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Corylus avellana L. modulates neurobehaviour and brain chemistry following high-fat diet

Anthony T Olofinnade[1, 3], Adejoke Y Onaolapo[2], Olakunle J Onaolapo[3], Olugenga A Olowe[4], Adriano Mollica[5], Gokhan Zengin[6], Azurra Stefanucci[5]

[1]Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Clinical Sciences, College of Medicine, Lagos State University, Ikeja, Lagos State, [2]Behavioural Neuroscience/Neurobiology Unit, Department of Anatomy, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria , [3]Behavioural Neuroscience/Neuropharmacology Unit, Department of Pharmacology, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria, [4]Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria, [5]Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy, [6]Department of Biology, Science Faculty, Selcuk University, Konya, Turkey

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1. ABSTRACT

Consumption of a high-fat diet has adverse impacts on metabolism, neurobehavioral, and neurochemical homeostasis in both humans and experimental animals. Here, we examined the effects of two different cultivars of Corylus avellana L. in a mouse model of metabolic syndrome. Corylus avellana L. reduced weight gain in mice that were treated with a high-fat diet, improved their behavioral parameters as exemplified by locomotion and rearing, working-memory, and reduced grooming and anxiety indices. Both *Corylus avellana* L. varieties reduced blood glucose levels and lipid peroxidation, improved lipid profile, and antioxidant status in mice placed on a high fat diet. Finally, brain acetylcholinesterase activity was also reduced, dopamine level was increased, while caspase-3 level was reduced. Thus, the *Corylus avellana L*. cultivars improve metabolic, behavioral, and neurochemical homeostasis in a diet with a high-fat content.

2. INTRODUCTION

Corylus avellana L. (genus *Corylus*) consists of more than a dozen species of shrubs and trees which produce edible nuts (1). A number of these species are of significant commercial importance due to the commercial value, while some are valued as ornamental plants (2). The common hazel (*Coryllus avellana*) produces fine oil that is used in soaps, perfumes and food products (3, 4).

Previous research had established the metabolic benefits of a diet that is supplemented with *Corylus avellana* (4, 5), showing that specific cultivars of Corylus avellana can be of benefit in combating diet-induced plasma lipid derangements and deleterious morphological changes in certain organs (4). However, while the beneficial effects of Corylus avellana on diet-induced plasma lipid derangements had been demonstrated, especially in experimental animals; its possible beneficial effects on the brain including neurobehavior, oxidative status, and brain neurochemical changes that are known to occur as consequences of high-fat diet is not well-researched. Research and historical records show that Corylus avellana had been used for its beneficial effects on the brain, with documentation of its use in Iranian traditional medicine for combating of Alzheimer-like dementia (6,7). Also, in rats, *Corylus avellana* dietary supplement had been shown to improve memory, reduce anxiety-related behavior, and also reduce the neuroinflammation/apoptosis induced by intrahippocampal amyloid beta injection (6). Therefore, *Corylus avellana* supplement was demonstrated to be neuroprotective against a number of the deleterious effects of intrahippocampal amyloid beta injection in rats. These results also highlight that while amyloid beta induced neurological deficit, *Corylus avellana* supplementation showed some beneficial effects.

In experimental animals, high-fat diet has been shown to be associated with deleterious changes in behavior, brain oxidative status, brain neurochemistry and possibly brain morphology (8, 9). Also, in humans, high dietary fat has been associated with changes cognition and anxiety indices, with anxiety and cognitive impairment being recognized as co-morbidities of obesity (8). Dietary components that can counteract such deleterious changes improve the health's maintenance, and central nervous system co-morbidities of obesity in humans. Therefore, considering the beneficial effects of *Corylus avellana* supplemented diet on metabolic indices in high-fat diet fed mice (4), evaluating its possible protective effects against high-fat diet in the brain is also noteworthy. Therefore, in this study, we investigated the effects of Corylus *avellana*-

