Ind. J. Pharm. Edu. Res., 2026; Krupapharmacon:123-132.
https://www.ijper.org

Original Article

Quercetin Role in Mitigating Insulin Resistance Mediated Anxiety in a High-Fat High-Fructose Diet Animal Model

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ABSTRACT

Background: Unhealthy dietary patterns, particularly high-fat high-fructose diets, are closely linked to metabolic conditions (resistance to insulin) that may lead to anxiety-related behaviors and neuro inflammation. A dietary bioflavonoid, Quercetin possess anti-inflammatory and antioxidant qualities that may provide protective action against such complications. **Objectives:** To discover the potential of quercetin in reducing metabolic changes in an HFHFrD animal model in turn ameliorating HFHFrD mediated anxiety. **Materials and Methods:** Rats were fed with HFHFrD for the induction of insulin resistance. 50 mg/kg and 100 mg/kg of quercetin and metformine respectively were evaluated in relation to insulin resistance mediated behavioral changes (anxiety). Behavioral parameters, serum lipid profile, antioxidant status and inflammatory cytokines were determined. **Results:** Rats administered with HFHFrD showed signs of oxidative stress, insulin resistance, lipid imbalance, neuroinflammation, and anxiety-related behavior. Treatment with quercetin and metformin significantly postponed the symptoms of anxiety by showing evident changes in neurobehavior and biochemical parameters. **Conclusion:** Quercetin can be used a preventive therapy in anxiety like disorders by reducing neuroinflammation, neurodegeneration, behavioural alterations and insulin related biochemical changes.

Keywords: Antioxidants, Anxiety, Insulin resistance, Metformin, Neuroinflammation, Quercetine.

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INTRODUCTION

Anxiety disorder is a common mental health condition that affects both young people and adults. It is characterized by excessive fear, motor tension, irritability, increased alertness and sympathetic hyperactivity.[1] Diet is one of the most accessible and adaptable lifestyle choices that affect overall health, including mental well-being. Obesity (westernised diet) increase the release of excess adipokines, which in turn raise levels of fatty acids (free) and amount of Oxygen-Derived Reactive Molecules (ROS). These Factors Cause Low-Grade Inflammation (TNF-α) and impair insulin signalling, which transcends to insulin resistance and promotes the detrimental effects of cytokines (pro - inflammatory), that has the ability to access the brain and stimulate microglia activation in the CNS. This neuroinflammation increases oxidative stress levels in regions of brain such as the hippocampus and prefrontal cortex leading to anxiety-related behaviours.[2] As obesity is frequently accompanied by neurobehavioral disturbances, increasing attention has been directed toward dietary approaches that can simultaneously address metabolic imbalance and anxiety-related symptoms. Under these circumstances, since flavonoids are exhibiting protective effects, we wanted to investigate the effect of Quercetin commonly identified in strawberries, grapes, citrus fruits, and tea. It suppresses enzymes producing ROS and prevents oxidative stress induced neuronal damage and possess anti-inflammatory property that was studied in animal models. Quercetin has shown potential effect by reducing IL-6, IL-1β, Cyclooxygenase-2 (COX-2), and Nuclear Factor-KappaB (NF-κB) amount in the brain.[3] Quercetin influence the reduction of Insulin Resistance (IR), which intimately linked to emergence of long-term low-grade inflammation. Quercetin may improve IR by reducing the generation and expression of pro-inflammatory cytokines.[4] This study was done to assess behavioural differences associated with HFHFrD intake. We examined the biochemical effects of HFHFrD-induced neuroinflammation, as well as the anxiolytic and neuroprotective effects of hesperidin.

MATERIALS AND METHODS

Reagents and Extract

1 mM of EDTA, 100 mM of phosphate buffer, Nitro Blue Tetrazolium (NBT), Sodium carbonate (Na_2CO_3), Hydroxylamine HCl (1 mM), Hydrogen peroxide (H_2O_2), Thio Barbituric Acid (TBA 0.6%), Phosphoric acid (H_3PO_4), n-Butanol, Sulfosalicylic acid (3%), $NADPH_2$, Glutathione reductase, GSH (Reduced

DOI: 10.5530/ijper.krupapharmacon.29

glutathione), Dimethyl Sulfoxide (DMSO), Glucose solution. Quercetin was purchased from Otto Chemi. Pvt. Ltd., India.

HFHFrD preparation and introduction of insulin resistance

High Fat High Fructose Diet (HFHFrD) was made by blending 390 g regular rat chow with 40 g of sour starch and 110 g of vegetable fat and dissolving in water at a concentration of 10 g per 100 mL in the form of pellets. The fructose diet (30 g fructose in 100 mL water) was given through feeding bottle. For induction of IR, the rats were fed with HFHFrD for a period of 42 days and resistance was determined using HOMA-IR.[5]

Assessment of OGTT (Oral glucose tolerance test)

OGTT was performed with the overnight fasted rats with free access to water. Glucose solution (2 g/kg p.o) was fed after half an hour of administration of extracts. The blood was withdrawn at time intervals of 0, 30, 60, 90, and 120 min by tail tip.[6] Glucose levels were estimated by standard glucometer (Accu-Chek, India).

