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RESEARCH ARTICLE

EPIGALLOCATECHIN-3-GALLATE ATTENUATED AUTOPHAGY EXACERBATED HIGH FAT DIET-INDUCED MEMORY AND TESTICULAR TOXICITY IN RATS: THE FUNCTION OF INFLAMMATORY AND MTOR SIGNALING PATHWAYS

Onyekweli Chinedu Charles, Ben-Azu Benneth, Nwangwa E. Kingsley, Oyovwi O. Mega

Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria
Department of Pharmacology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria
Department of Physiology, Faculty of Basic Medical Sciences, Adeleke University, Ede, Osun State, Nigeria
Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria
**Corresponding Author Email:anthonyoriginal26@gmail.com*

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ABSTRACT

The study evaluated the potential effects of epigallocatechin-gallate (EGCG) against high-fat diet (HFD)-induced memory decline and testicular abnormalities. Thirty-six (36) research rats divided into six groups with six rats each were utilized. DMSO (0.1 %) and EGCG (80 mg/kg) were administered to group 1 and 2, respectively. Groups 3 and 4 were treated with HFD and HFD plus rapamycin for 56 days. DMSO or EGCG (80 mg/kg) was given to groups 5 and 6 for 29-56 days, respectively. However, animals in groups 5–6 were treated with HFD and HFD plus rapamycin individually for 56 days. The study investigated the cognitive capacity of the rats using novel-object recognition tests. The adrenal gland and prefrontal cortex of the rat testes and brain were assessed for inflammatory, autophagic state, neurochemical, and histological alterations. All rats had their serum leptin, adiponectin, and corticosterone evaluated. The findings demonstrated that HFD consumption resulted in cognitive deterioration, an imbalance of inflammatory cytokines, an increase in the lee index, and neuronal death. But in the HFD-exposed rats, EGCG therapy reduced corticosterone, leptin, and lee index, improved cognitive impairment, regulated inflammatory, autophagic, and apoptotic status, elevated adiponectin, increased brain-testes weight, and protected neuronal atrophy. Accordingly, EGCG reduced the negative effects of an HFD-induced non-spatial memory and testicular dysfunctions, possibly by reducing hypercortisolism, controlling chemo-brain activity, regulating inflammatory, apoptotic, autophagic, and metabolic hormonal status, and preventing neuronal degeneration.

KEYWORDS

High-fat diet, Epigallocatechin-gallate, Metabolic hormone, Autophagy, Inflammation and Apoptosis

1. INTRODUCTION

One of the main public health issues in the globe today is obesity. Obesity is classified as a Body Mass Index (BMI) equal 30 kg/m2 or higher (Adegoke et al., 2021). Male and female obesity rates have dramatically increased over the previous 40 years, rising from 3.2% and 6.4% in 1975 to 27.44% and 38.06% in 2021, respectively, from 8% and 13.99%. (Chen et al., 2019; Liu and Ding, 2017). Obesity increases the risk of diverse illnesses, including cancer, diabetes, hypertension, cardiovascular disease, and male infertility (Craig et al., 2017). Male reproductive function has been shown to be impaired by obesity, which results from a high-fat diet (HFD), in both humans and animals (Palmer et al., 2012; Cho et al., 2016). High-fat diet has also been linked to neuropathological changes that cause obesity-related cognitive impairment and changes to the brain (Medic et al., 2016; Balistreri et al., 2010). Preclinical research has shown that a long-term high-fat diet is linked with cognitive decline and reproductive issues (Palmer et al., 2012; Cho et al., 2016; Medic et al., 2016;Miller and Spencer, 2014; Molteni et al., 2002). Learning, memory, and executive activities are the three important functions of the brain that an obesogenic diet has the greatest detrimental effects on (Park et al., 2014). These cognitive behaviors are principally controlled by the prefrontal cortex and hippocampus of the brain (Kim et al., 2016; Mu et al., 2017). Obesity's systemic effects on reproductive health and cognitive repercussions have drawn increased attention.

There is notable evidence suggesting that HFD-induced sperm deficiency and brain damage [15] in rodents are connected with autophagy dysfunction. Of note, autophagy, which is important biological machinery involved in cellular recycling and degradation of cellular elements, is regulated by lysosomal enzymes, and has been reported to play important role in reproductive biology, and neuronal degeneration (Chen et al., 2022; Raee et al., 2023; Oyovwi, M.O., Ben-Azu, B., Edesiri, T.P., Victor, E., Rotu, R.A., Ozegbe, Q.E., 2021). Notably, rapamycin-mediated autophagy is a popular preclinical rodent model for producing autophagy, through mechanisms related to increased apoptotic activity, p70SK expression, and stimulation of mechanistic target of rapamycin (mTOR) and adenosine monophosphate-activated protein (AMPK) phosphorylations (Raee et al.,2023). Although synthetic drugs have made remarkable progress in attenuating obesity-related reproductive and cognitive damage linked to autophagy, interesting findings have been reported from compounds with reproductive and psychotropic compounds naturally distributed in plants polyphenolic extracts of medicinal plants (Ben-Azu et al., 2019; Miyoshi et al., 2015).

Green tea (Theaceae) is usually made from the leaves and buds of the

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Camellia sinensis L. plant. The health advantages of green tea have been researched the most thoroughly among the several forms of tea, including black tea, oolong tea, and green tea (Carlson et al., 2007). The active polyphenol found in green tea (Camellia sinensis), epigallocatechin-3-gallate (EGCG), has attracted some interest for its potential health benefits, including those against cancer obesity diabetes (Unno et al., 2007; Unno rt al., 2004; Rezai-Zadeh et al., 2008) and neuroprotective (Rezai-Zadeh et al., 2005: Yokogoshi et al., 2017; Pervin et al., 2018; Xicota et al., 2017; Nanjo et al., 1996; Yang and Wang, 2016; Cavet et al., 2011; Xiang et al., 2016) agents (Yang et al., 2016; Lombo et al., 2016). The numerous health benefits of EGCG are strongly correlated with its antioxidant, metal chelating, anti-carcinogenic, pro-apoptotic, anti-inflammatory, anti-apoptotic, anti-apoptotic, and anti-apoptotic properties (Xiang et al., 2016; Shankar et al., 2007; Roghani et al., 2007; Opuwari and Monsees, 2020; Li et al., 2018). These include the prevention of neurodegenerative disorders, improvement of reproductive functions, and weight control via decreased energy absorption and improved fat burning (Ikpeme et al., 2016; Isbrucker et al., 2006; Wu et al., 2012). It's interesting to note that EGCG reduces reproductive toxicity and oxidative damage in rats exposed to cyromazine and chlorpyrifos (Pervin et al., 2019). Additionally, it has been suggested that drinking green tea is safe for reproductive, liver, and renal health and has been shown to improve testicular functions in male rats (Isbrucker et al., 2006). As a result, safety investigations showed that EGCG has no teratogenic effect and is also not hazardous to the reproductive system (Liu et al., 2016). Additionally, it has been demonstrated that EGCG reduces oxidative stress and neuroinflammation to improve learning and memory impairments in rats exposed to ischemia stress (Scarpace et al., 2016) and Alzheimer's disease (Ikpeme et al., 2016). In addition, pharmacokinetic research showed that EGCG's improved blood brain barrier (BBB) permeability relates to the supplement's potential to improve cognition in naturally aged rats (Kim et al., 2004). In this connection, it was suggested that EGCG supplementation in high fat induced obesity-related diseases of neurodegeneration and reproduction in male rats would have a positive effect.

