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Potential Effects of Epigallocatechin-Gallate against High-Fat Diet-Induced Memory Decline andTesticular Abnormalities in Male Wistar Rats

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ABSTRACT: The objective of this study was to evaluate the potential effects of epigallocatechin-gallate (EGCG) against high-fat diet (HFD)-induced memory decline and testicular abnormalities in male Wistar rats using appropriate standard techniques. The findings showed that HFD consumption led to cognitive deterioration, an imbalance in inflammatory cytokines, an increase in the Lee index, and neuronal death. However, in HFD-exposed rats, EGCG treatment reduced corticosterone, leptin, and the Lee index, improved cognitive impairment, regulated inflammatory, autophagic, and apoptotic processes, increased adiponectin levels, raised brain and testes weight, and protected against neuronal atrophy. Thus, EGCG mitigated the adverse effects of HFD-induced non-spatial memory decline and testicular dysfunction, potentially by reducing hypercortisolism, modulating chemo-brain activity, regulating inflammatory, apoptotic, autophagic, and metabolic hormonal status, and preventing neuronal degeneration.

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One of the main public health issues in the globe today is obesity. Obesity is classified as a Body Mass Index (BMI) equal 30kg/m2 or higher (Adegoke *et al.*, 2021). Male and female obesity rates have dramatically increased over the previous 40years, 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). 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 (Cho *et al.*, 2016*)*.A high-fat diet has been associated with neuropathological changes that lead to obesity-related cognitive impairment and alterations in the brain structure and function (Medic *et al.*, 2016). Preclinical studies have demonstrated that long-term consumption of a high-fat diet is linked to cognitive decline and reproductive issues (Molteni *et al.*, 2002). An obesogenic diet has the most significant detrimental effects on three key brain functions: learning, memory, and executive activities (Park *et al.*, 2014). These cognitive functions are primarily regulated by the brain's prefrontal cortex and hippocampus (Kim *et al.*, 2016). The systemic effects of obesity on reproductive health and its cognitive consequences have increasingly attracted attention.

There is notable evidence suggesting that HFD-

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induced sperm deficiency (Chen *et al.,* 2022) and brain damage (Raee *et al.,* 2023) in rodents are linked to autophagy dysfunction. Autophagy, a crucial biological process involved in cellular recycling and the degradation of cellular components, is regulated by lysosomal enzymes and has been reported to play a significant role in reproductive biology and neuronal degeneration (Oyovwi *et al.,* 2022). Notably, rapamycin-mediated autophagyis 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 mono phosphate-activated protein (AMPK) phosphorylations (Raee *et al.,* 2023). Although synthetic drugs have shown significant progress in mitigating obesity-related reproductive and cognitive damage associated with autophagy, compelling findings have emerged from polyphenolic extracts of medicinal plants, which contain naturally occurring compounds with reproductive and psychotropic properties (Ben-Azu *et al.,* 2019).

Green tea (Theaceae) is typically made from the leaves and buds of the Camellia sinensis L. plant. Among the various types of tea, including black tea, oolong tea, and green tea, the health benefits of green tea have been the most extensively researched (Carlson *et al.,* 2007). The active polyphenol in green tea, epigallocatechin-3-gallate (EGCG), has garnered significant interest for its potential health benefits, including its effects against cancer (Yang *et al.,* 2016), obesity (Lombo *et al.,* 2016; Meydani *et al.,* 2017), diabetes (Unno *et al.,* 2007), and as a neuro protective agent (Rezai-Zadeh *et al.,* 2008).The numerous health benefits of EGCG are strongly correlated with its antioxidant, metal chelating, anti-carcinogenic, pro-apoptotic, anti-inflammatory, anti-apoptotic, anti-apoptotic, andanti-apoptotic properties (Cavet *et al.,* 2011). These include the prevention of neurodegenerative disorders, improvement of reproductive functions, and weight control via decreased energy absorption and improved fat burning (Li *et al.,* 2016; Ikpeme *et al.,* 2016; Isbrucker *et al.,* 2006). It's interesting to note that EGCG reduces reproductive toxicity and oxidative damage in rats exposed to cyromazine and chlorpyrifos (Wu *et al.,* 2012). 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 (Ikpeme *et al.,* 2016). As a result, safety investigations showed that EGCG has no teratogenic effect and is also not hazardous to the reproductive system (Pervin *et al.,* 2006). Additionally, it has been demonstrated that EGCG reduces oxidative stress and neuro inflammation to improve learning and memory impairments in rats exposed to ischemia stress (Liu *et al.,* 2012) and Alzheimer's disease (Li *et al.,* 2018). 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 (Scarpace *et al.,* 2016). The objective of this study was to evaluate the potential effects of epigallocatechin-gallate (EGCG) against high-fat diet (HFD)-induced memory decline and testicular abnormalities in male Wistar rates.