Evaluation of Insulin Resistance (HOMA-IR Method)

It was evaluated in animals which were fasted for 12 hr with access to water. Glucose and insulin concentrations were determined using respective available commercial kits (Linco Research, United States, St. Charles, MO). The HOMA index of resistance towards insulin was evaluated using the equation:[7]

$$\text{HOMA} = \frac{\text{glucose (mM)} \times \text{insulin (}\mu\text{U/mL)}}{22.5}$$

Experimental Animals and Treatment Protocol

The study involves the use of male albino Wistar rats that weigh around 200 to 250 g. The approved protocols were obtained from the Institutional Animals Ethics Committee (Approval No: KCP/IAEC/PCOL/159/JULY-2024). The care and handling of animals met the animal welfare standards promulgated by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The rats were conditioned to an animal house maintained at 25±3ºC temperature, 55%-60% humidity and 12-hr dark/light photoperiod). Rats, permitted with unlimited water supply and maintained a standard laboratory diet prior to their dietary changes. We standardized the induction of the Insulin Resistance (IR) model in rats, prior initiating the experimental investigation based on the protocol described by Melo *et al.*, the rats were assigned into five groups. Each group consists of eight animals (n=8). Group I serves as a normal control and was maintained on a standard diet throughout the study whereas group II, IV and V were fed a HFHFrD for 6-weeks. This dietary model involved administering a high-fat diet as feed, accompanied by 30% fructose dissolved in drinking water and fed via water bottles. Treatments were given simultaneously with administration of HFHFrD from day one, group II (positive control) was fed with only HFHFrD, group IV received 50 mg/kg, p.o of quercetin, group V treated with 100 mg/kg of metformin and group III was given with quercetin alone (50 mg/kg) without HFHFrD. Behavioural parameters were assessed after the induction of IR and body weight of animals was tracked weekly.

Assessment of behavioural Parameters

Elevated Plus Maze (EPM)

Anxieties in the above rats were determined by using elevated plus maze. Maze is made of four arms arranged in a plus-shaped pattern, which is raised 50 cm above the floor having open and closed arms that were extending from a central platform. At the beginning of this model, each rat was positioned in central space and the anxiety was observed over a period of 5 min. The time explored and entries to both the arms were registered, greater amount of time spent in closed arms is considered a symptom of anxiety, whereas higher number of entries and prolonged time spent in open arms show anxiolytic activity.[8,9]

Open Field Test (OFT)

The anxiety and exploratory behaviour was determined by using open field test model an apparatus consisting of a square-shaped field measuring 70 × 70 × 60 cm, divided into 49 equal squares (each 10 × 10 cm). The test performed by placing animal in middle of the open field apparatus and test was initiated after 10 sec. Behaviour of rats was assessed over three sessions, each consisting of 5 min. The parameters recorded included: (A) time spent in the open field's center, (B) distance travelled in total, (C) duration of freezing behaviour.[10] Freezing time includes the period during which rat remains still, either lying or sitting with minimal movements.

Rotarod Test

The rotarod equipment consists of a 3 cm diameter horizontal rod (wooden or metallic) covered with rubber for grip. A motor that enabled rotation speed adjustment was attached to the rod. The rats were positioned on revolving rod at particular height that prevents the animals from voluntarily jumping off. The base of the apparatus is usually padded for a safe fall. The time for which the animal falls, will be measured.[11]

Serum lipid content

FFA, TG, HDL as well as serum TC concentrations were analysed using Semi auto analyser-Erba chem 5-plus (Erba Diagnostics, Germany) with standard enzymatic colorimetric kits following protocol.

Assessment of Biochemical Parameters in Brain Tissue

Biochemical parameters were assessed once the treatment period was completed, animal were fasted overnight and then sacrificed. The hippocampal region was dissected after the brain was promptly removed. Using an Elvehjem-Potter homogeniser, tissue

was homogenised in KH_2PO_4/K_2HPO_4 buffer (50 mM) at pH 7.4. The supernatant (after centrifugration and homogenation) was used to estimate the protein content, reactive oxygen species, reduced glutathione, and antioxidant enzyme activity. A separate portion of the hippocampus was processed following the method described by Gupta and Kaur for cytokine measurement.[12]

Assessment of Antioxidant Status

Protein levels were estimated by the Hartree method, which is modified adaptation of conventional Lowry method.[13] Lipid peroxidation evaluated by measuring reduced levels of Glutathione (GSH) based on the procedure stated by Sedlak and Lindsay.[14] whereas total sulfhydryl groups were measured using Ellman's method.[15] Glutathione peroxidase (GPx) activity in brain was analysed based on the Flohé and Günzler protocol.[16] Superoxide Dismutase (SOD) activity was measured using Fridovich and Misra method.[17] and Catalase (CAT) was evaluated based on method of Aebi.[18]

Assessment of Reactive Oxygen Species (ROS) and inflammatory mediators

H_2O_2 (hydrogen peroxide) levels in the hippocampus were estimated using Dingeon *et al.* method.[19] If peroxidase is present, H_2O_2 interacts with 4-aminoantipyrine and p-hydroxybenzoic acid and produce a pink complex of quinoneimine that is measured spectrophotometrically at 505 nm. The production of hydroxyl radicals was measured following Payá *et al.* procedure.[20] To create hydroxyl radicals, deoxyribose was oxidised using EDTA H_2O and the Fe^{3+} ascorbate system. The hippocampus homogenate was incubated with the reaction mixture for 1 hr at 37°C and stopped the reaction on addition of TCA (2.8%) and thiobarbituric acid (1%). After 20 min of boiling at 100°C, the absorbance at 532 nm was analysed in comparison to a blank consisting of buffer and deoxyribose. Radical superoxide levels were calculated using a slightly modified version of the Marklund and Marklund approach.[21] To start the reaction, homogenate was incubated in Tris-HCl buffer before addition of pyrogallol, then Hydrogen Chloride (HCl) was used to stop the reaction after a 4-min incubation period at 25°C. At 420 nm, absorbance was determined in relation to the blank.