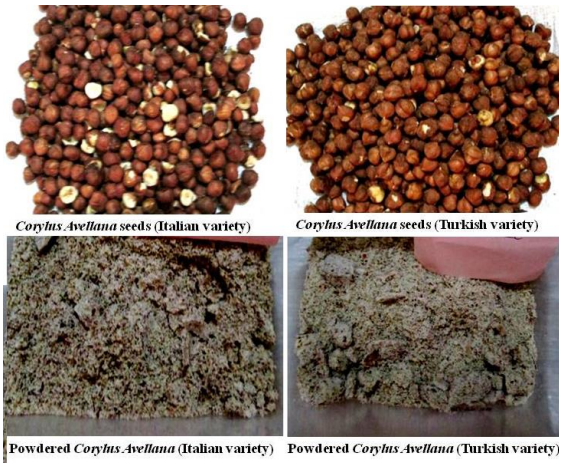

Figure 1. Figure showing seeds of *Corylus avellana* L (hazelnut) and powdered *Corylus avellana*.

supplemented diet on neurobehaviour, brain oxidative status, dopamine levels, caspase-3 induced apoptosis and acetylcholinesterase activity in mice treated with high-fat diet.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals

Assay kits for lipid peroxidation (malondialdehyde), superoxide dismutase, nitric oxide, dopamine, acetylcholinesterase and caspase-3 (Biovision Inc. Milpitas, CA, USA).

3.1.2. Corylus avellana L.

Dried *Corylus avellana L.*samples from two different regions in Europe (Turkey, Bartın, Black Sea Region: 41°36′44″N, 32°20′50″E) and (Italy, Cuneo province, Piedmonte, 44°28′46″N, 7°52′39″E) leftovers from a previous study (4) that had been stored in air tight containers in a cool dry room were ground into powder using an electric blender. Powdered *Corylus avellana L.* (Figure 1) was then incorporated into standard mouse chow or high-fat diet.

3.2. Animals

Healthy male mice obtained from Empire Breeders, Osogbo, Osun State, Nigeria were used for this study. Mice were housed in plastic cages that measured 12 x 9x 6 inches and housed in temperature-controlled (22.5°C ±2.5°C) quarters with 12 hours of light (Lights on at 7.00 am daily). Animals had free access to food and water except during behavioural tests.

3.3. Diet

All animals were fed commercially available standard mouse chow (29% protein, 11% fat, 58% carbohydrate) from weaning until the commencement of the study. At the beginning of the experimental periods animals were either fed standard mouse chow (29% protein, 11% fat, 58% carbohydrate) or high fat diet (18% protein, 42% fat, 36% carbohydrate) compounded from palm olein, and vegetable shortening (hydrogenated). The two *Corylus avellana L*.varieties were then incorporated into high fat diet at 2%, 4% and 8% (based on the results of a previous study by Mollica *et al.* (4) that had used lower concentrations of *Corylus avellana*.

3.4. Experimental methodology

Eighty adult male Swiss mice weighing 18-20 g were randomly assigned into eight groups of ten (n=10) mice each. Mice were grouped as follows, standard diet (SD) control, high fat control (HFD), three groups of Turkish and three groups of Italian *Corylus avellana* (hazelnut) were incorporated into high fat diet at 2 % (20 g/kg of feed), 4% (40 g/kg of feed) and 8% (80 g/kg of feed) respectively. Animals were allowed free access to food (standard diet, high fat diet or hazelnut/high fat diet) and water daily for eight weeks. Food consumption and body weight was assessed as previously described (4, 10). At the end of the experimental period, behaviors in the elevated plus maze, radial-arm maze, open field, and Y-maze paradigms were monitored and scored. Animals in all groups were then euthanised by cervical dislocation. Twenty-four hours after the last behavioural test, blood was taken from the tail vein after an overnight fast for estimation of glucose levels by the glucose oxidase method (11, 12). Mice in all groups were then euthanised by cervical dislocation. Blood taken via an intra-cardiac puncture was used for estimation of lipid profile. The brain was dissected, weighed and

homogenized for the assessment of, antioxidant status, acetylcholinesterase activity, lipid peroxidation, caspase-3 and dopamine levels.

3.5. Behavioral tests

On respective test days, animals were transported in their home-cages to the behavioral testing laboratory where they were allowed to acclimatize for 30 minutes before commencements of behavioral tests. Mice in all groups were exposed to the behavioral paradigm for 10 minutes for the open field and 5 minutes each for the elevated plus maze, Y-maze and radial arm maze. At the beginning of the tests, each mouse was placed in the apparatus and its behavior recorded. On completion of the behavioral tests, each mouse was then removed from the maze and then returned to its home cage; following which the interior surfaces of the maze was cleaned thoroughly with 70 % ethanol, and then wiped dry to remove traces of conspecific odor. The behavioral parameters were later scored by two independent observers who were blind to the groupings.