MATERIALSANDMETHODS

Animals: The study used 36 Wistar Rats, aged 6 to 8 weeks, housed in a controlled environment with a 12-hour light/dark cycle. The rats were acclimatized for 14 days before undergoing 56 days of treatment. The experiment followed NIH guidelines 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 (Kim *et al.,* 2004). Rapamycin was selected within the dose-response effect and previous investigation by Scarpace *et al.;* 2016.The doses used were in agreement with their commendations 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 their commendations for extrapolating human doses to animal doses. The daily doses were given orally between the hours of Balistreri *et al.,* 2010 and Miller et al., 2014.

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

Typical rat chow diet: As described in Kim et al., 2004, the typical rat chow diet was created. 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 (50g). 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., 2004*, the high-fat diet (HFD) consisted of 50 g of vitamins, minerals, and fiber, 300 g of concentrates, 350 g of corn, and 300 g of beef tallow. The diet composition

included 20% crude protein, 35% fat, 40% carbohydrates (35% starch and 5% sugar), and 5% vitamins, minerals, and fiber. 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 (LI) was calculated (1980).

$$LI\ (\%) = \sqrt[3]{\frac{Body\ weight\ (g)}{Nose\ to\ anus\ length\ (cm)}}\ x\ 1000 \quad (1)$$

Rats were deemed obese and used in the study if their Lee obesity index value was greater than310g/cm, which is similar to a BMI of 30 in humans.

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 (Kim *et al.,* 2004).*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 1mg/kg body weight orally for 56 days to induced autophagic flux (Ben-Azu *et al.,* 2020). *Group V***:** This group was treated with HFD for 56 days plus EGCG at 80 mg/kg body weight orally for28 days starting from day 29-56. *Group VI***:** This group was treated with HFD plus Rapamycinfor56 days plus EGCG at 80 mg/kg body weight from day 29-56.

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 cleaning the arena and objects with 70% v/v ethanol to eliminate scent cues. A short-term memory (STM) test was conducted five minutes after the trial phase, during which each rat was presented with identical objects, except one was replaced with a different one. Object placement was counterbalanced to avoid bias, with half of the rats encountering the new object on the left side. To test long-term memory (LTM) using the Novel Object Recognition (NOR) task, a wash-out period of five days was allowed before testing. The LTM test followed the same procedure as the STM test, but the test phase occurred 24 hours after the trial phase. During both STM and LTM tests, the time spent investigating each object was manually recorded using a stopwatch. A rat was considered to be exploring an object when its head was oriented within 2 cm of the object or when its nose made contact with it. 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% (Lillie *et al.,* 1976).

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-kappaß, nitrite, Beclin-1, mammalian target of rapamycin, glutamate, dopamine, no repinephrine) 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.

Testicular inflammation markers test: Using the ELISA kit bought from IL-1ß) (R & D systems, USA and Thermo Fisher Scientific respectively, testicular cells were used to assess and quantify (pg/mg protein) pro inflammatory 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 My Bio Source, BioVision, or Abbexa was used to measure the expression levels of

BECLIN-1 and mTOR in testicular and brain homogenates, respectively.

Estimation of dopamine concentration: There action mixture used in the study, consisting of 0.05mL of 0.4MHCl, 0.1mL 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 Na_2SO_3 was added to stop the reaction. After 1.5 minutes, 0.1 mL of acetic acid was added (Novelli *et al.*, 2007). After 6 minutes of heating to 100°C, a spectrofluorimeter's excitation and emission spectra are read. No repinephrine measurements are made at 395–485 nm, while dopamine readings are made between 330–375nm.

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 what man No.1 chromatography paper. After that, the paper was put inside a solvent chamber. After being dried and coated within hydrin reagent, the first and second papers were baked at 100°C for four minutes. $CuSO_4$ in 75% ethanol was used to elude the glutamate-carrying parts of the sample. A spectrophotometer was used to test their absorption.

Estimation of no repinephrine level: The process involved adding iodine (0.1ml) solution, 0.05ml of 0.4M HCl, 0.1ml of EDTA/Sodium acetate buffer (PH6. 9). 0.1 ml of Na_2SO_3 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 at330-375 nm and 395-485nm.

Histopathological examination of brain: The brain slices were stained with Haematoxylin and eosin stain (Picklo *et al.*, 2017). The Olympus microscope was used to analyze the stained sections.

Statistical analyses: With the aid of the post hoc Bonferroni'st-test and an ANOVA, differences between groups were found using the Graph Pad PRISM 8 program. P values less than 0.05 were used to evaluate statistical significance, along with mean and SEM.

