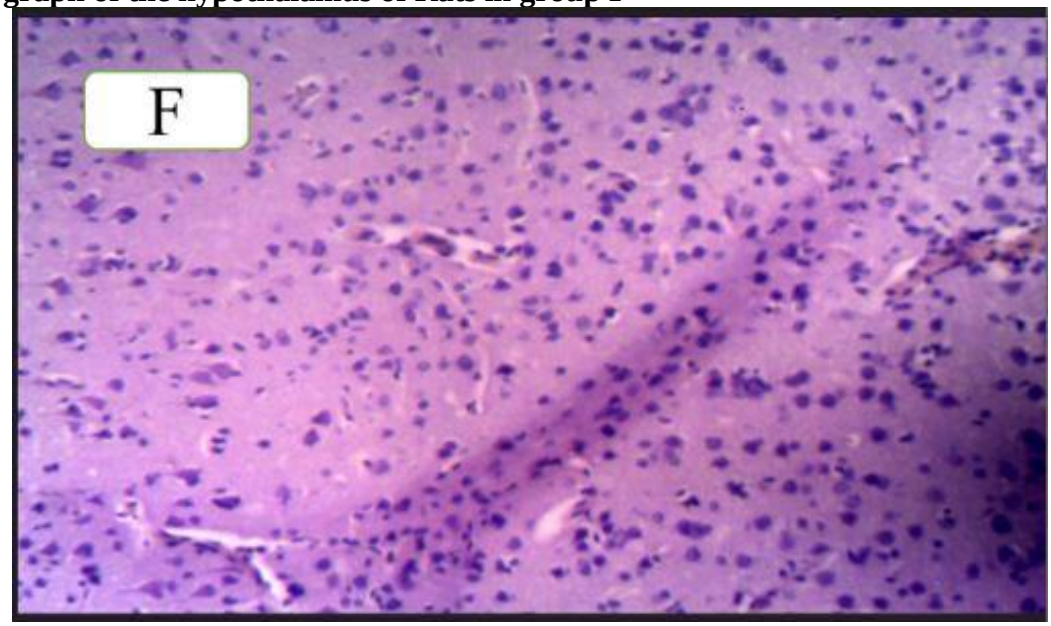


Plate F: Photomicrograph of group (F) Diabetic treated with Quercetin high dose (50mg/kg/day) shows a reduced number of apoptotic cells from the administration of Diabetes (STZ-Nicotinamide) which led to swollen, distorted hypothalamic cells and cellular apoptosis. The destructive effect of Diabetes (STZ-Nicotinamide) was likely to be reversed by Quercetin to a larger extent.