2. Materials and Methods

2.1 Materials

Animals: 36 Wistar rats, aged 6 to 8 weeks, were used in the study. They were housed in a controlled setting with a 12-hour light/dark cycle. They underwent acclimatization for 14 days and therapy for 56 days. The experiment adhered to NIH standards and received ethical approval from the Faculty Animal Care and Use Research Ethics Committee (REC/FBMS/DELSU/22/147).

Preparations of drugs: Sigma-Aldrich Chemical Company (St. Louis, MO, USA) provided the EGCG, which was produced in 0.1% DMSO and administered orally in accordance with previous dosing (Ben-Azu et al., 2020). Rapamycin was selected within the dose-response effect and previous investigation by Scarpace, et al.(Schlumpf et al., 1974). The doses used were in agreement with the recommendations for converting human doses to those for animals. The length of time EGCG was administered was determined by the spermatogenesis' end point. The doses selected were in line with the recommendations for extrapolating human doses to animal doses. The daily doses were given orally between the hours of 8 and 9.

Diet formulation: In this study, two different types of diets-a specific high fat diet (HFD) (35%) intended to induce obesity in rats and cause cognitive and reproductive impairments were used.

Typical rat chow diet: As described in Kim et al., the typical rat chow diet was created (Lillie and Fullmer,1976). The typical normal rat diet contains 65% CHO, 5% fat, 20% crude protein, and 5% fibers. It is also made up of 350g concentrate, 600g maize, calcium carbonate, dicalcium phosphate, sodium chloride, magnesium oxide, and vitamins (50 g). The diet's metabolic energy was 2813 kcal/kg, with 8% of it derived from fat.

The high fat diet: According to Kim et al., it contained 50 g of vitamins, minerals, and fibers along with 300 g of concentrates, 350 g of corn, and 300 g of beef tallow (Lillie and Fullmer, 1976). Crude protein made up 20% of the HFD, followed by fat at 35%, CHO at 40% (starch at 35% and sugar at 5%), and vitamins, minerals, and fiber at 5%. This diet metabolic energy was 5130 kcal/kg, with 61% of it coming from fat. The HFD, consisting of lard, sunflower oil, and starch, was created by adding 30% lard or beef tallow and 5% sunflower to the control diet.

Each rat's body weight was measured in grams using a weighing balance after every seven days during the entire experiment. The Lee index was used to define the obesity index. According to Lee's formula, the Lee index was calculated (1980).

$$\text{Lee index } (\%) = \sqrt[3]{\frac{\text{Body weight (g)}}{\text{Nose to anus length (cm)}}} \times 1000.$$

Rats were deemed obese and used in the study if their Lee obesity index value was greater than 310g/cm, which is similar to a BMI of 30 in humans.

2.2 Methods

2.2.1 Experimental protocol

Six experimental groups of six animals each were created using 36 experimental rats. **Group I:** This group served as control. Rats were treated with normal rat chow diet plus 0.1% DMSO for 56 days. **Group II:** Rats in this group were treated with EGCG alone at a dose of 80 mg/kg daily dissolved in 0.1% DMSO orally for 28 days (45). **Group III:** This group was treated with HFD plus 0.1% DMSO daily for 56 days. **Group IV:** This group was treated with HFD plus Rapamycin at 1 mg/kg body weight orally for 56 days to induced autophagic flux (46). **Group V:** This group was treated with HFD for 56 days plus EGCG at 80 mg/kg body weight orally for 28 days starting from day 29-56. **Group VI:** This group was treated with HFD plus Rapamycin for 56 days plus EGCG at 80 mg/kg body weight from day 29-56.

2.2.2 Cognitive function test

NORT performance: The habituation, trial, and test phases made up the object recognition (NOR) task. Plastic made up the arena, which was 43 cm by 31 cm by 16 cm. During the trial phase, each rat was left alone in an open field for five minutes with two identical objects (A1 and A2). After that, the rat was put back in its usual cage. The study involved washing the arena and objects with 70% v/v ethanol to remove smell cues. A short-term memory (STM) test was conducted five minutes after the trial phase, with each rat repositioned with identical objects swapped with a different one. The object placement was counterbalanced to prevent bias, with half of each group seeing the new object on the left side. Before using the NOR task to test a rat's long-term memory (LTM), a wash-out time of five days was permitted. The process followed the same steps as for STM, with the exception that rats were exposed to the test phase 24 hours following the trial phase. Using a stopwatch, we manually kept track of how much time was spent investigating each object in each phase. When a rat's head was pointed in the direction of an object within 2 cm of it or when the nose made contact with the object, the rat was rated as exploring. Measured variables included the amount of time (in seconds) spent examining the familiar object (Tf), the novel object (Tn), and the combined amount of time (Tn + Tf). The following equation was used to calculate the percentage of discrimination index (%DI): %DI = Tn divide by Tn + Tf multiply by 100% (Novelli et al., 2007).

2.2.3 Blood and tissues preparation

At the conclusion of the trial, the animals were fasted for the night before being put to sleep by cervical dislocation. Blood was then drawn through heart punctures and tested for leptin and adiponectin using ELISA techniques. The testes and the brain were dissected out for biochemical assay (B cell lymphoma-2, Caspase 3, tumor necrotic factor-α, interleukin-1ß, necrotic factor- kappa ß, nitrite, Beclin-1, mammalian target of rapamycin, glutamate, dopamine, norepinephrine) and histological studies including that of the adrenal gland. The reproductive organ was harvested, freed from adherent tissues, and weighed on an electronic weighing balance.

Estimations of leptin, adiponectin and corticosterone in serum: Using an ELISA kit from Cayman Ltd. in the United States in accordance with the manufacturer's instructions, the levels of leptin, adiponectin, and corticosterone in serum were measured.

2.2.4 Testicular inflammation markers test

Using the ELISA kit bought from IL-1ß) (R & D systems, USA and Thermo Fisher Scientific espectively, testicular cells were used to assess and quantify (pg/mg protein) proinflammatory cytokines in the testes, including nuclear factor kabba (NF-kB), tumor necrosis factor (TNF), cyclooxygenase-2 (COX-2), and interleukin-1.

Examination of testicular apoptotic markers: The expression of Bcl-2 and caspase-3 in testicles was evaluated using commercial ELISA kits from Sigma-Aldrich and BioVision, Inc., following manufacturer's recommendations.

Evaluation of autophagic related protein markers in testicular and brain homogenate: A commercial ELISA kit from MyBioSource, BioVision, or Abbexa was used to measure the mechanistic expressions of BECLIN-1 and mTOR in testes and brain homogenate, respectively.

Estimation of dopamine concentration: The reaction mixture used in the study, consisting of 0.05 mL of 0.4 M HCl, 0.1 mL of EDTA, 6.9 pH of sodium acetate buffer, and 0.1 mL of iodine solution, is used to calculate the amounts of dopamine in the brains. After 2 minutes, 0.1 mL of Na2SO3 was added to stop the reaction. After 1.5 minutes, 0.1 mL of acetic acid was added (Picklo Sr et al., 2017). After 6 minutes of heating to 100°C, a spectrofluorimeter's excitation and emission spectra are read. Norepinephrine measurements are made at 395–485 nm, while dopamine readings are made between 330–375 nm.