RESULTS AND DISCUSSION

Effect of EGCG on rapamycin exacerbated HFD-induced non-spatial memory impairment in male Wistar rats: Using novel object recognition test (Figures 1), 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

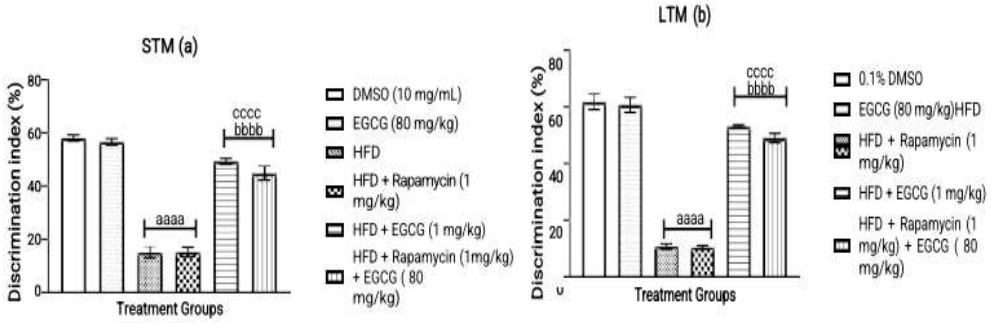

Fig. 1: EGCG prevents rapamycin exacerbated HFD-induced memory impairment in male Wistar rats using novel object recognition performance: short-term memory (STM) (a). Bars depicts the mean and S.E.M(n=6)$^{aaaa}p < 0.0001$ was used as comparism to control group;$^{bbbb}p < 0.0001$ was used as comparism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

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 2. HFD, as well as rapamycin treated-HFD produced significant ($p < 0.05$) increase in Lee index values of rat's body weight (Figure2a) 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.

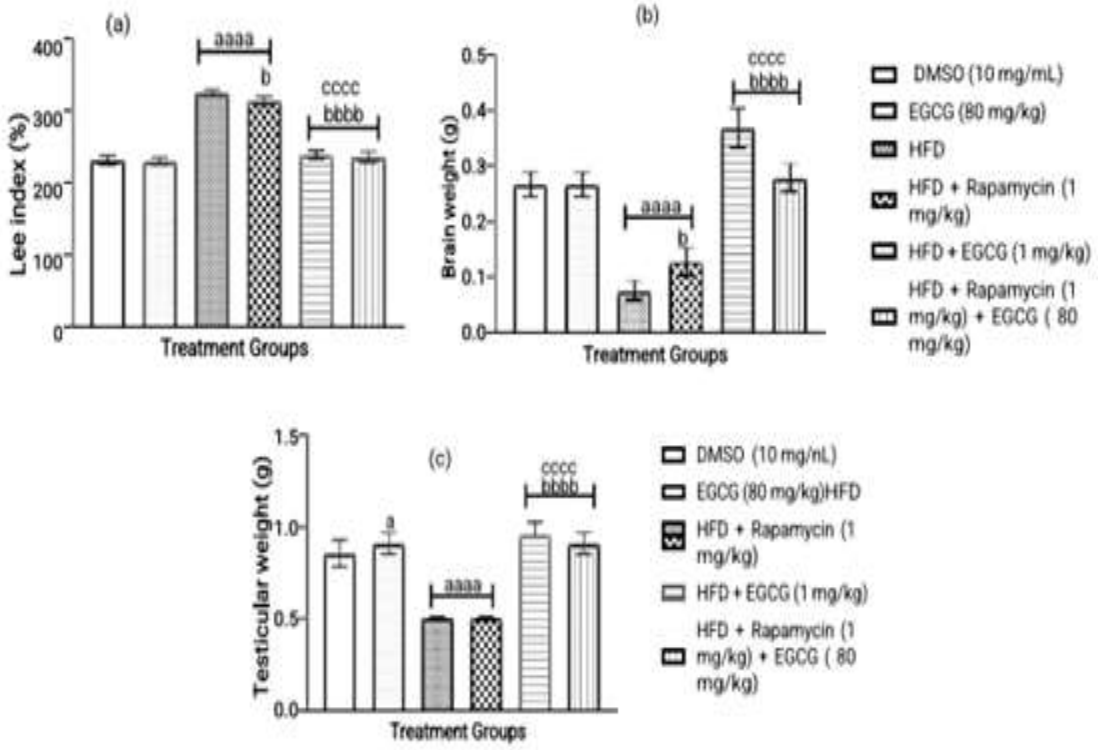

Fig. 2: Effect of EGCG on rapamycin mediated HFD-induced increase in Lee index. Bars depicts the mean and S.E.M (n=6).$^{aaaa}p < 0.0001$ was used as comparism to control group; $^{bbbb}p < 0.0001$ Vs HFD; $^{cccc}p < 0.0001$ Vs rapamycin plus HFD group (One way ANOVA was applied following Bonferroni's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

Effect of EGCG on rapamycin enhanced HFD-induced changes on leptin, adiponectin and corticosterone concentrations in rats: Figure 3 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 (Figure 3a). Compared to the HFD group or the HFD plus rapamycin group, EGCG therapy significantly reduced the increase in leptin levels caused by both treatments (Figure 3).