Statistical evaluation

All data are presented as Mean±Standard Error of the Mean (SEM). Statistical comparisons were carried out among groups, by employiong unpaired Student's t-test as well as One-Way Analysis of Variance (ANOVA), after confirmation of data normality distribution across all measured parameters. p value ≤0.05 was regarded as statistically significant. Analyses were carried out using GraphPad Prism (version 8.0)

RESULTS

HFHFrD - Effect on body weight, brain weight, and abdominal fat weight

HFHFrD showed a significant increase in weights of body, brain and abdominal fat. Adding quercetin and metformin to the regular diet attenuated the raise much significantly ($p<0.05$). Pronounced effect was observed when these were treated with metformin ($p<0.01$) Table 1.

OGTT and resistance towards insulin

Progressive impairment of intolerance towards glucose was observed in HFHFrD-fed rats. Post six weeks treatment with this diet, these rats exhibited significant impaired glucose tolerance (data not shown) and still worsened on continuation of HFHFrD. HOMA-IR results showed additional confirmation that these rats had severe insulin resistance when compared to rats fed with normal diet. Among the treated groups, the rats treated with the conventional antidiabetic agent (metformin) had produced significant improvement in declining the insulin resistance (Figure 1).

Behavioral Parameters

Influence on open field test

The symptoms of anxiety was clearly evident when the rats were fed with HFHFrD. When tested for their behaviour response, a significant reduction was seen to explore the central zone and the distance travelled was decline when compared to normal fed chow rats, in turn causing an increase in freezing episodes. Treated groups showed better reversal in these behaviour responses. Off which, metformin had better response than quercetin (Figures 2-4).

Effect on elevated plus maze

Quercetin treatment to normal rats had no much impact on its behaviour. Quercetin treated HFHFrD rats exhibited an elevated period spent in open arms which also reflects decline in time spent in closed arms when compared to HFHFrD rats that were untreated. HFHFrD rats administered with metformin had also

Table 1: Fat weight. Data expressed here are in Mean±SEM (n=8), * $p<0.05$ vs HFHFrD, ##$p<0.01$ vs normal.

Parameter (g)	Normal	HFHFrD	HFHFrD + Quercetin	HFHFrD + Metformin
Brain weight	1.42±0.08	1.86±0.06 ##	1.60±0.05 *	1.51±0.02**
Body weight	268.46±2.67	324.70± 24.00 ##	285.24± 10.20 *	262.96±8.35**
Abdominal weight	10.24±0.99	26.20±3.60 ##	16.65±2.90 *	13.92±2.52**

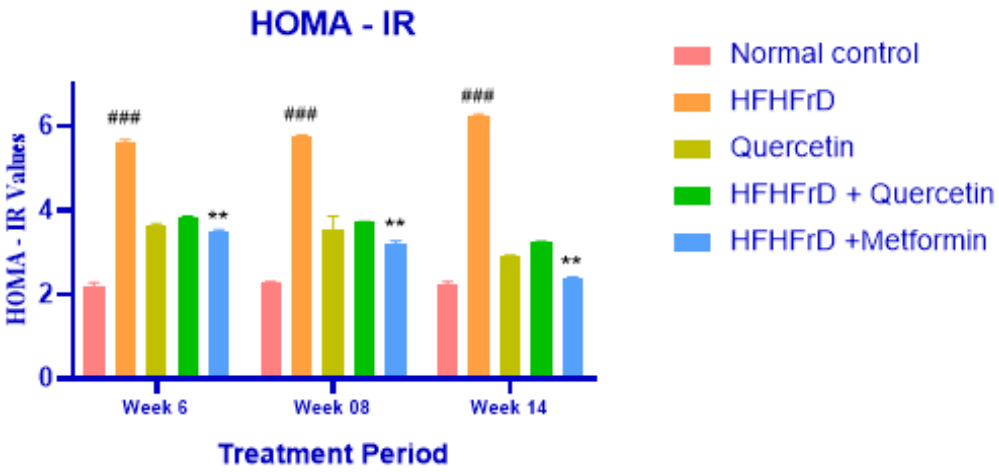


Figure 1: HOMA-IR. Data expressed here are in Mean±SEM (*n*=8), ***p*<0.01 vs HFHFrD, ### *p*<0.001 vs normal.