Measurement of glutamate concentration: The supernatant of the brain homogenate (1 mL) was evaporated and then reconstituted in distilled water. Using glutamate and GABA solutions, the sample was spotted on Whatman No. 1 chromatography paper. After that, the paper was put inside a solvent chamber. After being dried and coated with ninhydrin reagent, the first and second papers were baked at 100°C for four minutes. CuSO4 in 75% ethanol was used to elute the glutamate-carrying parts of the sample. A spectrophotometer was used to test their absorption.

Estimation of norepinephrine level: The process involved adding iodine (0.1 ml) solution, 0.05 ml of 0.4 M HCl, 0.1 ml of EDTA/Sodium acetate buffer (PH6. 9). 0.1 ml of Na2SO3 solution was added after 2 minutes to stop the process. 1.5 minutes later, 0.1 mL of acetic acid was added to the aqueous phase. After heating to 100°C for 6 minutes, the spectrofluorimeter's excitation and emission spectra were recorded, with readings for nor-adrenaline collected at 330-375 nm and 395-485 nm.Histopathological examination of brain: The brain slices were stained with Haematoxylin and eosin stain (Howes and Houghton, 2003). The Olympus microscope was used to analyze the stained sections.

Statistical analyses: With the aid of the post hoc Bonferroni's t-test and an ANOVA, differences between groups were found using the GraphPad PRISM 8 program. P values less than 0.05 were used to evaluate statistical significance, along with mean and SEM.

3. Interpretation of Results

3.1 Effect of EGCG on rapamycin exacerbated HFD-induced non-spatial memory impairment in male Wistar rats

Using novel object recognition test (Figures 1a–b), the study investigated the effects of EGCG on HFD-induced memory impairment. In comparison to the control group, EGCG had no discernible impact on the discrimination index in either short- or long-term memories. However, both the short-term (Figure 1a) and long-term (Figure 1b) discrimination index in the HFD- treated rats and the combination of HFD and rapamycin exposure were lower. In rats exposed to HFD and those receiving HFD and rapamycin, EGCG markedly improved the discrimination index.

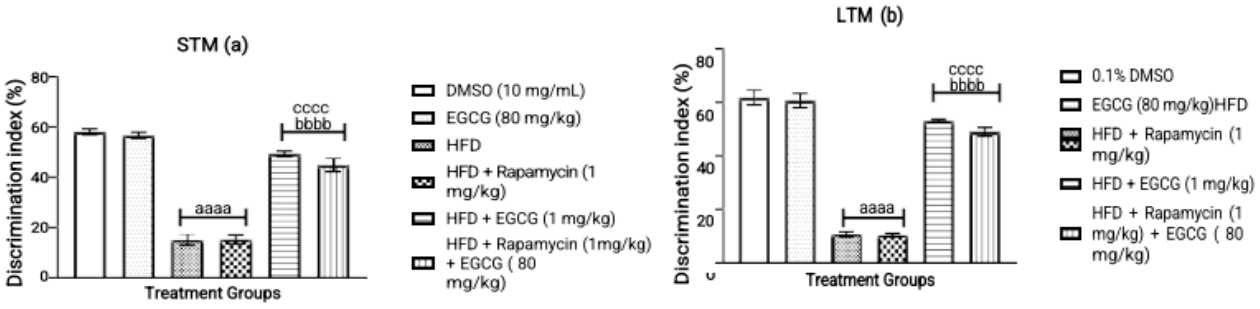

Figure 1a,b: EGCG prevents rapamycin exacerbated HFD-induced memory impairment in male Wistar rats using novel object recognition performance: short-term memory (STM) (a). Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$ was used as comparism to control group; $^{bbbb}p < 0.0001$ was used as comparism to HFD; $^{cccc}p < 0.0001$ Vs rapamycin plus HFD group (One way ANOVA was applied following Bonferroni's post-hoc).

3.2 Effect of EGCG on rapamycin mediated HFD-induced weight changes and obesity as indicated by Lee index in rats.

The effect of EGCG on HFD and rapamycin-induced increase in body weight and obesity as indicated by Lee index in rats are shown in Figures 2a-c. HFD, as well as rapamycin treated- HFD produced significant ($p < 0.05$) increase in Lee index values of rat's body weight (Figure 2a) however with marked decrease in brain (Figure 2b) and testicular (Figure 2c) weights. But treatment with EGCG (80mg/kg p.o) significantly ($p < 0.05$) reversed HFD or HFD in combination with rapamycin-induced obesity as indicated by decrease in Lee index values (Figure 2a) as well as increased brain (Figure 2b) and testicular (Figure 2c) weights relative to HFD or HFD plus rapamycin-treated rats alone. In EGCG treated HFD alone, there was no significant difference as compared to EGCG treated HFD plus rapamycin.

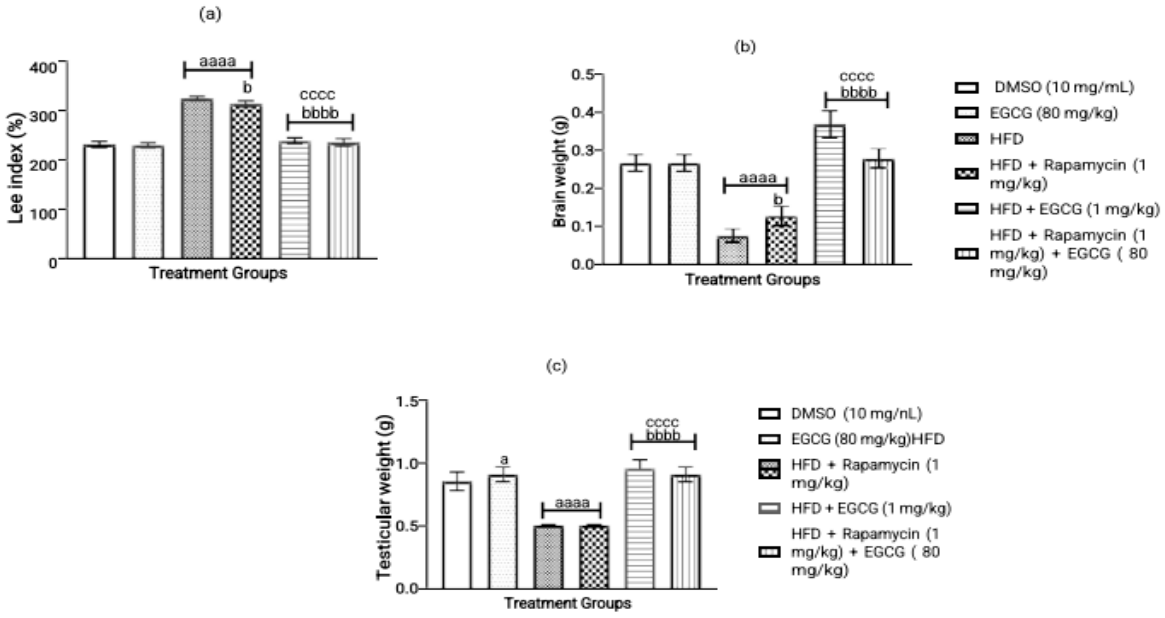

Figure 2a-c: Effect of EGCG on rapamycin mediated HFD-induced increase in Lee index. Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$ was used as comparism to control group; $^{bbbb}p < 0.0001$ Vs HFD; $^{cccc}p < 0.0001$ Vs rapamycin plus HFD group (One way ANOVA was applied following Bonferroni's post-hoc).