One-way ANOVA and Bonferroni's post-hoc test revealed that HFD and HFD plus rapamycin significantly lowered adiponectin concentrations compared to the normal control group (Figure 3b), with a significant difference ($p < 0.05$). EGCG (80 mg/kg b.w.) therapy notably corrected the decrease in adiponectin levels induced by HFD and HFD plus rapamycin (Figure 3b).

Additionally, rats treated with HFD or co-treated with HFD and rapamycin exhibited a significant increase in corticosterone levels ($p < 0.05$). EGCG (80 mg/kg, p.o.) significantly reduced the corticosterone rise caused by HFD and rapamycin, compared to the HFD and HFD plus rapamycin groups (Figure 3c).

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 (Figure 4a), dopamine (Figure 4b), and noradrenaline (Figure 4c) levels compared to the normal control group. None the less, 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 shown in Figure 4.

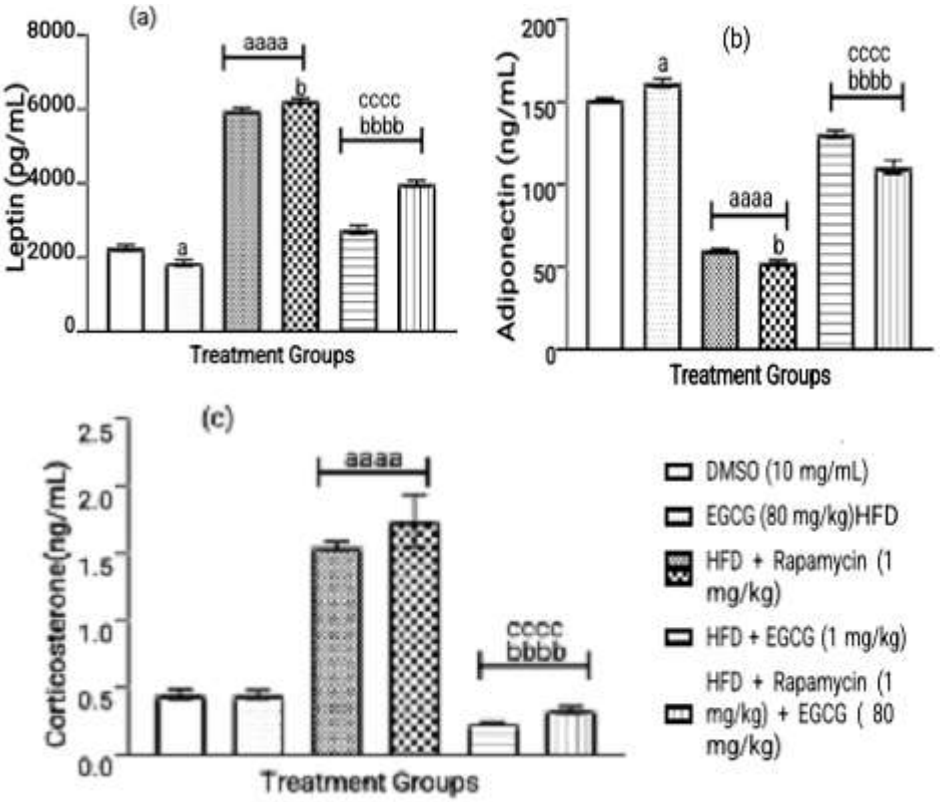

Fig. 3: EGCG abates rapamycin enhanced HFD-induced changes in leptin, adiponectin and corticosterone levels. Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$, $^{a}p < 0.05$ Vs control group;$^{bbbb}p < 0.0001$VsHFD; $^{cccc}p < 0.0001$Vs rapamycin plus HFD group.