3.6. Anxiety model: Elevated plus-maze

The elevated plus-maze is a plus-shaped apparatus validated for use in rodents for the assessment of anxiety-related behaviors. It has four arms, two open arms measuring 25 x 5 x 0.5 cm lying across from each other and perpendicular to two closed arms measuring 25 x 5 x 16 cm with a center platform. The elevated plus-maze relies on the rodents' proclivity for dark, enclosed spaces (approach) and an unconditioned fear of open spaces (avoidance) or heights. Mice were placed in the central platform facing a closed arm, and their behaviors recorded for 5 min. The criterion for arm visit is considered only when the animal decisively moved all its four limbs into an arm. Anxiety behaviors were scored as the percentage time spent in the closed arm or open arm as previously described (13, 14).

3.7. Open field behaviors

Locomotor activity (horizontal locomotion and rearing) of rodents are central behaviors that are indicative of the animal's exploratory ability. The open-field arena is a rectangular box, with a hard floor made of white painted wood measuring 36 x 36 x 26 cm. It's floor is divided into 16 equal squares with permanent red markings. Animals are then allowed to explore the open field arena for ten minutes during which locomotor activity, (number of floor units entered with all paws), rearing (number of times the animal stood on its hind legs either with its fore arms against the walls of the observation cage or free in the air) and grooming frequency (number of body cleaning with paws, picking of the body and pubis with the mouth and face washing actions) were observed for 10 minutes and scored as previously described (15, 16).

3.8. Memory tests (Y maze and radial-arm maze)

The Y-maze and the radial-arm maze are used to measure spatial working-memory and general activity in rodents. Spatial working-memory measures the tendency of rodents to alternate conventionally non-reinforced choices of the arms of the Y-maze or radial-maze on successive alternations. The Y-maze is an arena with three equally spaced arms (120°, 41cm long, 15 cm high and 5 cm wide) made of white painted wood. The floor is also made of painted white wood. At the beginning of the tests each mouse is placed in one of the arms and allowed to move freely until its tail completely entered another arm. The sequence of arm entries is then recorded. An alternation is defined as consecutive entry into all three arms. The percentage alternation was calculated from the number of sequential arm entries into the three arms (actual alternation arm entry) divided by the total arm entry after a factor of two has been subtracted from it as previously described (17, 18).

The radial-arm maze apparatus has eight equidistantly spaced arms with each arm measuring 33 cm long. All arms radiate from a small circular central platform. Each mouse is placed on the central platform of the maze and allowed to move freely through the arm for 5 minutes following which the behaviours are scored. Working memory is scored when the

mouse enters each arm a single time. Re-entry into the arms is scored as a working memory error. Spatial working memory was assessed as alternation index, which is defined as the ratio of sequential arm entries before error as previously described (17, 18).

3.9. Blood collection

Blood was collected from each mouse 24 hours after the last behavioral tests via intra-cardiac puncture after an overnight fast. Samples were collected into universal bottles, allowed to clot, centrifuged at 3,500 rpm for 10 minutes using a general centrifuge (Uniscope SM112, Surgifriend Medicals, England) to allow separation as previously described (19, 20). The serum was assayed either immediately or stored at -20ºC.

3.10. Brain tissue homogenization

Within 24 hours of the completion of the behavioral tests, animals were sacrificed by cervical dislocation, the brain was dissected, blotted dry and weighed. A 10 % homogenate was then prepared with ice-cold phosphate buffered saline using a Teflon-glass homogenizer. The homogenate was centrifuged at 5,000 rpm (4 °C) for 15 min, and used to measure biochemical parameters.

3.11. Biochemical parameters

3.11.1. Lipid profile

Total cholesterol, triglycerides, HDL-C and LDL-C in serum were also analyzed using commercially available kits following the instructions of the manufacturer.

3.11.2. Lipid peroxidation (Malondialdehyde)

The lipid peroxidation kit was used to assess the level of malondialdehyde (MDA) in samples by measuring thiobarbituric acid-reactive species (TBARs). Reactive substances of the thiobarbituric acid react with free MDA in tissue to produce a colored complex (TBAR-MDA adducts) which is measured at an absorbance of 532 nm. The MDA concentration was expressed as nmol/L (21).