Photomicrograph of the hypothalamus of Rats in group G

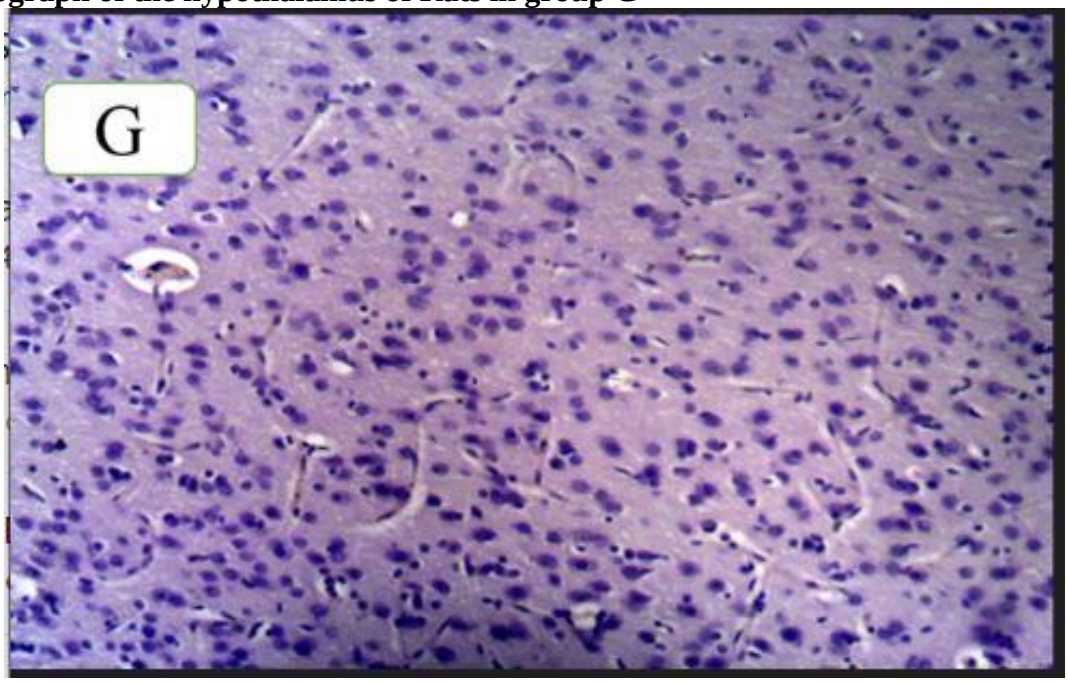


Plate G Photomicrograph of Metformin only treated group of 50 mg/kg (Group G) shows no feature of significant inflammation or degenerative changes on the hypothalamic cells

Photomicrograph of the hypothalamus of Rats in group H

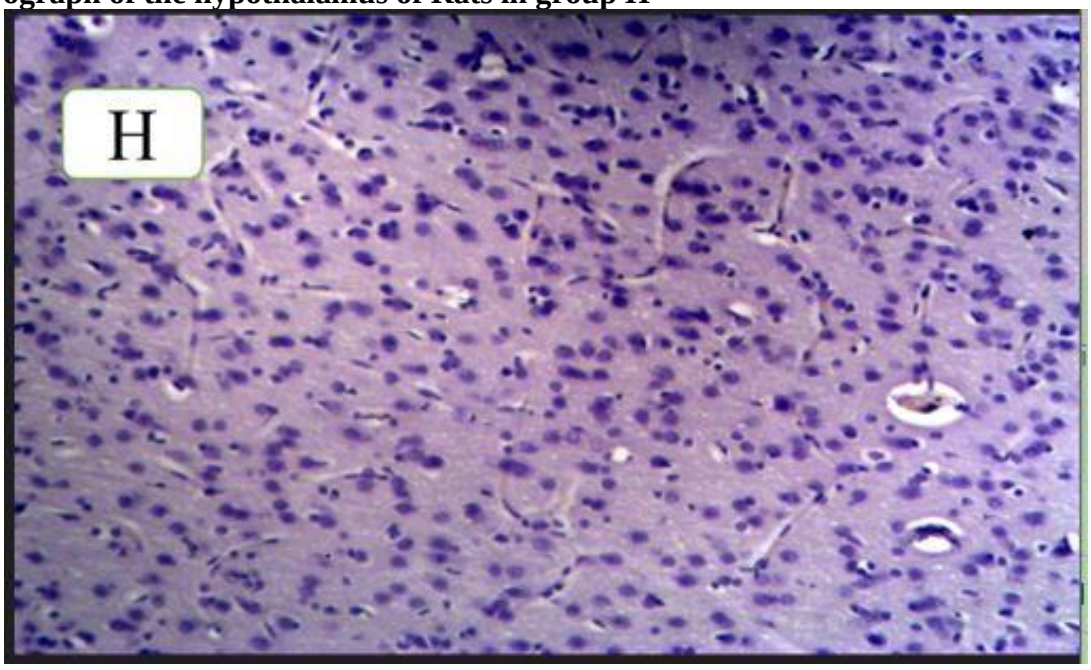
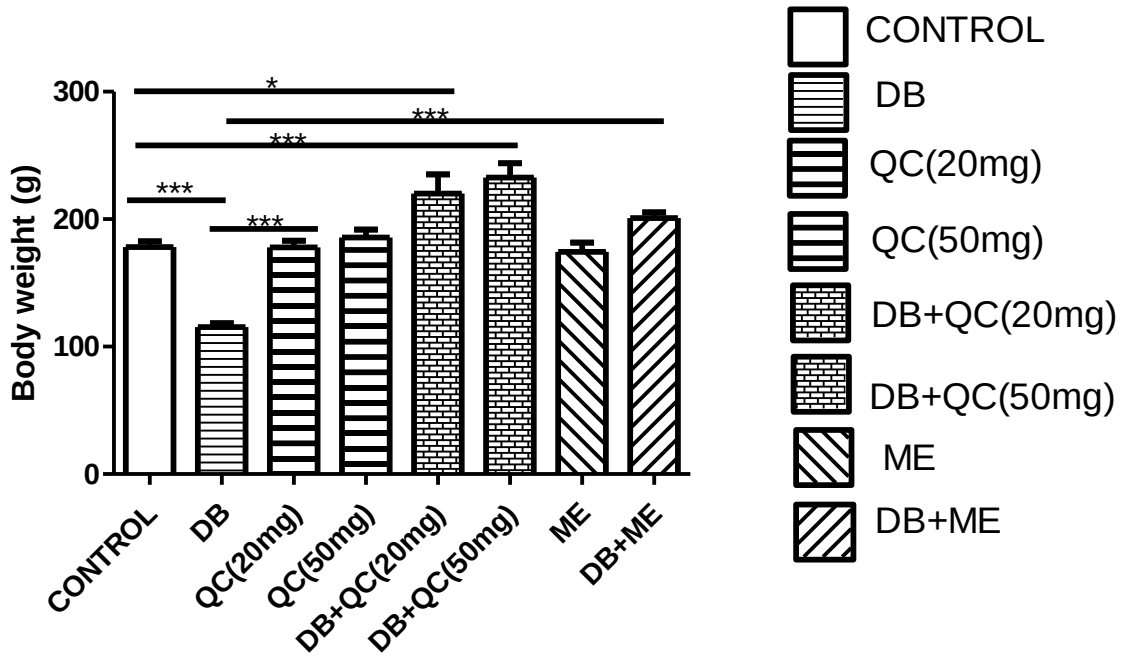