3.3 Effect of EGCG on rapamycin enhanced HFD-induced changes on leptin, adiponectin and corticosterone concentrations in rats

Fig. 3a-b depicts the effects of EGCG HFD and rapamycin-induced alterations in leptin and adiponectin. Following the results of the Bonferroni post-hoc test, which showed that HFD or HFD with rapamycin significantly (p less than 0.05) raised leptin concentration in comparison to the normal control group, one-way ANOVA was used (Fig. 3a). In comparison to HFD group or HFD plus rapamycin group, EGCG therapy significantly reduced the increase in leptin level caused by HFD and HFD plus rapamycin (Fig. 3). When compared to the normal control group, HFD or HFD + Rapamycin significantly (p less than 0.05) lowered adiponectin concentration, according to one-way analysis of variance (ANOVA) and Bonferroni's post-hoc test results (Fig. 3b). When compared to the HFD group or the HFD plus rapamycin group, EGCG (80mg/kg b.w.) therapy significantly corrected the HFD and HFD plus rapamycin-induced decrease in adiponectin levels (Fig. 3b). Furthermore, rats treated with HFD or HFD rats co-treated with rapamycin showed a substantial (p less than 0.05) rise in corticosterone concentration. In comparison to HFD and HFD + rapamycin-treated rats, EGCG (80 mg/kg, p.o.) significantly (p less than 0.05) reduced the corticosterone rise caused by HFD and rapamycin (Fig. 3c).

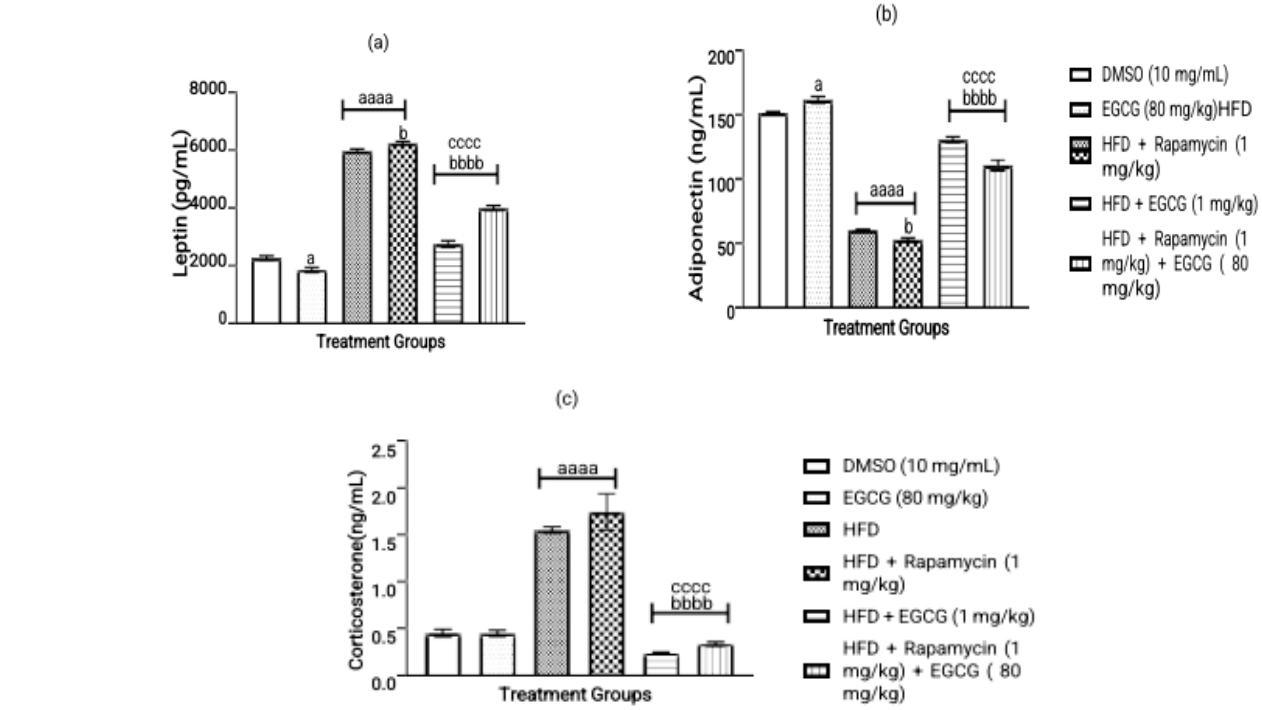

Fig 3a-c: EGCG abates rapamycin enhanced HFD-induced changes in leptin, adiponectin and corticosterone levels. Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$, $^{a}p < 0.05$ Vs control group; $^{bbbb}p < 0.0001$ Vs HFD; $^{cccc}p < 0.0001$ Vs rapamycin plus HFD group.

3.4 Effect of EGCG on rapamycin-HFD-induced changes on neurochemical concentrations in rat brains

The study found that HFD or HFD plus rapamycin significantly increased glutamate (Fig 4a), dopamine (Fig 4b), and noradrenaline (Fig 4c) levels compared to the normal control group. Nonetheless, EGCG (80mg/kg b.w.) treatment significantly reversed HFD and HFD plus rapamycin-induced decrease in serotonin, glutamate, dopamine and noradrenaline concentrations when compared with HFD or HFD plus rapamycin group respectively (Fig 4a-c).

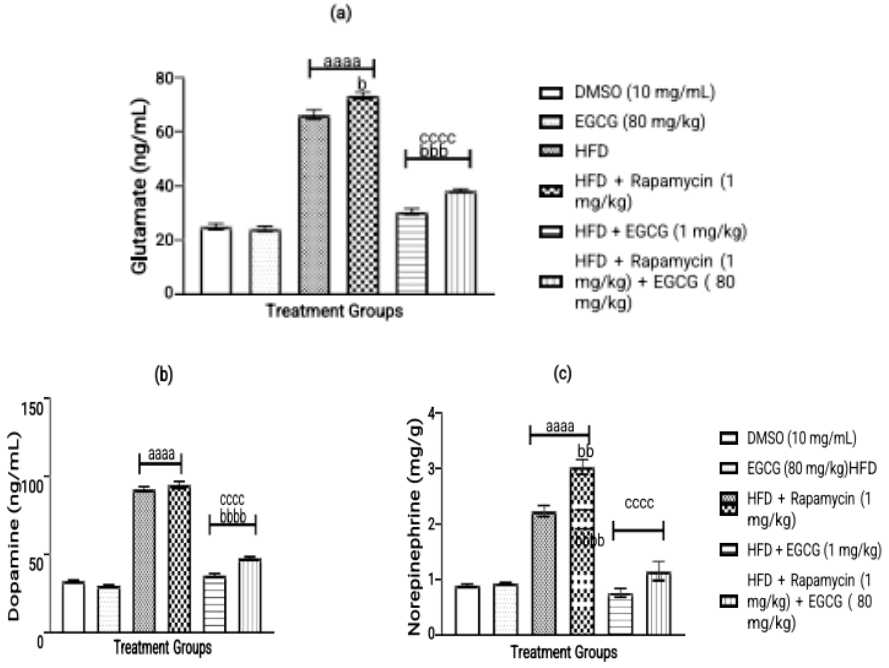

Fig 4a-c: EGCG reverses HFD and rapamycin-induced alteration in neurochemical concentrations in rats: a) glutamate, b) dopamine, c) noradrenaline. Bars depicts the mean and