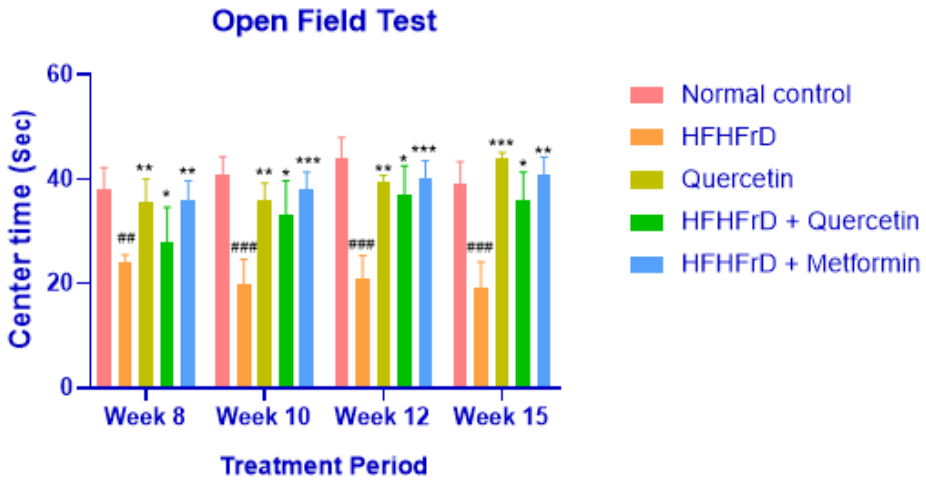


Figure 2: Open field test-centre time (s). Data expressed here are in Mean±SEM (*n*=8), ****p*<0.0001, ***p*<0.01, **p*<0.05 vs HFHFrD, ### *p*<0.001, ##*p*<0.01 vs normal.

showed improved duration of period spent in the arm that is open in an EPM (Figures 5, 6).

Effect on Rotarod

The rats fed with HFHFrD diet exhibited significant less fall in time from 10th week onward when tested with rotarod apparatus, which indicates a progressive decline in motor coordination. As the weeks progressed the fall time was much more significantly declining when compared to normal as well as quercetin only treated normal rats. On treatment with quercetin and metformin to the rats fed with HFHFrD diet showed significant improvement in motor coordination (Figure 7).

Antioxidant profile of rat's brain

Normal rats when fed with HFHFrD, showed a significant decline in antioxidant enzyme levels and subsequent increase in LPO concentration. On treatment with quercetin and metformin resulted in reversal of all these biochemical markers towards normal but effect of

quercetin was less effective than that of metformin (Table 2).

Effect on lipid profile

Serum lipid profile

An abnormal lipid profile was observed in HFHFrD fed rats. However, on treatment with quercetin and metformin, these abnormal levels were reverted back towards normal but efficacy of quercetin was less when compared with metformin treated rats (Table 3).

Effects on brain oxidative free radicals and inflammatory markers

In hippocampal tissue, excessive concentrations of oxidative free radicals as well as inflammatory markers were observed in the rats fed with HFHFrD and all these abnormalities were reversed

by treating the rats with quercetin and metformin but metformin treatment showed better response (Figures 8A-8C).

DISSCUSION

In the current study, HFHFrD has shown to have deleterious results on metabolic, neurochemical, and behavioural parameters. Whereas the treatment with quercetin and metformin has successfully reversed the detrimental effects of HFHFrD, especially in context with the neuroinflammation and delay in anxiety-like behaviours. In our animal model, prolonged consumption of HFHFrD led to Insulin Resistance (IR), as indicated by elevated insulin levels that were validated by HOMA-IR and disturbed glucose regulation. These outcomes are in accordance with previous research showing that diets rich

Table 2: Effect on brain antioxidant status. All data here are expressed as Mean±SEM (*n*=8), ### *p*<0.001 vs normal, * *p*<0.001, ** *p*<0.01 vs HFHFrD.**

Groups	SOD (U/mg)	CAT (U/mg)	GSH (U/mg)	GPx (U/mg)	LPO (nmol/mg)
Normal	22.54±2.40	42.2±8.3.6	23.24±4.2	13.24±1.2	1.94 ±0.27
HFHFrD	10.84±4.2 ###	20.0±1.8 ###	8.2±0.7 ###	4.24±3.2 ###	6.42±0.59 ###
Quercetin	23.10±2.2 ***	38.8±1.8 ***	21.8±0.7 ***	12.98±0.9 ***	2.14± 0.23 ***
HFHFrD + Quercetin	16.2±2.0 **	32.5±1.5 **	14.2±0.7 **	8.42±1.9 **	4.45±0.22 **
HFHFrD + Metformin	19.6±2.0 ***	35.2±1.5 ***	20.9±0.7 ***	13.12±0.9 ***	1.98± 0.18 ***

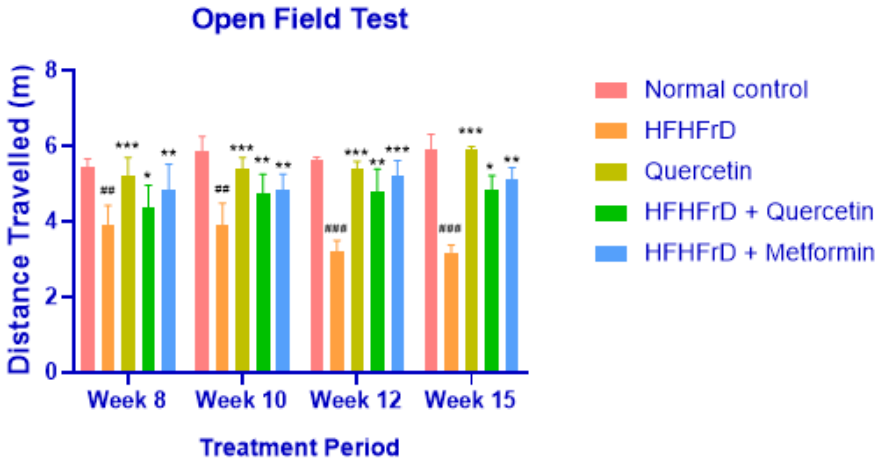


Figure 3: Open field test-Distance travelled (m). Data expressed here are in Mean±SEM (*n*=8), ***p<0.001, **p<0.01, *p<0.05 vs HFHFrD, ##p<0.01, ### p<0.001 vs normal.