Plate H Photomicrograph of Diabetic (STZ) co-administered with Metformin of 50 mg/kg (Group H) shows slightly reduced number of apoptotic hypothalamic cells and small remnant of free fatty acids

Table 2: Effect of Administered Drug on Testosterone, Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Glutathione and MDA levels

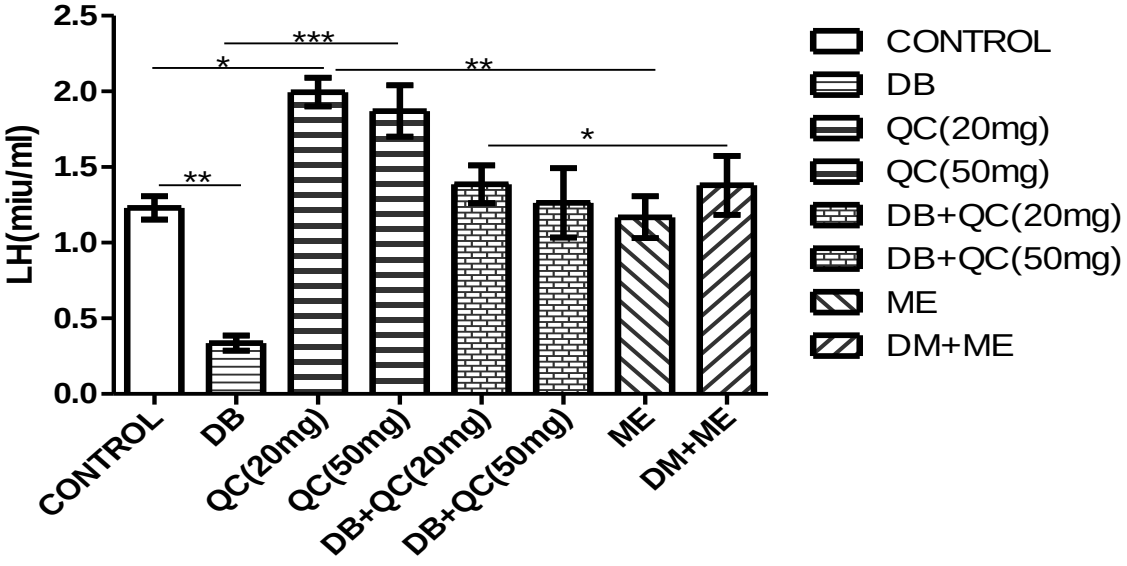
Parameters	Normal Control	Diabetic Control	Quercetin Low dose	Quercetin High dose	Diabetic+ Quercetin low dose	Diabetic + Quercetin high dose	Metformin only	Diabetic + Metformin
Testosterone	0.94±0.20	0.28±0.03	$1.23\pm0.10^{\#\#}$	$1.38\pm0.16^{\#\#\#}$	0.94±0.13	$1.08\pm0.17^{\#}$	0.96±0.14	$1.04\pm0.21^{\#}$
FSH	1.63±0.10	0.38±0.03***	$1.80\pm0.19^{\#\#\#}$	$1.85\pm0.14^{\#\#\#}$	$1.41\pm0.17^{\#\#\#}$	$1.23\pm0.07^{\#\#}$	$1.50\pm0.19^{\#\#\#}$	$1.45\pm0.13^{\#\#\#}$
LH	1.23±0.08	0.34±0.05**	$2.00\pm0.09^{*\#\#\#}$	$1.87\pm0.17^{\#\#\#}$	$1.39\pm0.13^{\#\#\#}$	$1.26\pm0.23^{\#\#\alpha}$	$1.17\pm0.14^{\#\#\alpha\alpha\beta}$	$1.38\pm0.20^{\#\#\#}$
Glutathione	1.59±0.77	0.35±0.02***	$2.39\pm0.24^{\#\#\#}$	$2.13\pm0.32^{\#\#\#}$	$1.18\pm0.10^{\#\#\#}$	$1.25\pm0.72^{\#\#}$	$1.5\pm0.14^{\#\#\#}$	$1.11\pm0.29^{\#\#\#}$
MDA	1.88±0.36	5.84±0.29***	$1.97\pm0.14^{\#\#\#}$	$2.011\pm0.20^{\#\#\#}$	$3.42\pm0.30^{*\#\#\#\alpha\beta}$	$2.99\pm0.25^{\#\#\#}$	$1.90\pm0.31^{\#\#\#\gamma}$	$3.30\pm0.40^{*\#\#\#\delta}$