S.E.M (n = 6). aaaap < 0.0001 was used as comparism to control group; bbbbp < 0.0001 was used as comparism to HFD; ccccp < 0.0001 was used as comparism to rapamycin plus HFD group

3.5 Effect of EGCG on rapamycin exaggerated HFD-induced pro-inflammatory cytokines in rats brains

In line with Fig 5a-d, when compared to control mice, HFD and HFD + rapamycin exposure significantly increased the levels of TNF-α (Fig. 5a), IL-1β (Fig. 5b), NF-kß (Fig. 5c), and COX- 2 (5d). According to the study, EGCG administration dramatically decreased the high levels of pro-inflammatory cytokines in rats when compared to the group that received only HFD treatment. (Fig 5a-d).

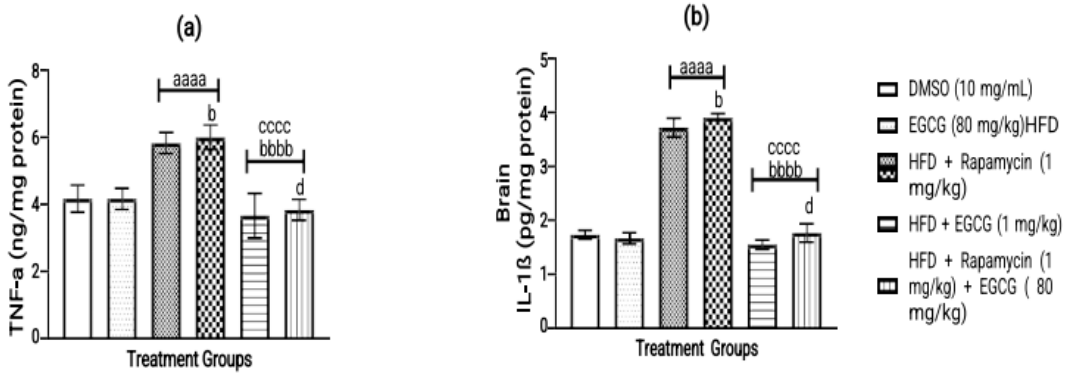

Fig 5a-d: EGCG inhibits HFD-induced release of pro-inflammatory cytokines in rat's brain. a) Tumor necrotic factor-alpha TNF-α, b) interleukin-1β (IL-1β). Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$, $^{a}p < 0.05$ Vs control group; $^{bbbb}p < 0.0001$, $^{b}p < 0.05$ Vs HFD; $^{cccc}p < 0.0001$ Vs rapamycin plus HFD group; $^{dd}p < 0.01$ Vs HFD + EGCG group.

3.6 EGCG inhibits HFD-induced release of pro-inflammatory cytokines in rat testes

According to Fig. 6a-c when compared to control animals, both HFD and HFD + rapamycin treatment demonstrated a substantial increased TNF- α (Fig. 6a), IL-1β (Fig. 6b), levels. However, compared to rats receiving HFD therapy alone, EGCG post- treatment significantly reduced the elevated pro-inflammatory cytokine levels.

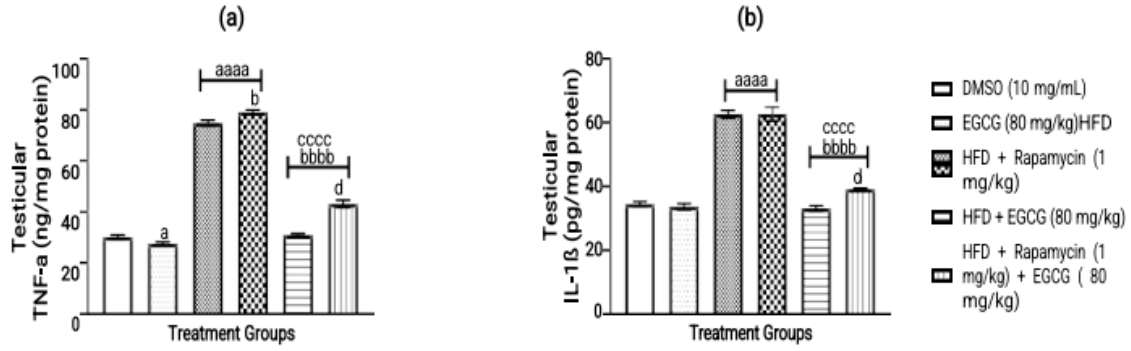

Fig 6a-c EGCG inhibits HFD-induced release of pro-inflammatory cytokines in rat testes. a) tumor necrotic factor-alpha (TNF-α), b) interleukin-1β (IL-1β). Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$, $^{a}p < 0.05$ Vs control group; $^{bbbb}p < 0.0001$, $^{b}p < 0.05$ Vs HFD; $^{cccc}p < 0.0001$ Vs rapamycin plus HFD group; $^{dd}p < 0.01$ Vs HFD + EGCG group

3.7 Effect of EGCG on HFD-induced alteration in testicular autophagy in rat

The research demonstrates that EGCG counteracts the effects of HFD on testicular autophagy- related protein levels in rats, restoring decreased mTOR (Fig. 7a) and higher BECLIN-1 (Fig. 7b) levels, but not significantly changing mTOR or BECLIN-1 levels compared to normal control groups.

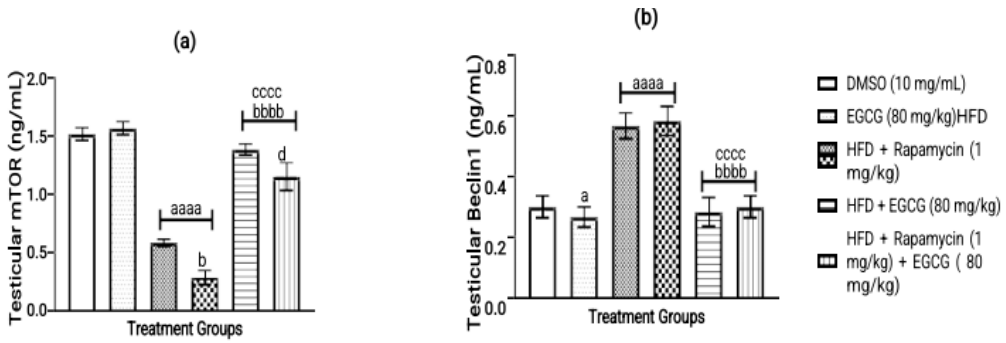

Fig 8a-b: EGCG counteracts the effects of HFD on testicular autophagy-related protein levels in rats. a) Mammalian Target of rampamycin (mTOR) and b) autophagy (Atg-7) activities. Bars depicts the mean and S.E.M (n = 6). aaaap < 0.0001, ap < 0.05 Vs control group; bbbbp < 0.0001, bp < 0.05 Vs HFD; ccccp < 0.0001 Vs rapamycin plus HFD group; ddp < 0.01 Vs HFD + EGCG group

3.8 Effects of EGCG on HFD and rapamycin-mediated pathological alteration of the adrenal glands of rats

Plate's 1a-f shows how EGCG affected the histopathological alterations brought on by rapamycin and HFD in the rat adrenal gland. EGCG alone did not alter the adrenal gland's architecture, revealing a normal cortex zonation pattern and a normal medulla layer compared to a normal control group. As opposed to the normal controls, rats given HFD or rapamycin in this study displayed minor vascular congestion within the medulla, which was remedied by EGCG given at a level of (80 mg/kg/day).