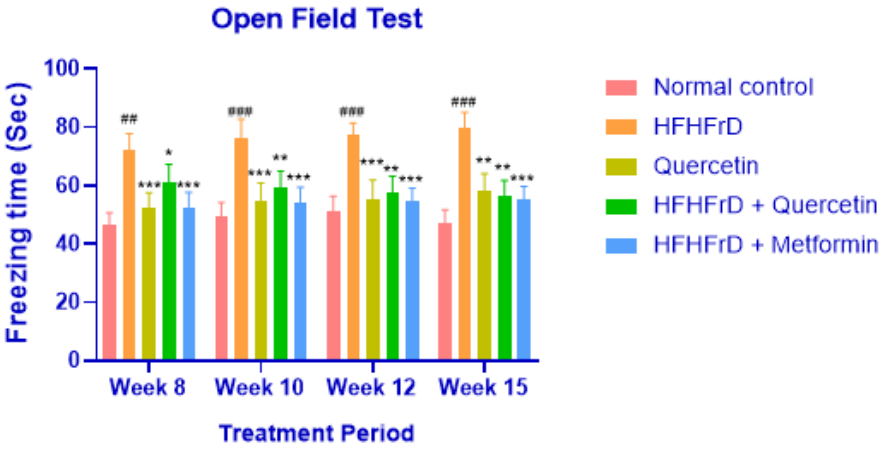


Figure 4: Open field test-Freezing time (s). Data expressed here are in Mean±SEM (*n*=8), ***p<0.001, **p<0.01, *p<0.05 vs HFHFrD, ### p<0.001, ##p<0.01 vs normal

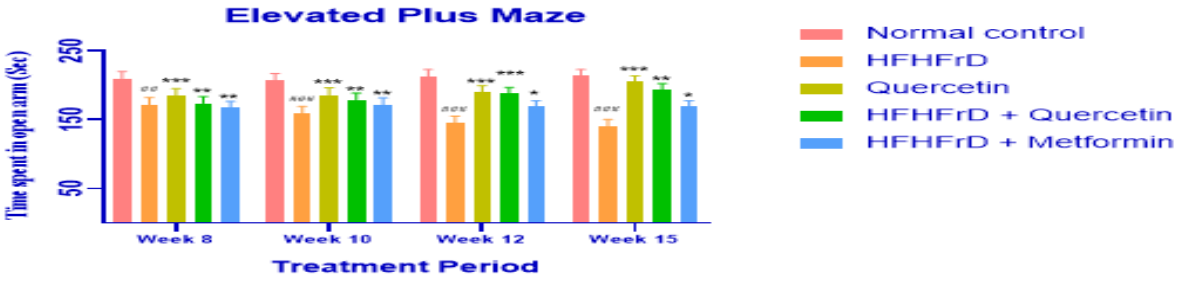


Figure 5: Elevated plus maze- time spent in open arm (s) Data showed are in Mean±SEM (*n*=8), * *p*<0.05 vs HFHFrD, ** *p*<0.01 vs HFHFrD, *** *p*<0.001 vs HFHFrD. ## *p*<0.01 vs normal, ### *p*<0.001 vs normal.

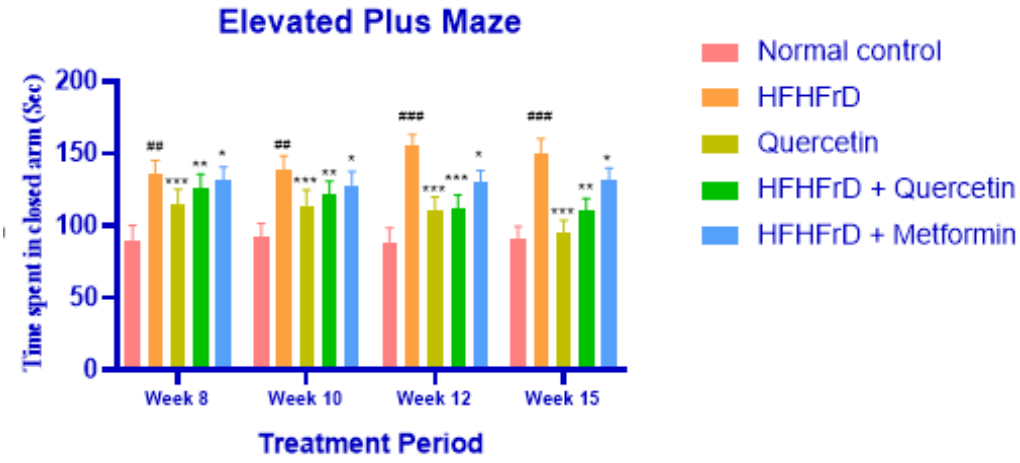


Figure 6: Elevated plus maze- time spent in closed arm (s) Data showed are in Mean±SEM (*n*=8), *p<0.05 vs HFHFrD, **p<0.01 vs HFHFrD, ***p<0.001 vs HFHFrD. ##p<0.01 vs normal, ### p<0.001 vs normal.