Values are expressed as Mean ± SEM, n=5 (numbers of animals in each group). One-way analysis of variance (ANOVA) was used followed by Tukey's post hoc test. Statistical differences between control and different groups: *; P<0.05,**; P<0.01, ***; P<0.001. Statistical differences between Diabetic control and different groups: $^{\#}$; P<0.05,$^{\#\#}$; P<0.01, $^{\#\#\#}$; P<0.001. Statistical differences between Quecertin low dose and different groups: $^{\alpha}$; P<0.05,$^{\alpha\alpha}$; P<0.01, $^{\alpha\alpha\alpha}$; P<0.001. Statistical differences between Quecertin high dose and different groups: $^{\beta}$; P<0.05,$^{\beta\beta}$; P<0.01, $^{\beta\beta\beta}$; P<0.001. Statistical differences between Diabetic + Quecertin low dose and different groups: $^{\gamma}$; P<0.05,$^{\gamma\gamma}$; P<0.01, $^{\gamma\gamma\gamma}$; P<0.001. Statistical differences between Diabetic + Quecertin high dose and different groups: $^{\theta}$; P<0.05,$^{\theta\theta}$; P<0.01, $^{\theta\theta}$; P<0.001. Statistical differences between Metformin and different groups: $^{\delta}$; P<0.05, $^{\delta\delta}$; P<0.01, $^{\delta\delta\delta}$; P<0.001. P value<0.05 was considered statistically significant compared to the control group or other groups.



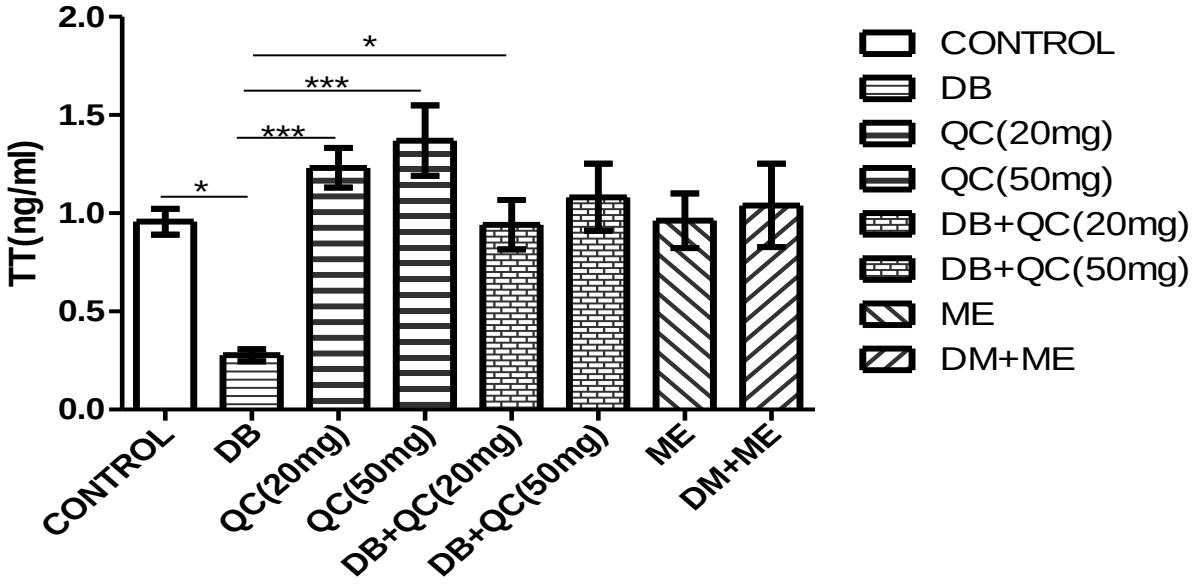
KEY: DB- Diabetes, QC- Quercetin, ME- Metformin