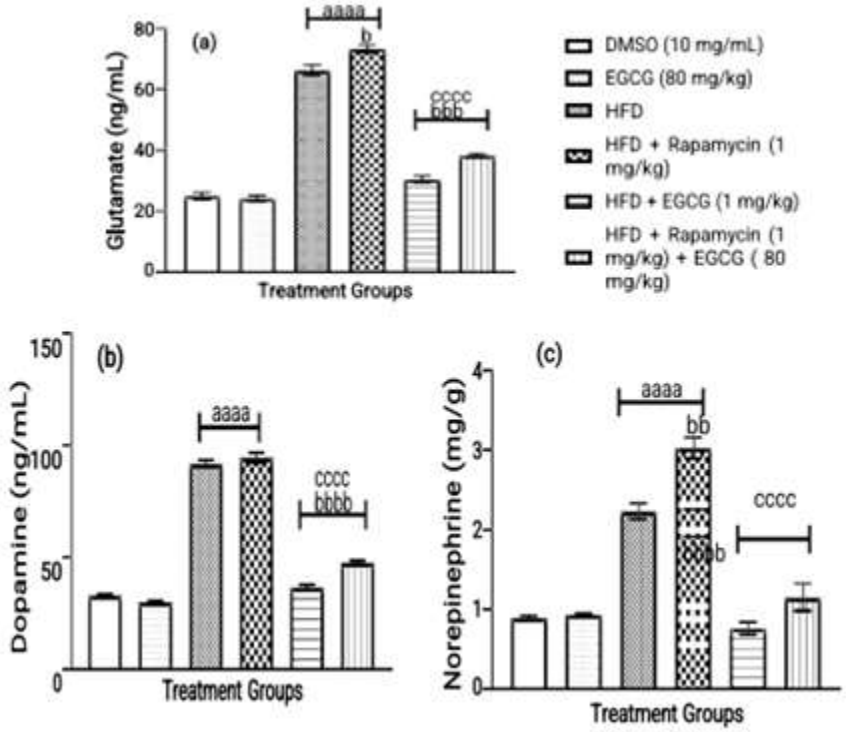

Fig.4: EGCG reverses HFD and rapamycin-induced alteration in neurochemical concentrations in rats: a) glutamate, b) dopamine, c) nor adrenaline. Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$ was used as comparism to control group;$^{bbbb}p < 0.0001$ was used as comparism to HFD;$^{cccc}p < 0.0001$was used as comparism to rapamycin plus HFD group

Effect of EGCG on rapamycin exaggerated HFD-induced pro-inflammatory cytokines in rats brains: In line with Figure 5, when compared to control mice, HFD and HFD + rapamycin exposure significantly increased the levels of TNF-α (Figure 5a), IL-1β (Figure 5b), NF-kß (Figure 5c), and COX-2 (Figure 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 as shown in Figure 5.

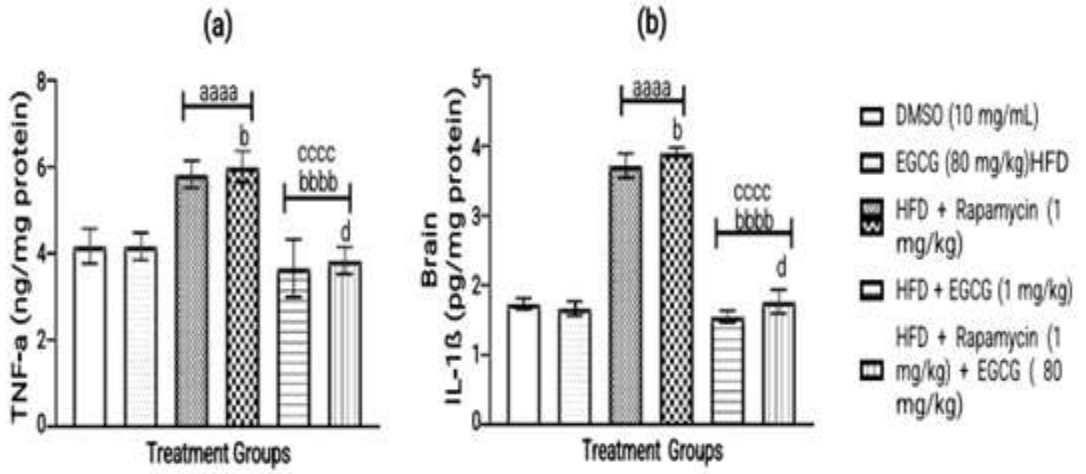

Fig. 5: EGCG inhibits HFD-induced release of pro-inflammatory cytokines in rat's brain. (a) Tumor necrotic factor-alpha TNF-α, (b) interleukin-1β (IL-1β). Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$,$^{a}p < 0.05$ Vs control group; $^{bbbb}p < 0.0001$, $^{b}p < 0.05$ Vs HFD; $^{cccc}p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

EGCG inhibits HFD-induced release of pro-inflammatory cytokines in rat testes: According to Figure 6 when compared to control animals, both HFD and HFD + rapamycin treatment demonstrated a substantial increased TNF- α (Figure 6a), IL-1β (Figure 6b) levels. However, compared to rats receiving HFD therapy alone, EGCG post-treatment significantly reduced the elevated pro-inflammatory cytokine levels.