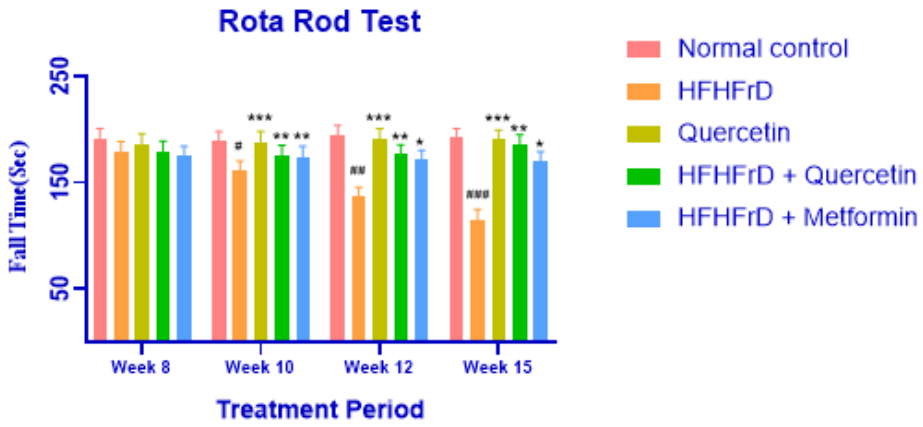
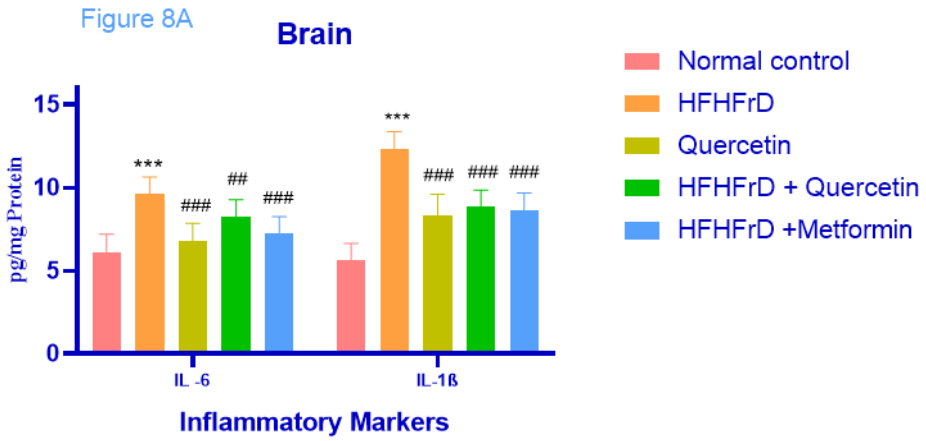
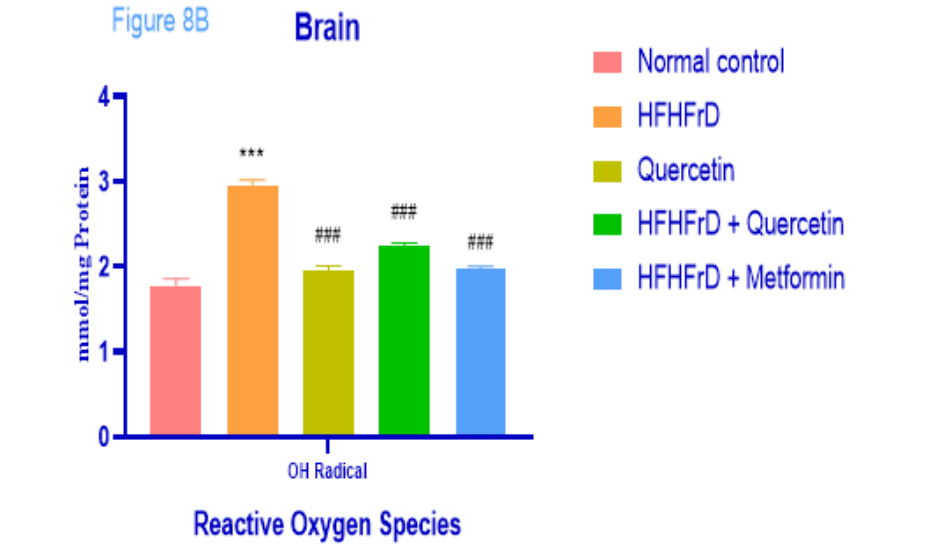


Figure 7: Rotarod Test - Fall time (seconds). Data showed are in Mean±SEM (*n*=8), *p<0.05, **p<0.01, ***p<0.001 vs HFHFrD, #p<0.05, ##p<0.01, ###p<0.001 vs Normal.

in fructose and saturated fats cause metabolic disorders because of high energy density, which encourages overconsumption of calories and further led to obesity.[22-24] The current study confirmed the involvement of HFHFr diet in metabolic dysfunction by showing that six weeks of exposure to HFHFrD dramatically changed body weight and glucose metabolism in rats. Significantly, quercetin and metformin treatments effectively decreased weight gain and almost restored normal glucose and insulin levels, indicating their potential to increase insulin sensitivity and control systemic metabolism. Additionally, the results showed a pattern of anxiety-like behaviour in HFHFrD fed animals that got worse over time and became statistically significant by the eighth week, which was demonstrated by behavioural tests like EPM and OFT. The animals treated with quercetin have delayed the onset of anxiety-like behavioural changes, by showing only mild anxiety-like effects at week 8.