Figure 1: Showing comparison in Body weight among groups. Body Weight was significantly ($p < 0.05$) reduced in all rats induced with Diabetes prior to treatment compared with normal control group while body weight was significantly ($p < 0.05$) increased in groups treated with Quercetin compared to the normal control group.



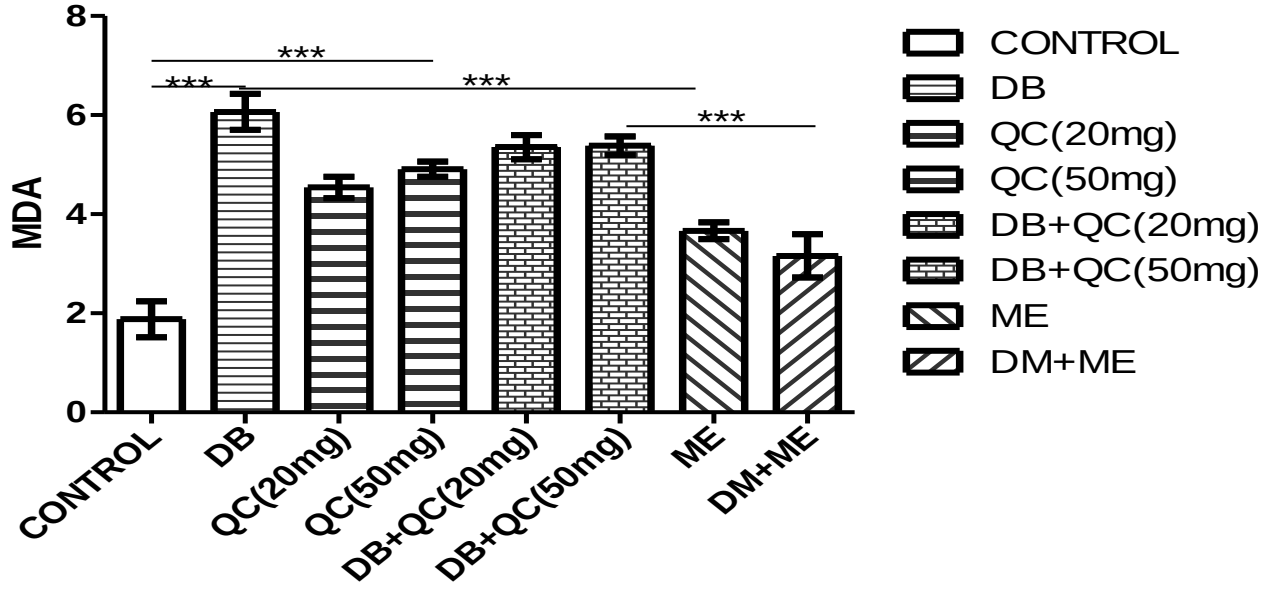
KEY: DB- Diabetes, QC- Quercetin, ME- Metformin,

Figure 2. Showing comparison in Luteinizing hormone (LH) level among groups**.** LH levels were significantly ($p < 0.05$) reduced in all rats induced with diabetes prior to treatment compared to the normal control group, while LH levels were significantly ($p < 0.05$) increased in groups treated with Quercetin and Metformin compared to the normal control group.