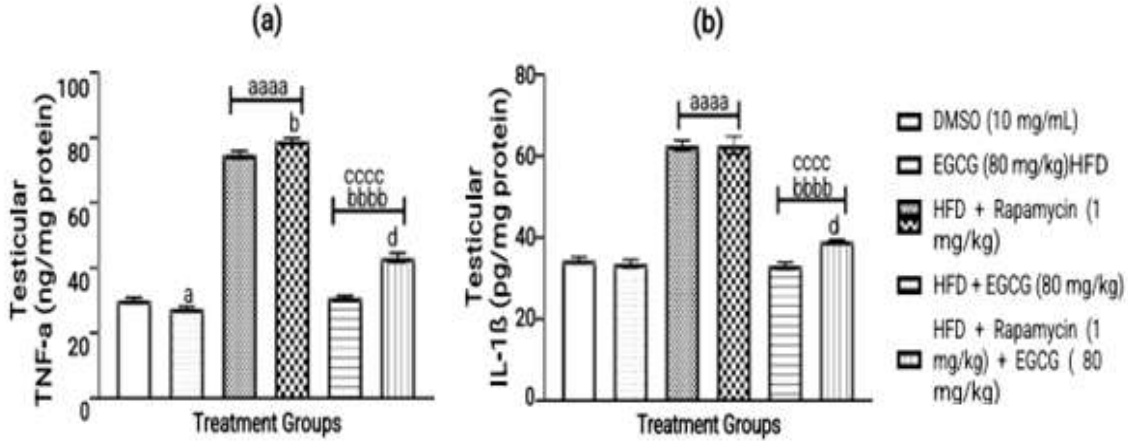

Fig. 6: EGCG inhibits HFD-induced release of pro-inflammatory cytokines in rat testes. (a) Tumor necrotic factor-alpha (TNF-α), (b) interleukin-1β (IL-1β). Bars depicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$,$^{a}p < 0.05$ Vs control group; $^{bbbb}p < 0.0001$, $^{b}p < 0.05$ Vs HFD; $^{cccc}p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

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 (Figure 7a)and higher BECLIN-1(Figure 7b) levels, but not significantly changing mTOR or BECLIN-1 levels compared to normal control groups.

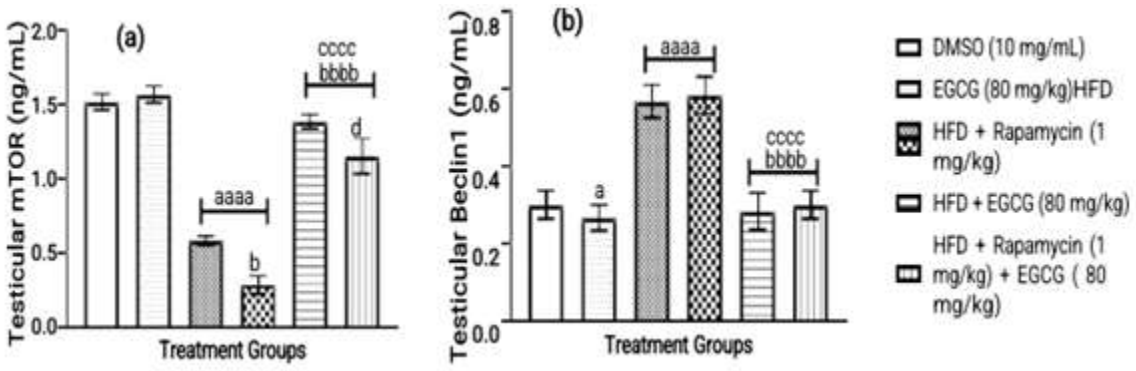

Fig. 8: EGCG counteracts the effects of HFD on testicular autophagy-related protein levels inrats. (a) Mammalian Target of rampamycin (mTOR) and (b) autophagy (Atg-7) activities. Barsdepicts the mean and S.E.M (n = 6). $^{aaaa}p < 0.0001$, $^{a}p < 0.05$ Vs control group; $^{bbbb}p < 0.0001$,$^{b}p < 0.05$ Vs HFD; $^{cccc}p < 0.0001$ Vs rapamycin plus HFD group; $^{dd}p < 0.01$ Vs HFD + EGCG group

Effects of EGCG on HFD and rapamycin-mediated pathological alteration of the adrenal glands of rats: Plate 1: 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 glands 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). The zonation pattern of the cortex was clearly visible on slides A and B for plate 2, showing columns of clear cells in the zonafasciculata, clusters of stainable cells in the zonaglomerulosa, and cells with acidophilic cytoplasm in the zonareticularis. 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 of0.01 mm (10 µm) and an original magnification of x 100.