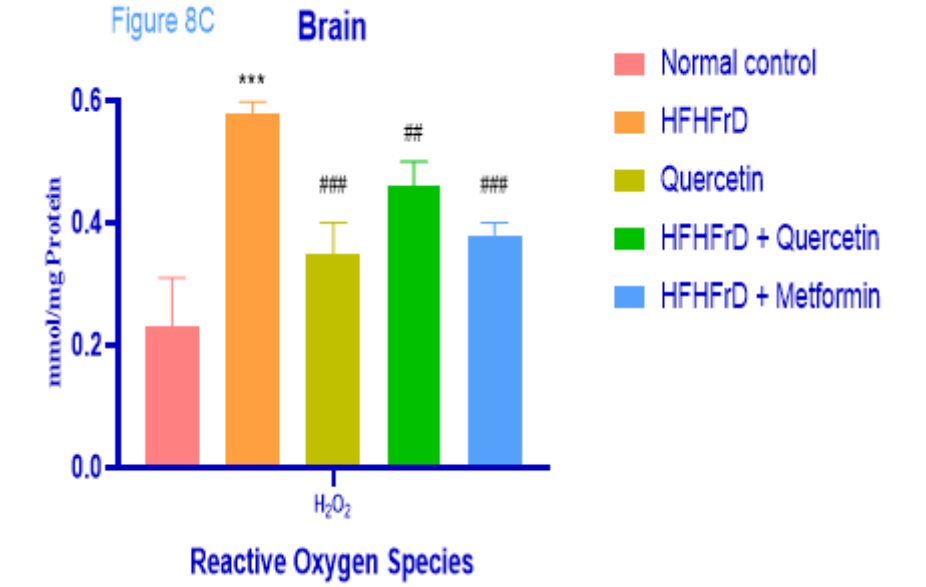


Figure 8: Brain ROS and inflammatory mediators A) Interleukin-6 (IL-6), Interleukin-1 beta (IL-1β), B) Hydroxyl radical (OH), C) Hydrogen peroxide (H_2O_2). Data showed are in Mean±SEM (*n*=8), ***p<0.001 vs Normal, ##p<0.001, ##p<0.01 vs HFHFrD.

Table 3: Effect on serum lipid profile. All data here are expressed as mean±SEM (*n*=8), ###*p*<0.001 vs normal, * *p*<0.001, **p<0.01, vs HFHFrD.**

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	FFA (mg/dL)
Normal	122.0±4.5	78.0±4.0	42.0±3.0	18.25±2.05
HFHFrD	244.68±16.0 ###	168.4± 10.2 ###	28.0±2.5 ###	48.24±8.10 ###
Quercetin	130.12±6.0 ***	70.24±6.6 ***	38.5±2.8 ***	16.26±1.08 ***
HFHFrD + Quercetin	146.82±5.0 **	58.5±5.6 **	32.2±2.9 **	24.98±2.06 **
HFHFrD + Metformin	128.32±5.8 ***	72.34±6.2 ***	39.0±2.9***	16.56±0.07 ***

In comparison to HFHFrD group, metformin treated group demonstrated a slow tendency toward improvement, but did not approach statistical significance, whereas it is consistent with the anxiolytic effect. Along with the metabolic disruptions, HFHFrD also affected the brain's lipid profile by increasing triglycerides, total cholesterol levels and while HDL levels remain low, it seems lipid peroxidation play significant part in mediating diet-induced metabolic alterations on behavioral disorders.[25] This lipid dysregulation has harmful effects on brain due to its limited ability to self-regulate lipid substrates, and chronic accumulation leads to systemic and organ-specific damage.[26-28] Our results support these findings and we can see that quercetin mitigates hypercholesterolemia and restoring lipid homeostasis. Oxidative stress is a key mechanism that links HFHFrD to neurological impairment, which occurs when ROS generation surpasses the capability of defensive antioxidant status. In this present study, the rats fed with HFHFrD has showed decreased activity of antioxidants such as CAT, SOD, and GPx, including non-enzymatic Glutathione (GSH) antioxidants as an indicator of lipid peroxidation. These disturbances were effectively reversed by quercetin and metformin, as exhibited by increased concentrations of CAT, SOD, GPx. Therefore, restoration of antioxidant enzyme activity towards normal levels confirms potent antioxidant role of quercetin that would have mitigated the oxidative stress induced neurobehavioral changes such as anxiety.

Quercetin exhibits antimicrobial, antiviral, antioxidant, and anti-inflammatory properties. It enhances cellular antioxidant defences by activation of the Nrf2 pathway. Animal studies indicate that quercetin reduces oxidative tissue damage in organs such as the brain and heart during ischemia-reperfusion injury and under conditions of oxidative stress. Elik *et al.* (2007) demonstrated its antidiabetic potential, showing protection against oxidative injury in the heart, brain, liver, aorta, and kidneys of diabetic rats over mid- to long-term exposure. Quercetin is also known to inhibit enzymes such as cyclooxygenases and protein kinases, thereby influencing processes related to cell proliferation and apoptosis.[29] In our research it was observed that quercetin and metformin mitigated ROS levels in brain as compared to HFHFrD fed groups. More notably our findings showed how treatment with quercetin and metformin reduced proinflammatory cytokines in rat's brain. HFHFrD induced insulin resistance mediated anxiety prominently causes neuroinflammation identified by production of proinflammatory mediators like IL-6, TN-α which known to activate glial cells, mainly microglia and astrocytes that results in neuroinflammatory cascades and gliosis.[30] This research resonates with the previous studies where HFHFrD-induced elevations in Free Fatty Acids (FFAs) activates microglia and disrupts hypothalamic signalling and further anxiety-like behaviour.[31] Rats fed a HFHFrD showed elevated Free Fatty Acids (FFAs) and increased neuroinflammatory markers in the brain, both of which were markedly reduced by quercetin and metformin treatment.