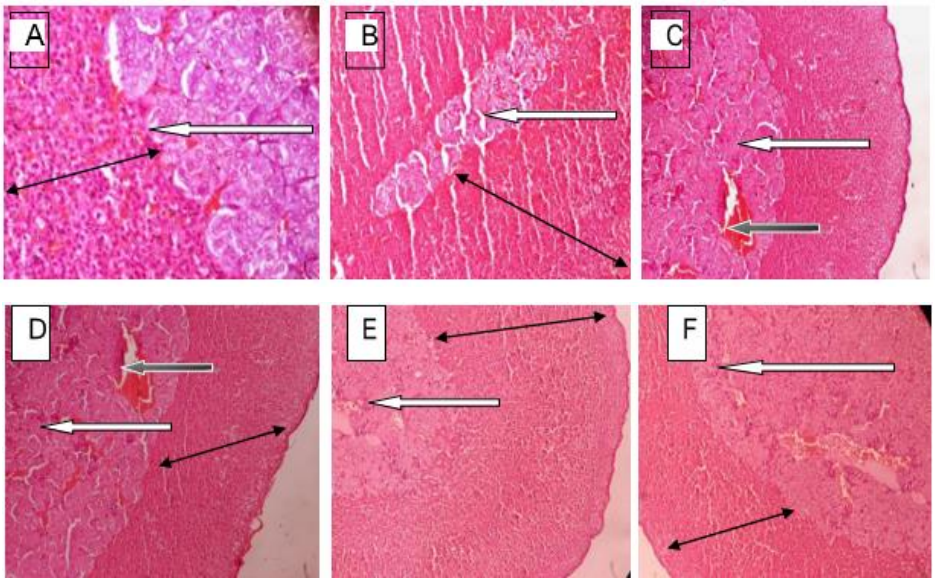

Plate 1a-f: Photomicrographs demonstrating the impact of EGCG on the histopathological alterations brought on by rapamycin and HFD in the rat adrenal gland. Control (0.1% DMSO), EGCG (80 mg/kg), HFD, HFD + Rapamycin (1.0 mg/kg), HFD + EGCG (80 mg/kg), and HFD + rapamycin (1.0 mg/kg) + EGCG (80 mg/kg) are shown in the following order: A, B, C, D, and F. The zonation pattern of the cortex was clearly visible on slides A and B for plate 2, showing columns of clear cells in the zona fasciculata, clusters of stainable cells in the zona glomerulosa, and cells with acidophilic cytoplasm in the zona reticularis. Lysed red cells were visible on slides C and D among medullar cells with vascular congestion in the medulla. Slides E and F are known for displaying the typical anatomy of the adrenal glands. The arrows in this image, which are black and white, respectively, denote vascular congestion and lysed red blood cells, a normal zonation pattern of the cortex, and a normal medulla layer. For all plates, the H and E stain was applied using a calibration bar of 0.01 mm (10 µm) and an original magnification of x100.

3.9 Effects of EGCG on HFD and rapamycin-induced histological alteration of the prefrontal cortex of rats

In plate 2a-f, the effects of EGCG on rapamycin- and HFD-induced histological alterations in the rat prefrontal cortex are shown. When compared to the normal control group, rats treated with EGCG alone showed abnormalities in the prefrontal cortex's laminae, neuronal cells, and architecture. As seen by degenerated, hyalinized neuronal cells and dilated capillaries (Plates C and D) when compared with normal controls, rats treated with HFD and rapamycin, respectively, showed reduced neuronal cells of the prefrontal cortex. However, EGCG therapy reduced HFD- induced hyalinization and cell degeneration in prefrontal neuronal cells in comparison to HFD groups. In addition, rats given EGCG displayed less degenerating neural cells than those given HFD.

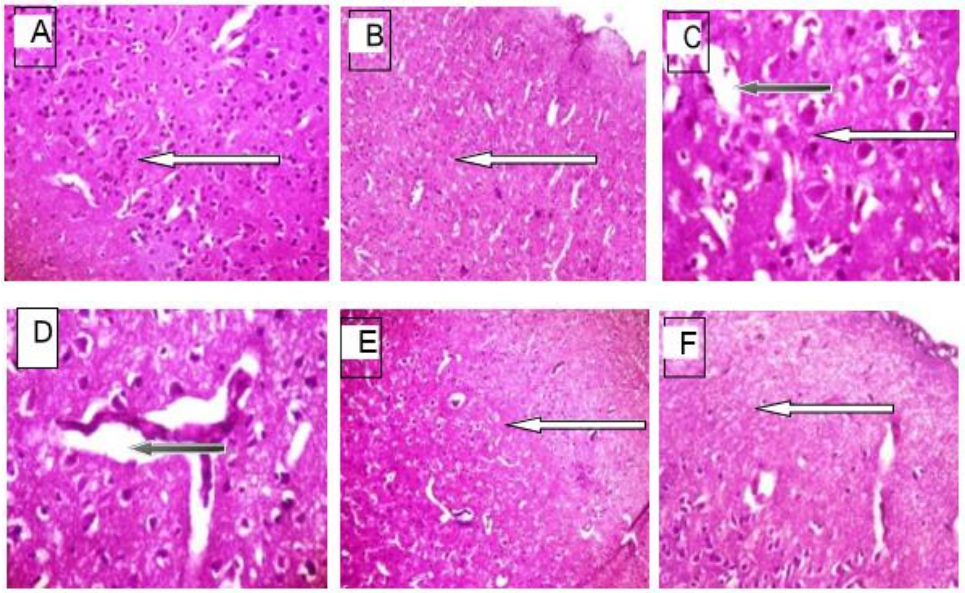

Plate 2: Photomicrographs displaying the impact of EGCG on rats' prefrontal brain alterations caused by the HFD. Control (0.1% DMSO), EGCG (80 mg/kg), HFD, HFD + Rapamycin (1.0 mg/kg), HFD + EGCG (80 mg/kg), and HFD + rapamycin (1.0 mg/kg) + EGCG (80 mg/kg) are shown in the following order: A, B, C, D, and F. Normal neuronal cells on a normal stroma were visible on slides A and B. Several severely deteriorated neuronal cells with dilated capillaries were visible on slides C and D. (Black arrow). Normal neuronal cells on a normal stroma were shown on slides E and F. Black arrow indicates necrosis on normal stroma; white arrow indicates normal neural cells. Original magnification of haematoxylin-eosin stain: x100; calibration bar: 0.01 mm (10 m); for all figures.