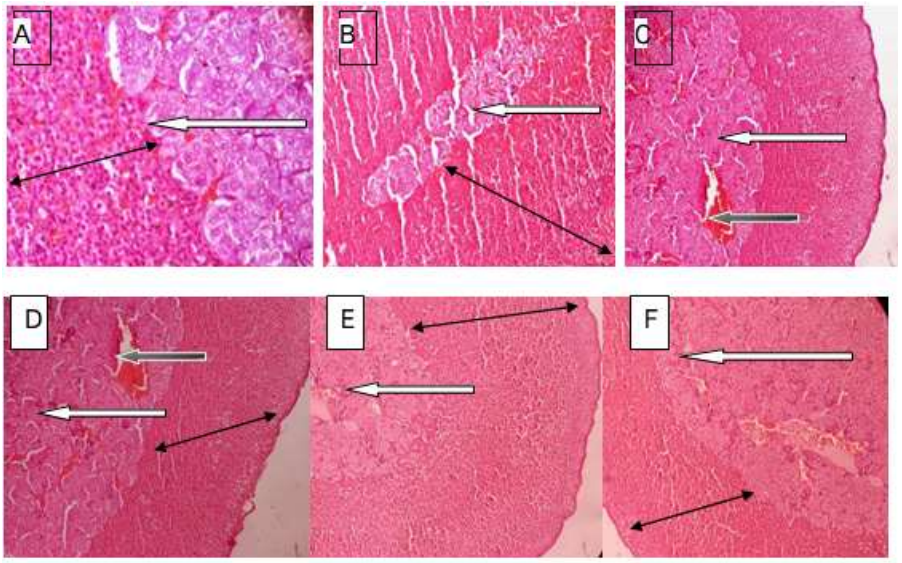

Plate1: Photomicrographs demonstrating the impact of EGCG on the histopathological alterations brought on by rapamycin and HFD in the rat adrenal gland. Control (0.1% DMSO),EGCG (80mg/kg), HFD, HFD+Rapamycin (1.0mg/kg), HFD+EGCG (80mg/kg), and HFD+rapamycin (1.0mg/kg)+EGCG (80mg/kg) are shown in the following order: A, B, C, D and F.

Effects of EGCG on HFD and rapamycin-induced histological alteration of the prefrontal cortex of rats: In plate 2,the effects of EGCG on rapamycin- and HFD-induced histological alterations in the rat prefrontal cortex are shown. Compared to the normal control group, rats treated with EGCG alone exhibited abnormalities in the laminae, neuronal cells, and overall architecture of the prefrontal cortex.

Specifically, EGCG-treated rats showed signs of degeneration, hyalinization of neuronal cells, and dilated capillaries (as shown in slides C and D). In contrast, rats treated with HFD and rapamycin displayed reduced neuronal cell density in the prefrontal cortex, with similar degeneration and hyalinization. However, EGCG therapy effectively reduced HFD-induced hyalinization and cell degeneration in prefrontal neuronal cells when compared to the HFD groups. Slides A and B depict normal neuronal cells within a normal stroma. Slides C and D show severely deteriorated neuronal cells with dilated capillaries (black arrow). Slides E and F display normal neuronal cells within a normal stroma, with necrosis indicated by black arrows and normal neural cells marked by white arrows. The original magnification of the hematoxylin-eosin stain is ×100, with a calibration bar of 0.01 mm (10 µm).

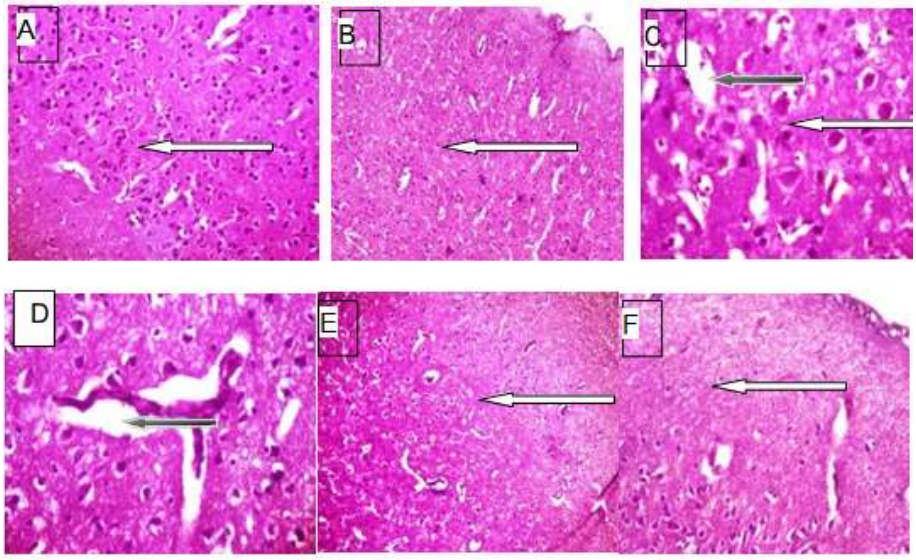

Plate 2: Photomicrographs displaying the impact of EGCG on rats' prefrontal brain alterations caused by the HFD. Control (0.1% DMSO), EGCG (80 mg/kg), HFD, HFD + Rapamycin (1.0mg/kg), HFD + EGCG (80 mg/kg), and HFD + rapamycin (1.0 mg/kg) + EGCG (80 mg/kg) are shown in the following order: A, B, C, D, and F.