3.11.3. Antioxidant activity

Superoxide dismutase activity was determined using commercially-available assay kit. Colour changes were measured at an absorbance of 560 nm as described previously (22). Nitric oxide assay was carried out using the Nitric oxide kit as previously described (17). The change in absorbance at 540 nm over 5 minutes was measured.

3.11.4. Dopamine level

Level of dopamine in the homogenate was assayed using commercially-available dopamine assay kit following the instructions of the manufacturer and as previously described (17, 23). Color change was measured at an absorbance of 450 nm as specified by the manufacturer.

3.11.5. Acetylcholinesterase activity

Brain acetylcholinesterase activity was determined using the commercially available acetylcholinesterase assay kit (Biovision Inc. Milpitas, CA, USA), as previously described (18). Colour change was measured at an absorbance of 412 nm and expressed as nmol per microgram (nmol/mg) of brain tissue.

3.11.6. Assessment of caspase-3 concentration

The concentration of caspase-3 in whole brain homogenate was assessed using caspase-3 assay kit as previously described (24)

3.12. Statistical analysis

Data was analysed using Chris Rorden's ezANOVA for windows. Hypothesis was tested using analysis of variance (ANOVA). Tukey (HSD) test was used for post-hoc analysis. Results were expressed as mean ± S.E.M, and $p< 0.05$ considered significant.

4. RESULTS

4.1. Effect of *Corylus avellana L*. on body weight and food intake

Effect of Italian (ITA) and Turkish (TKY) *Corylus avellana L* (hazelnut) varieties on body weight (upper panel) measured as change in body

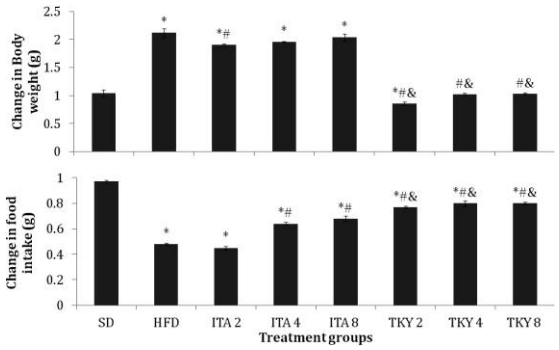

Figure 2. Effect of Italian and Turkish *Corylus avellana* L. varieties on change in body weight (upper panel) and change in food intake (lower panel). Each bar represents Mean ± S.E.M, *p<0.05 vs. SD, $^{\#}$p<0.05 vs. HFD, $^{\&}$p<0.05 ITA vs. TKY, number of mice per treatment group =10. SD: standard diet HFD: High fat diet, TKY: Turkish, ITA: Italian

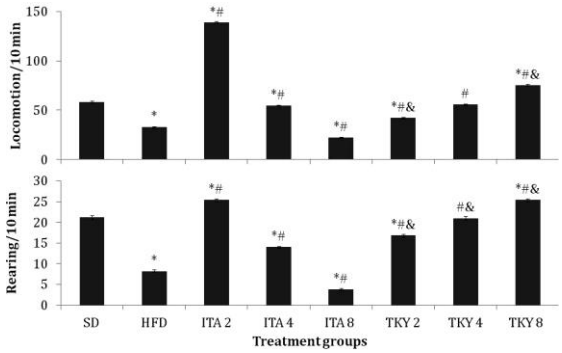

Figure 3. Effect of Italian and Turkish *Corylus avellana L*. varieties on locomotor (upper panel) and rearing (lower panel) activity. Each bar represents Mean ± S.E.M, *p<0.05 vs. SD, $^{\#}$p<0.05 vs. HFD, $^{\&}$p<0.05 ITA vs. TKY, number of mice per treatment group =10. SD: standard diet HFD: High fat diet, TKY: Turkish, ITA: Italian.

weight, and food intake (lower panel) measured as change in food intake is shown in Figure 2. There was a significant ($F(7, 72) = 151$, $p < 0.001$) increase in body weight with HFD and ITA at 2, 4 and 8% and a decrease with TKY at 2% compared to standard diet (SD). Compared to HFD, there was a significant decrease in body weight with ITA at 2% and TKY at 2, 4 and 8%. Dose for dose comparison revealed a significant decrease in body weight with TKY at 2, 4 and 8% compared to corresponding ITA groups.