4. Discussion

Studies on both humans and animals have revealed that HFD-induced obesity is connected to testicular dysfunction and non-spatial memory problems (Palmer et al., 2012; Medic et al., 2016). Notably, obesity has been linked to poor molecular and functional neuronal homeostasis, which increases the risk of brain injury, behavioral problems, and cognitive deficiencies (Balistreri et al., 2010). The cognitive decline and neurochemical changes caused by HFD are produced by significantly more complex molecular pathways than are now understood, despite the considerable progress made in this field of neuropathophysiology. To combat non-spatial memory impairment and anomalies in the testicles caused by HFD, the possible neurotherapeutic potential of EGCG was examined. In the current work, rats with non-spatial memory deficiencies and testicular abnormalities brought on by HFD were treated with EGCG therapy. Treatment with EGCG was able to reverse the cognitive deficit and the elevated norepinephrine, glutamate, corticosterone, dopamine, and leptin levels in the brains of HFD-treated rats. Additionally, compared to rats treated with EGCG, treatment with HFD increased levels of the Lee index, Beclin-1, caspase-3, NF-kB, IL- 1ß, and TNF while decreasing levels of adiponectin, mTOR, the discriminating index, testicular/brain weight, and Bcl-2. However, EGCG corrected the non-spatial memory and

testicular deficits brought on by HFD. As evidenced by a higher Lee index, the high-fat diet used in the current study was effective in encouraging obesity. The observed greater lee index in HFD rats is consistent with Novelli et al. (Malafaia et al., 2013). The findings of Picklo et al., who demonstrated the obesogenic effect of a saturated fat diet in an animal model, are consistent with this observation (Vigueras-Villaseñor et al., 2011). The increasing Lee obesity index supported the findings that long-term consumption of high-fat meals led to obesogenic conditions. It has been determined that the obesity index is the most accurate predictor of intra-abdominal fat in rats and, consequently, of central obesity (Aizawa-Abe, et al., 2000). There is a correlation between the Lee index and fat mass. Although the naso-anal length in rats is only a somewhat reliable indicator of fat-free mass, the Lee index is currently employed as a quick and reliable tool to detect obesity in rodents that have undergone a weight gain procedure (Power and Schulkin, 2008). By boosting satiety and energy expenditure, the hormone leptin, which is mostly produced by adipocytes, helps to regulate body weight (Mayes and Watson, 2004; Lin et al., 2000). According to Power and Schulkin (Mzhelskaya et al., 2020), leptin has both stimulatory and inhibitory effects on the reproductive system. According to Mayes and Watson, the leptin concentration is correlated with the amount and distribution of body fat, so that in rodents and humans, the higher the body weights, the higher the leptin concentration (Balistreri et al., 2010;Lin et al., 2000). Increased fat accumulation is likely the cause of the higher serum leptin levels found in the current study. According to previous research in other studies in the literature that showed high leptin levels in models of rodent diet induced obesity (DIO), which demonstrated that Obesity induced by high-fat diets (HFDs) occurs in three stages: early response due to exogenous leptin sensitivity, increased food intake, and brain changes, with significant leptin increase (Duszka et al., 2020; Duszka et al., 2020;). This conclusion is supported by Mzhelskaya et al., who discovered that leptin loses its anorexigenic activity on hypothalamic neurons in HFD-induced obesity, increasing hunger and the creation of fat mass (Aouichat et al., 2020).

However, EGCG therapy reduced the negative changes in leptin levels caused by HFD consumption. These findings suggested that lower plasma leptin levels following EGCG medication could be attributed to decreased lipid formation in white adipose tissue. We measured the levels of serum adiponectin in rats that had undergone an HFD in order to better understand the physiological mechanisms by which EGCG exerts its therapeutic intervention on levels of insulin and blood glucose. Adipokines have functions in insulin sensitization, immunology, neuroendocrine function, glucose and lipid metabolism regulation, energy homeostasis, anti-inflammatory, anti-atherogenic, and cardiovascular function (Dwaib et al., 2021; Saad et al., 2017; Saad et al., 2017;Landrier et al., 2017; Chijiokwu et al., 2022). In research, it was found that adiponectin influences the sensitivity of diabetic mice to insulin (Yamauchi et al., 2002). People with type 2 diabetes, coronary artery disease, and obesity brought on by the HFD had low levels of adiponectin (Kogel et al., 2021). As previously reported this study's HFD-treated rats showed a substantial drop in serum adiponectin levels (Kogel et al., 2021). Insulin resistance, poor insulin sensitivity, and the emergence of obesity have all been associated with lower blood levels of adiponectin (Kim et al., 2004; Kogel et al., 2021;Fasshauer and Paschke, 2003). The suppression of gluconeogenesis and an increase in lipid oxidation caused by adiponectin have been found to promote AMP-activated protein kinase (AMPK), which regulates glucose metabolism and improves insulin sensitivity (Cialdella-Kam et al., 2017). Type 2 diabetes, coronary artery disease, and obesity brought on by the HFD were all associated with low levels of adiponectin (Kogel et al., 2021). A considerable decrease in serum adiponectin was seen in this investigation in the HFD- treated rats, as was previously reported (Kogel et al., 2021). Insulin resistance, poor insulin sensitivity, and the emergence of obesity have all been associated with lower blood levels of adiponectin (Kogel et al., 2021; Wu et al., 2012;Fasshauer and Paschke, 2003). Improved insulin sensitivity and glucose metabolism regulation have been seen as a result of adiponectin's stimulation of AMP-activated protein kinase (AMPK) by reducing gluconeogenesis and enhancing lipid oxidation (Cialdella-Kam et al., 2017). Inflammation, which is connected to hyperglycemia, is one of the key pathogenic aspects of HFD-induced obesity (Kogel et al., 2021; He et al., 2017). As demonstrated below, the chronic inflammatory flux that ensues may result in the development of insulin resistance in tissues (Fasshauer and Paschke, 2003). Notably, it has been demonstrated that fat accumulation in adipocytes increases brain-testicular TNF- production and that TNF- causes insulin resistance in obese animal models (Niu et al., 2019). TNF-α, and IL-1, levels in the brain were considerably greater in the HFD-treated rats than in the control group. EGCG therapy lowered brain-testicular TNF-α, and IL-1 levels to levels comparable to the control group.