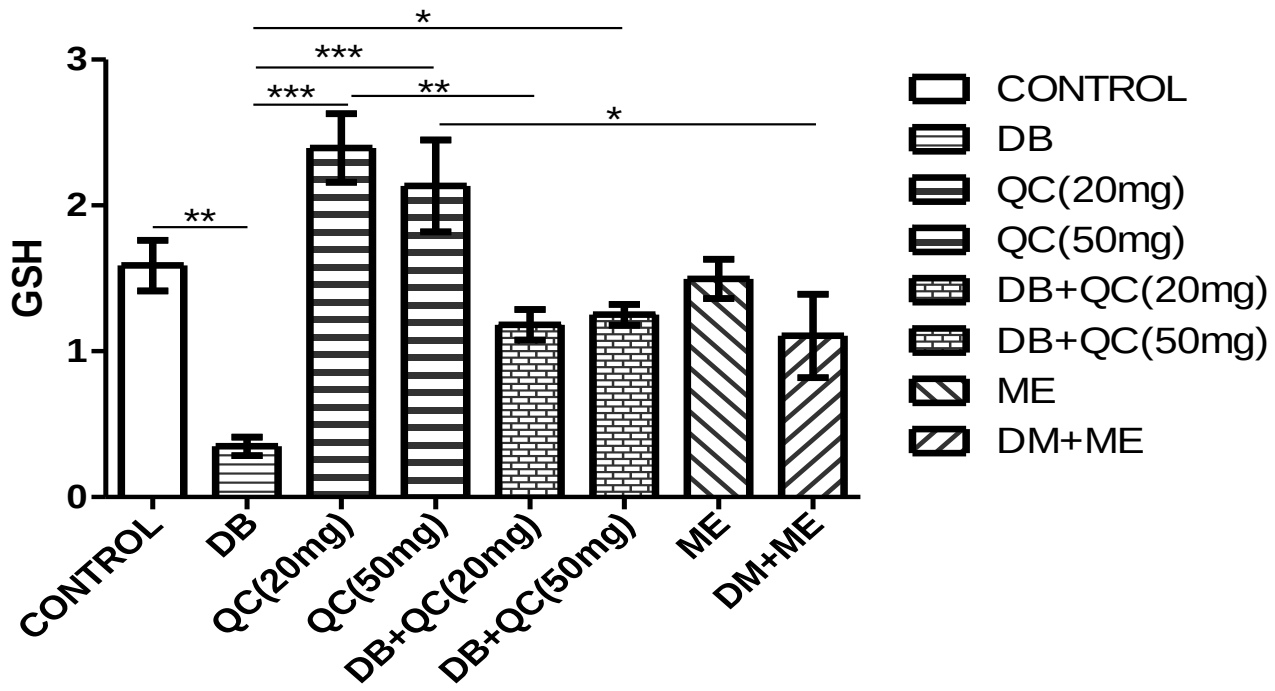
KEY: DB- Diabetes, QC- Quercetin, ME- Metformin

Figure 3: Showing comparison in Testosterone hormone level among groups. Testosterone levels were significantly ($p < 0.05$) reduced in all rats induced with diabetes prior to treatment compared to the normal control group, while testosterone levels were significantly ($p < 0.05$) increased in groups treated with Quercetin and Metformin compared to the normal control group.



KEY: DB- Diabetes, QC- Quercetin, ME- Metformin,

Figure 5. Showing comparison in Malondialdehyde (MDA) level among groups. MDA levels were significantly ($p < 0.05$) increased in all rats induced with diabetes prior to treatment compared to the normal control group, while MDA levels were significantly ($p < 0.05$) reduced in group treated with Quercetin compared to the normal control group.



KEY: DB- Diabetes, QC- Quercetin, ME- Metformin

Figure 6. Showing comparison in Glutathione (GSH) level among groups. GSH levels were significantly ($p < 0.05$) reduced in all rats induced with diabetes prior to treatment compared to the normal control group, while GSH levels were significantly ($p < 0.05$) increased in group treated with Quercetin compared to the normal control group.

DISCUSSION

The results show that quercetin was able to decrease elevated blood glucose associated with diabetes. This is consistent with previous studies (Ji-Hye *et al.,* 2011; Chakravarthy *et al.,* 1981) that reported consumption of Quercetin by STZ-treated rats significantly suppressed the elevation of plasma glucose. Kwon *et al*., 2007 showed a robust inhibition of Quercetin in glucose and fructose transport by GLUT2 in Caco-2E intestinal cells. However, the two other major intestinal sugar transporters, GLUT5 and SGLT1, were unaffected by this flavonoid. It appears that quercetin as an antioxidant and a free radical scavenger prevents autopoly (ADPribosyl)- ation of Poly (ADP-ribose) polymerase, thereby stabilizing regenerating gene transcriptional complex and resulting in the regeneration β-cells and protection of pancreatic islets against STZ (Szkudelski, 2001).