Food intake decreased significantly ($F(7, 72) = 219$, $p < 0.001$) with HFD, ITA at 2, 4 and 8% and TKY at 2, 4 and 8% compared to SD. Compared to HFD, there was a significant increase in food intake with ITA at 4 and 8% and TKY at 2, 4 and 8%. Dose for dose comparison revealed a significant increase in food intake with TKY at 2, 4 and 8 % compared to corresponding ITA groups.

4.2. Effect of *Corylus avellana L*. on locomotor and rearing activity

Figure 3 shows the effect of Italian (ITA) and Turkish (TKY) *Corylus avellana L* (hazelnut) varieties on locomotor (upper panel) and rearing (lower panel) activity in the open field arena. There was a significant ($F(7, 72) = 1023$, $p < 0.001$) decrease in locomotor activity with HFD, ITA (4 and 8%) and TKY at 2% and an increase with ITA at 2% and TKY at 8% compared to SD. Compared to HFD, there was a significant increase in locomotor activity with ITA at 2 and 4% and TKY at 2, 4 and 8% while with ITA at 8% a significant decrease was observed. Dose for dose comparison revealed a significant decrease in locomotor activity with TKY at 2 and 8% compared to corresponding ITA groups.

Rearing activity decreased significantly ($F(7, 72) = 491$, $p < 0.001$) with HFD, ITA (4 and 8%) and TKY at 2% and an increase with ITA at 2% and TKY at 8% compared to SD. Compared to HFD, there was a significant increase in rearing activity with ITA at 2 and 4% and TKY at 2, 4 and 8% while with ITA at 8% a significant decrease was observed. Dose for dose comparison revealed a significant decrease in rearing activity with TKY at 2% and an increase with TKY at 4 and 8% compared to corresponding ITA groups.

4.3. Effect of *Corylus avellana L.* on grooming behavior

Figure 4 shows the effect of Italian (ITA) and Turkish (TKY) *Corylus avellana L* (hazelnut) varieties on grooming behavior measured as number of body-licking or face-washing episodes/10 min. There was a significant ($F(7, 72) = 26.2$, $p < 0.001$) decrease in grooming with HFD, ITA (2, 4 and 8%) and TKY at 2, 4 and 8 % compared to SD. Compared to HFD, there was a significant decrease in grooming with ITA at 2 and 8 % and TKY at 2, 4 and 8 %. Dose for dose

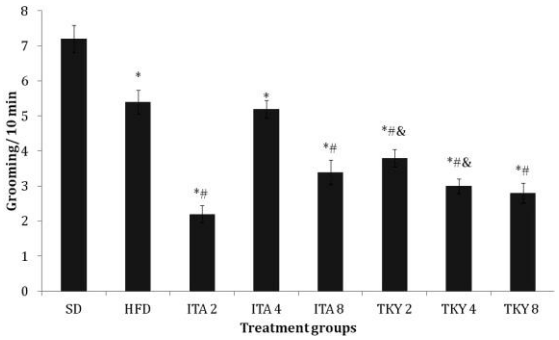

Figure 4. Effect of Italian and Turkish *Corylus avellana L*. varieties on grooming behaviour. Each bar represents Mean ± S.E.M, *p<0.05 vs. SD, #p<0.05 vs. HFD, &p<0.05 ITA vs. TKY, number of mice per treatment group =10. SD: standard diet HFD: High fat diet, TKY: Turkish, ITA: Italian.

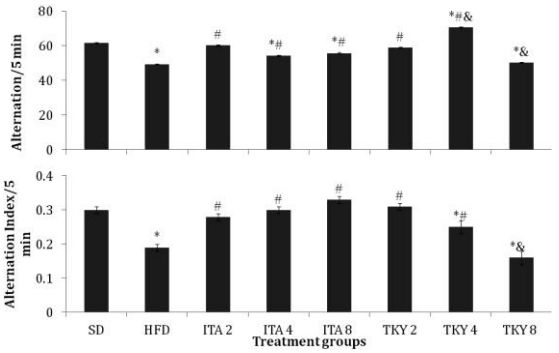

Figure 5. Effect of Italian and Turkish *Corylus avellana L*.varieties on spatial working memory in the Y-maze (upper panel) and radial-arm maze (lower panel). Each bar represents Mean ± S.E.M, *p<0.05 vs. SD, #p<0.05 vs. HFD, &p<0.05 ITA vs. TKY, number of mice per treatment group =10. SD: standard diet HFD: High fat diet, TKY: Turkish, ITA: Italian.

comparison revealed a significant increase in grooming with TKY at 2 % and a decrease with TKY at 4 % compared to corresponding ITA groups.