Recently, it was shown that EGCG can reduce inflammation and increase insulin sensitivity (Makaronidis and Batterham, 2018). In response to growth factors, cytokines, and pro-inflammatory substances, the inducible isoform COX-2 quickly expresses itself in a variety of cell types. Hormonal cues and IL1 regulate testicular COX-2 expression in these somatic cells (Ben-Azu et al., 2019). Decreased hormonal input and increased IL1 may therefore be the root of the abnormal rise in COX-2 expression. The role of COX-2 in inflammatory reactions in peripheral tissues has recently come to light. The brain's production of COX-2 has been associated with pro-inflammatory actions that are hypothesized to play a part in the neurodegenerative processes of a variety of acute and chronic disorders. According to previous research, HFD-induced obesity is linked to abnormal adrenal cortical function as seen by elevated corticosterone levels (Ghosh and Mukherjee, 2018; Wang et al., 2019). These data show that HFD exposure has a significant and long-term impact on the development of neuro-metabolic regulating mechanisms. EGCG, on the other hand, prevented the HFD-induced rise in corticosterone in rats. Male rats with HFD-induced obesity had alterations in testis and brain weights. These findings are consistent with the study's findings (Carlini, 2011). Numerous earlier studies have demonstrated that consuming HFD might result in histological abnormalities in the brain and reduced neuro-reproductive organ weights (Francis and Stevenson, 2013; Antunes and Biala, G, 2012). The current research discovered that HFD-induced brain-testicular damage was accompanied by a reduction in the relative weights of the testes and brain, which may be the result of hypercorticism and excessive apoptosis in the brain-testicular structure. The negative effects of HFD exposure, on the other hand, were mitigated by EGCG treatment. In this work, a new object recognition task (NORT) was employed to assess HFD- induced non spatial memory deficit. However, as object identification studies rely on spontaneous exploratory behavior, they do not completely rule out the possibility that some animals have a preference for a given object that is not influenced by its novelty or familiarity (Shoelson et al., 2007). As a result, the capacity to detect novel items is thought to be one of the most important tests for measuring an animal's aversion to new objects, which influences working or learning memory. A high-fat diet has previously been demonstrated to impair non-spatial memory as judged by the NORT paradigm (Piero et al., 2012). However, there was a noticeable increase in the amount of time spent exploring new items in the EGCG-treated HFD rats. The NORT, which is based on rats' natural tendency to investigate strange objects more thoroughly than they do familiar ones. This identification memory test is non-rewarding, well-validated, and significant to ethology (Bélanger et al., 2004). This paradigm was employed in this study to evaluate a memory-improving drug's effectiveness against memory impairments brought on by HFD.

In the object identification test, the effects of EGCG on memory impairment were further examined. Between all EGCG treated groups and the control group, there was no statistically significant difference in the total amount of time spent examining two objects, suggesting no variation in visual recognition abilities. In this experiment, poor eyesight, dilated pupils, and impaired lens adaptation may all be brought on by HFD treatment. Because of insulin sensitivity, prolonged HFD use is frequently linked to hyperglycemia (Cavalheiro et al., 2022). One of the metabolic effects of chronically high blood sugar levels is retinal disease, which reduces vision (Ben-Azu et al., 2018). Rats in the HFD group performed poorly, which could be attributed to their poor vision, which causes them to improperly interpret the surrounding external cues needed to explore the novel object. These results corroborated those of Bélanger et al. (Biyong et al., 2021), who reported that diabetic ZDF rats that were left untreated for eight weeks developed cataracts, which affected their ability to perform well in the labyrinth. By analyzing the data as a percentage discrimination index, the current study demonstrated the effectiveness of EGCG treatment to prevent and reverse HFD- induced memory loss of novel object recognition performance. The percentage discrimination index of the EGCG-treated rats was similar to that of the healthy control group, indicating that EGCG can alleviate memory impairment brought on by HFD. It should be noted that HFD's influence on both short-term and long-term memory impairment in memory loss rats is most likely caused by glutamate excitoxicity of neuronal cells caused by enhanced N-methyl-d-aspartate receptor (NMDAR) activation, clarifying its probable neurodegenerative process. This finding also suggested that neurotoxic effects of HFD could be used to assess neurochemical changes linked to the pathogenesis of neurodegenerative and developmental illnesses (Fritz et al., 2018; Guo and Xia, 2013). The neurochemical pathways behind neurological diseases are frequently studied using animal models. According to Ben-Azu et al. the intricacy of the wide range of neurological symptoms associated with neurodegenerative diseases makes it impossible to reproduce important aspects of the disease (Guo and Xia, 2013).

According to a modest body of epidemiological data, HFD exposure has lately been related to the development of a wide range of learning difficulties and neurodevelopmental disorders, including autism, ADHD, and schizophrenia (Osna et al., 2011). According to Fritz et al.'s 2018 study, mice given a HFD have longer excitatory postsynaptic currents because their glutamate buffering is reduced, and their glutamate

receptors are muted (Osna et al., 2011). This confirms the findings of the study that obesity is associated with altered glutamate transmission and enhanced dopamine transmission in the dorsal striatum. The effects of high fat consumption on brain functions and the possible importance of these mechanisms in aggravating nonhomeostatic eating are now better understood as a result of these results. However, in rats with HFD-induced obesity, EGCG treatment raises neurotransmitter levels.

Furthermore, autophagy has been linked to the development of various diseases, including cancer liver disease, kidney disease, reproductive disease [90], and neurological disease [91] (Zhang et al., 2012; Banerjee et al., 2010; Aparicio et al., 2016; Wang et al., 2020). A recent study revealed that autophagy and apoptosis jointly cause germ cell death during mouse spermatogenesis, and autophagy has also been connected to sperm survival (Wang et al., 2013). As is well known, mTOR is an important gatekeeper that negatively controls autophagy (Chen et al., 2022; Raee et al., 2023; Oyovwi et al., 2021). It is important to remember that mTOR is necessary prior to autophagy during oxidative stress (Wang et al., 2013). We consequently proposed that one main mechanism by which HFD-induced obesity increased potential autophagy and produced reproductive harm was the oxidative stress-mediated mTOR signaling pathway.

However, the current study found that HFD triggered autophagy in rats which was further exacerbated by rapamycin exposure, as evidenced by increased Beclin-1 protein levels and decreased mTOR. This observation is consistent with the findings of Mu et al., who stated that autophagy is overactivated in male mice with HFD-induced spermatogenesis deficit (Chen et al., 2022). Rapamycin-treated HFD rats, on the other hand, showed larger changes in autophagic related protein (Beclin-1 and mTOR). Interestingly, EGCG treatment of HFD rats improved protein mTOR levels and inhibited autophagy as measured by decreased Beclin-1 in the tests. These findings suggest that inhibiting excessive autophagy may protect against HFD- induced impairment in reproductive functioning. Apoptosis and autophagy interact in a complicated manner in general. The independent occurrence of both processes; apoptosis and autophagy, which can either promote or inhibit one another (Chen et al., 2022; Raee et al., 2023; Oyovwi et al., 2021). Numerous studies have shown that autophagy triggers programmed cell death in Caenorhabditis worms, and that autophagy activity is a cell death trigger in other organisms as well. Similar to prior findings, our findings showed that HFD-induced obesity could cause apoptosis, as evidenced by increases in caspase-3 and decreases in Bcl-2, as well as changes in cellular ultrastructure (Fasshauer and Paschke, 2003). EGCG, on the other hand, substantially corrected HFD- induced apoptosis and ultrastructural damage. Notably, EGCG protected against HFD-induced cell death by decreasing autophagy flux as measured by Beclin-1, which suggests that EGCG demonstrate enhancing spermatogenic activity in rat testes.

5. Conclusion

Finally, we found that suppressing autophagy and apoptosis corrected HFD-induced non-spatial memory and testicular deficits in rats. Our findings provide crucial insights into the use of EGCG in the treatment of HFD-induced brain-testicular impairments via protective effect against adrenal gland, brain, and testicular damage caused by a high fat diet via modulation neurochemicals, and inhibition of inflammatory-, apoptotic-autophagy-dependent mechanisms.

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Author Contributions: conceptualization:

Recommendations

- The use of another method for the preparation should be studied carefully because it may change the release pattern of the drug.
- The preparation of different formulations should be studied under sterilization conditions, as the biodegradable lipid base antibiotic will be used during surgery to prevent post-operative infection.

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