The hypothalamus is responsible for secretion of gonadotropin-releasing hormone (GnRH) that stimulate the pituitary to produce FSH and LH which control testosterone production in males (Hauger *et al.,* 2022). The amplitudes of the LH and FSH pulses that are generated are dependent upon the GnRH pulse frequency (Wildt *et al.,*1981). This infers that the hypothalamic function can be deduced using the LH, FSH and Testosterone level present in the plasma. From this research, the marked decrease in the circulatory concentration of LH, FSH and Testosterone level was observed just after induction of diabetes using STZ-Nicotinamide. This significant decrease in plasma LH, FSH and Testosterone could result in male infertility associated with hypothalamic dysfunction due to induction of diabetes (Huang *et al.,* 2024).

After treatment of diabetes mellitus with quercetin, there was a significant increase in the circulatory concentration of LH, FSH and Testosterone level in Rats that were induced with diabetes and treated with quercetin showing the ameliorative effect of

quercetin as a treatment regimen for diabetes mellitus. This is consistent with previous studies (Osman *et al.,* 1998). There was no significant ($p>0.05$) difference between animals treated with high dose (50mg/kg/day) and those treated with low dose (20mg/kg/day). When animal groups that were not induced with diabetes but were administered with quercetin only (Quercetin only 50mg/kg/day and 20mg/kg/day) were compared with animal groups that were induced with diabetes and the treated with low dose and high dose of quercetin (Diabetes+ Quercetin 50mg/kg/day and Diabetes+ Quercetin 20mg/kg/day), it is seen that there is a significant increase of LH, FSH and Testosterone level in animals that were only administered with quercetin as compared with animals that were induced with diabetes prior to treatment with quercetin. This suggest that quercetin could be used as a dietary supplement to increase the hypothalamic stimulation of GnRH, thereby increasing plasma LH and FSH level, and finally increasing plasma Testosterone level.

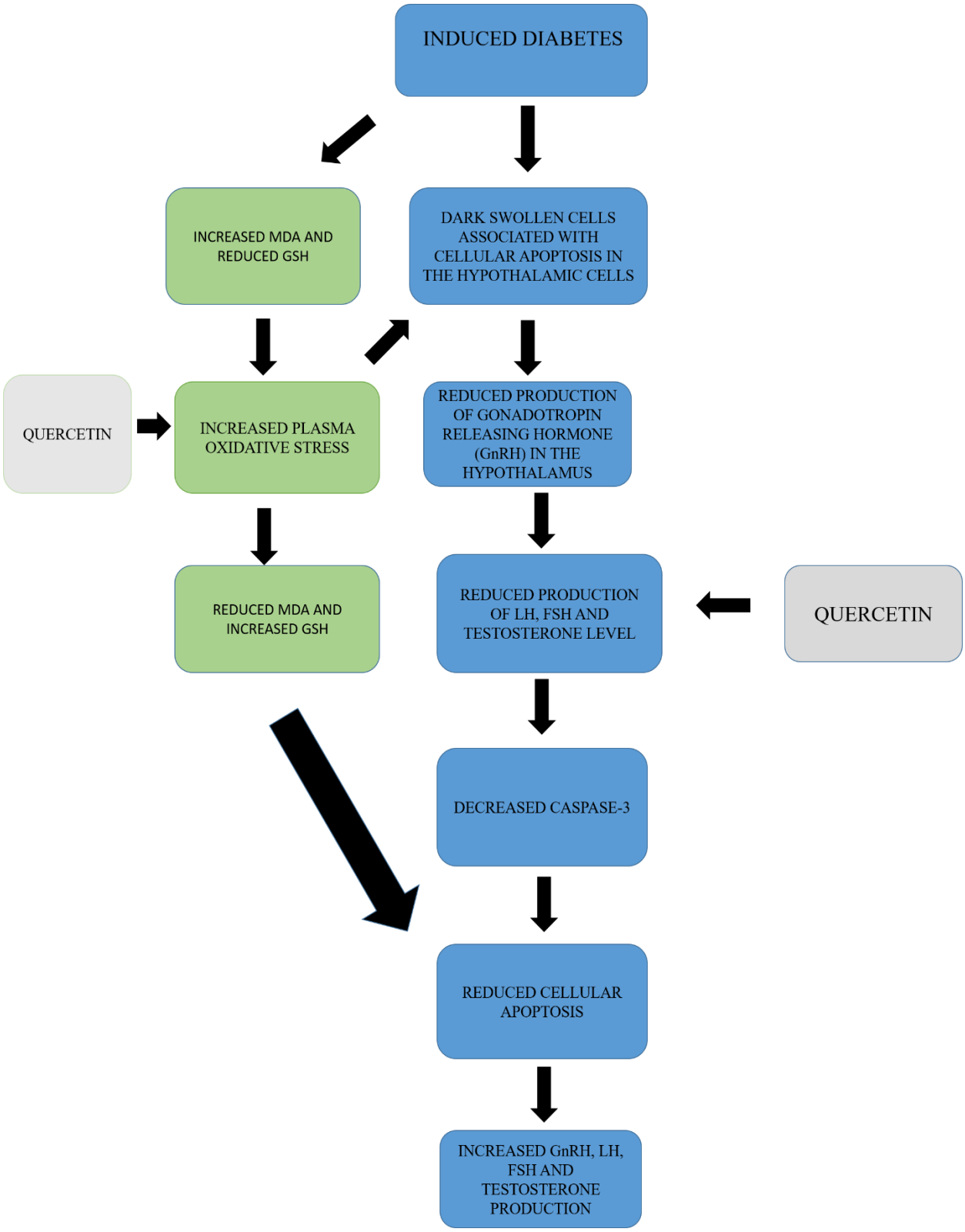
Histological observations of the hypothalamus tissue of rats in group B showed swollen, distorted hypothalamic cells and cellular apoptosis of hypothalamic nuclei. This is consistent with previous studies (Taylor *et al.,* 2004). Photomicrography of normal control group, groups treated with quercetin and metformin had similar features of cellular delineation, histoachitectural assortment and no feature of significant inflammation or degenerative changes on the hypothalamus