The anxiety-like actions were assessed using behavioral parameters such as OFT, and EPM based on previous studies.[32] Rats fed with HFHFrD showed anxiety-like behavior, which include reduced period of stay in the central region of OFT, increased entries to the closed arms than open arms. These behavioral changes are frequently observed following consumption of high-fat diet and that are considered to interfere with neurotransmitter function, retard synaptic plasticity, and cause neuroinflammation.[33-34] Our study confirms the anxiogenic effects in HFHFrD fed rats, and treatment with quercetin and metformin demonstrated anxiolytic effect by restoring exploratory activity and reversed behavioral parameters. Based on already conducted research, quercetin may modulate neurotransmitters and receptors interactions to produce anxiolytic effects. It includes serotonin GABAergic and glutamatergic, key chemical messengers present in central nervous system, perform key role in neuronal alteration.[35] These pathways are essential in anxiety regulation, and although current study did not specifically evaluate the neurotransmitter levels, but the reversal of anxiety-like behavior suggests possible participation of these systems. Our data suggest that quercetin has multiple therapeutic benefits, including the ability to prevent the progression of insulin resistance related anxiety.

CONCLUSION

The present study showed a close association between insulin resistance, neuroinflammation and anxiety-like behaviours, with species of reactive oxygen playing important part in the detrimental effects of HFHFrD. HFHFrD fed rats developed Insulin Resistance (IR) and glucose intolerance within six weeks, accompanied by anxiety-like behaviours. These metabolic and behavioural changes were associated with reduced antioxidant enzyme activities (GPx, SOD, CAT), glutathione depletion, elevated lipid peroxidation, excessive formation of reactive oxygen species (H_2O_2 and OH•), and increased proinflammatory

cytokines (IL-1β, IL-6). Quercetin administered from the start of the diet effectively mitigated these alterations, delaying the onset of anxiety-like behaviours and providing significant protection. Overall, quercetin prevented progression of insulin resistance, preserved antioxidant defences, lowered neuroinflammation, and improved behavioural outcomes, supporting its potential as a preventive and anxiolytic agent that links metabolic dysfunction and anxiety through oxidative, lipid and inflammatory pathways.

ACKNOWLEDGEMENT

We acknowledge the Department of Pharmacology at the Krupanidhi Group of Institutions; their scientific committee.

ABBREVIATIONS

HFHFrD: High-Fat High-Fructose Diet; **ROS:** Reactive Oxygen Species; **TNF-α:** Tumor Necrosis Factor-alpha; **CNS:** Central Nervous System; **IL-6:** Interleukin-6; **IL-1β:** Interleukin-1 beta; **COX-2:** Cyclooxygenase-2; **NF-κB:** Nuclear Factor-kappaB; **IR:** Insulin Resistance; **EDTA:** Ethylenediaminetetraacetic acid; **NBT:** Nitro Blue Tetrazolium; **TBA:** Thiobarbituric Acid; **GSH:** Reduced Glutathione; **DMSO:** Dimethyl Sulfoxide; **HOMA-IR:** Homeostatic Model Assessment for Insulin Resistance; **OGTT:** Oral Glucose Tolerance Test; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animals Ethics Committee; **EPM:** Elevated Plus Maze; **OFT:** Open Field Test; **FFA:** Free Fatty Acids; **TG:** Triglycerides; **HDL:** High-Density Lipoprotein; **TC:** Total Cholesterol; **SOD:** Superoxide Dismutase; **GPx:** Glutathione Peroxidase; **CAT:** Catalase; **TCA:** Trichloroacetic Acid; **SEM:** Standard Error of the Mean; **ANOVA:** Analysis of Variance; **LPO:** Lipid Peroxidation; **Nrf2:** Nuclear factor erythroid 2-related factor 2.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study had determined the preventive role of quercetin, a bioflavonoid, in mitigating insulin resistance and anxiety-related behaviour induced by HFHFrD in male Wistar rats. Dietary exposure to high-fat high-fructose regimen resulted in significant insulin resistant as demonstrated by raised metabolic parameters, as well as the development of anxiety-related behaviours identified by behavioural models. Quercetin administration from the start of the study considerably reduced these metabolic changes, increased antioxidant defence enzyme activity, and decreased neuroinflammation indicators. Furthermore, administration of quercetin delayed the progression of anxiety-related symptoms, with significant protective results found in this study. Overall, our findings indicate that quercetin has a protective impact against diet-induced metabolic and neurobehavioral complications, through mechanism such as improved antioxidant status, anti-inflammatory response, and neuroprotective property.

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Cite this article: Reddy KP, Nanjundan PK, Shireesha KR, Boruah G, Chhetri P. Quercetin Role in Mitigating Insulin Resistance Mediated Anxiety in a High-Fat High-Fructose Diet Animal Model. Indian J of Pharmaceutical Education and Research. Krupapharmacon 2026:123-32.