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 neuropath physiology. 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 nor epinephrine, 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.,* 2007. The findings of Picklo *et al.,* 2017, who demonstrated the obesogenic effect of a saturated fat diet in an animal model, are consistent with this observation. 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 (Vigueras-Villasenor *et al.,* 2011). 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 (Aizawa-Abe *et al.,* 2000).By

boosting satiety and energy expenditure, the hormone leptin, which is mostly produced by adipocytes, helps to regulate body *weight* (Power *et al.,* 2008, Mayes *et al.,* 2004). According to Power *et al.,* 2008, leptin has both stimulatory and inhibitory effects on the reproductive system. According to Mayes *et al.,* 2004), 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 (Mayes *et al.,* 2004). 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*)* (Mzhelskaya *et al.,* 2020, Duszka *et al.,* 2020), 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. This conclusion is supported by Liang *et al.,* 2021, which discovered that leptin loses it sanorexigenic activity on hypothalamic neurons in HFD-induced obesity, increasing hunger and the creation of fat mass.

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, neuro endocrine function, glucose and lipid metabolism regulation, energy homeostasis, anti-inflammatory, anti-atherogenic, and cardio vascular function (Aouichat *et al.,* 2020, Dwaib *et al.,* 2021, 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.,* 2022). People with type 2 diabetes, coronary artery disease, and obesity brought on by the HFD had low levels of adiponectin (Kogel *et al.,* 2020). As previously reported this study's HFD-treated rats showed substantial drop in serum adiponectin levels (Kogel *et al.,* 2020). Insulin resistance, poor insulin sensitivity, and the emergence of obesity have all been associated with lower blood levels of adiponectin (Wu *et al*., 2012; Kogel *et al.,* 2020; Fasshauer *et al.,* 2003). The suppression of gluconeogenesis and an increase in lipid oxidation caused by adiponect in 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.,* 2020). A considerable decrease in serum adiponectin was seen in this investigation in the HFD-treated rats, as was previously reported (Kogel *et al.,* 2020). Insulin resistance, poor insulin sensitivity, and the emergence of obesity have all been associated with lower blood levels of adiponectin (Wu *et al.,* 2012; Kogel *et al.,* 2020; Fasshauer *et al.,* 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.,* 2020, He *et al.,* 2017). As demonstrated below, the chronic inflammatory flux that ensues may result in the development of insulin resistance in tissues (Fasshauer *et al.,* 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 *et. al.,* 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 *et al.,* 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 *et al.,* 2011). Numerous earlier studies have demonstrated that consuming HFD might result in histological abnormalities in the brain and reduced neuro-reproductive organ weights (Francis *et al.,* 2013; Antunes *et al.,* 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 (Belanger *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 cronically 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.,* 2004, 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 develop mental illnesses (Fritz *et al.,* 2018; Guo *et al.,* 2013). The neurochemical pathways behind neurological diseases are frequently studied using animal models. According to Ben-Azu *et al.,* 2018, the intricacy of the wide range of neurological symptoms associated with neurodegenerative diseases makes it impossible to reproduce important aspects of the disease.

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 neuro developmental disorders, including autism, ADHD, and schizophrenia (Osna *et al.,* 2011). According to Fritz *et al.,* 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 effect so high fat consumption on brain functions and the possible importance of these mechanisms in aggravating non homeostatic 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 (Zhang *et al.,* 2010), liver disease (Banerjee *et al.,* 2010), kidney disease (Aparicio *et al.,* 2016), reproductive disease (Wang *et al.,* 2020), and neurological disease (Wang *et al.,* 2013). 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.

The current study observed that HFD triggered autophagy in rats, which was further exacerbated by rapamycin exposure. This was evidenced by increased levels of Beclin-1 protein and decreased levels of mTOR. These findings are consistent with those of Mu *et al.,* 2017, who reported that autophagy is over activated in male mice with HFD-induced spermatogenesis deficits. Rapamycin-treated HFD rats exhibited more pronounced changes in autophagy-related proteins, including Beclin-1 and mTOR. Interestingly, EGCG treatment of HFD rats improved protein mTOR levels and inhibited autophagy as measured by decreased Beclin-1in the tests. These findings suggest that inhibiting excessive autophagy may protect against HFD-induced impairment in reproductive functioning. Apoptosis and autophagy interactina 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 ultra-structure (Fasshauer *et al.,* 2003). EGCG, on the other hand, substantially corrected HFD-induced apoptosis and ultra-structural 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.

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.

Declarations of interest: The authors declare no conflict of Interest

Data Availability Statement: Data are available upon request from the first author or corresponding author or any of the other authors.

Abbreviations
NE - norepinephrine, GLUT – glutamate, CORT-corticosterone, DA–dopamine
NORT–Novel object recognition task
NF-kB-Nuclear fact or kappa-light-chain-enhancer of activated B cells,
TNF-α– tumor necrotic factor alpha,
IL-1ß – interleukin 1 beta,
BCL-2–B-celllymphoma
Atg7 – autophagy related protein 7
mTOR–mammalian target of rapamycin
PUFAs-Poly unsaturated fatty acids

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