Oxidative stress biomarkers measured are Malondialdehyde(MDA) and Reduced Gluthathione (GSH). Oxidative stress results from an imbalance between prooxidants (free radical species) and the body's scavenging ability (antioxidants) (Agarwal *et al.,* 2005). It has been studied that hyperglycemia exhibit its deteriorating effects on beta cells of pancreatic islets by inducing the oxidative stress within the beta cells of the pancreatic islets. Results from this study show that STZ-nicotinamide induced diabetes increased the lipid peroxidation index (MDA). This is in tandem with previous studies (Domingo *et al.,* 1987 Dua and Gill, 2001, Anane and Creppy, 2001). A significant ($p<0.05$) increase was observed in the MDA level of diabetic untreated rats compared to other groups treated with quercetin owing to hyperglycemia associated with induced diabetes. Also, a significant ($p<0.05$) decrease was observed in the GSH level of diabetic untreated rats compared to other groups treated with quercetin owing to hyperglycemia associated with induced diabetes. After the administration of quercetin, the level of MDA and GSH were normalized showing the antioxidant enhancing effect of quercetin against oxidative stress resulting from hyperglycemia.

When animal groups that were not induced with diabetes but were administered with quercetin only (Quercetin only 50mg/kg/day and 20mg/kg/day) were compared with animal groups that were induced with diabetes and the treated with low dose and high dose of quercetin (Diabetes+ Quercetin 50mg/kg/day and Diabetes+ Quercetin 20mg/kg/day), a significant increase was seen in the GSH level of animals that were only administered with quercetin as compared with animals that were induced with diabetes prior to treatment with quercetin but when animal groups that were not induced with diabetes but were administered with quercetin only (Quercetin only 50mg/kg/day and 20mg/kg/day) were compared with animal groups that were induced with diabetes and then treated with low dose and high dose of quercetin (Diabetes+ Quercetin 50mg/kg/day and Diabetes+ Quercetin 20mg/kg/day), a significant decrease was seen in the MDA level of animals that were only administered with quercetin as compared with animals that were induced with diabetes prior to treatment with quercetin.

CONCLUSION

The result obtained in this present study suggest that Quercetin was able to ameliorate the effect of STZ-Nicotinamide (Diabetes) in the hypothalamus of an adult male Wister rat.


INDUCED DIABETES
INCREASED MDA AND REDUCED GSH
DARK SWOLLEN CELLS ASSOCIATED WITH CELLULAR APOPTOSIS IN THE HYPOTHALAMIC CELLS
QUERCETIN
INCREASED PLASMA OXIDATIVE STRESS
REDUCED PRODUCTION OF GONADOTROPIN RELEASING HORMONE (GnRH) IN THE HYPOTHALAMUS
REDUCED MDA AND INCREASED GSH
REDUCED PRODUCTION OF LH, FSH AND TESTOSTERONE LEVEL
QUERCETIN
DECREASED CASPASE-3
REDUCED CELLULAR APOPTOSIS
INCREASED GnRH, LH, FSH AND TESTOSTERONE PRODUCTION

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