4.4. Effect of *Corylus avellana L.* on spatial working memory

Figure 5 shows the effect of Italian (ITA) and Turkish (TKY) *Corylus avellana L* (hazelnut) varieties on spatial working memory in the Y- maze (upper panel) and radial-arm maze (lower panel). There was a significant ($F(7, 72) = 20.0$, $p < 0.001$) decrease in Y-maze spatial working memory with HFD, ITA (4 and 8%) and TKY at 8% and an increase with TKY at 4 % compared to SD. Compared to HFD, there was a significant increase in Y maze spatial working memory with ITA at 2, 4 and 8 % and TKY at 2, 4 and 8%. Dose for dose comparison revealed a significant increase in Y- maze spatial working memory with TKY at 4% and a decrease with TKY at 8% compared to corresponding ITA groups.

Radial arm maze spatial working memory decreased significantly ($F(7, 72) = 7.18$, $p < 0.002$) with HFD, and TKY at 4 and 8 % compared to SD. Compared to HFD, there was a significant increase in radial-arm maze spatial working memory with ITA at 2, 4 and 8 % and TKY at 2, 4 and 8%. Dose for dose comparison revealed a significant decrease in radial-arm maze spatial working memory with TKY at 8% compared to corresponding ITA group.

4.5. Effect of *Corylus avellana L.* on behaviors in the elevated-plus maze

Figure 6 shows the effect of Italian (ITA) and Turkish (TKY) *Corylus avellana L* (hazelnut) varieties on time spent in the open arm (upper panel) and closed arm (lower panel) of the elevated plus maze. There was a significant ($F(7, 72) = 61.5$, $p < 0.001$) decrease in open arm time with HFD and an increase with ITA and TKY at 2, 4 and 8 % compared to SD. Compared to HFD, there was a significant increase in open arm time with ITA and TKY at 2, 4 and 8 % respectively. Dose for dose comparison revealed a significant decrease in open arm time with TKY at 2, 4% and 8% compared to corresponding ITA groups.

Closed arm time increased significantly ($F(7, 72) = 12.5$, $p < 0.001$) with HFD, ITA (2, 4 and 8 %) and TKY at 2, 4 and 8 % compared to SD. Compared to HFD, there was a significant increase in closed arm time with ITA and TKY at 2, 4 and 8 % respectively. Dose for dose comparison revealed a significant increase in closed arm time with TKY at 2, 4% and 8% compared to corresponding ITA groups.

4.6. Effects of *Corylus avellana L.* on blood glucose levels and Lipid profile

Table 1 shows the effect of Italian (ITA) and Turkish (TKY) *Corylus avellana L* (hazelnut) varieties

Table 1. Effect of *Corylus avellana L*.on blood glucose levels and serum lipid profile

Groups	Glucose (mg/dl)	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
SD	76.25±4.92	1.47±0.05	1.42±0.03	1.21±0.02	1.23±0.20
HFD	146.61±4.23[1]	7.68±2.05[1]	2.62±1.17[1]	1.34±0.11[1]	3.50±0.15[1]
ITA 2	65.60±3.221,2	2.00±0.20[1,2]	0.90±0.10[1,2]	1.68±0.09[1,2]	1.40±0.16[1,2]
ITA 4	75.11±2.25[2]	1.81±0.20[1,2]	0.80±0.10[1,2]	1.48±0.11[1,2]	1.35±0.12[1,2]
ITA 8	102.60±3.35[2]	1.76±0.21[1]	0.76±0.42[1]	1.45±0.10[1,2]	1.28±0.23[1,2]
TKY 2	70.60±2.41[1,2,3]	3.94±1.17[1,2,3]	1.20±0.23[1,2]	1.68±0.190[1,2,3]	1.40±0.28[1,2]
TKY 4	62.60±3.40[1,2,3]	4.30±1.12[1,2,3]	1.32 ±0.20[1,2,3]	1.70±1.10[1,2,3]	1.65±0.23[1,2]
TKY 8	60.40±3.51[1,2,3]	5.14±1.43[1,2,3]	1.40±0.31[2,3]	2.41±1.08[1,2,3]	1.32±0.08[1,2,3]
Values are presented as Mean ± S.E.M, [1]p<0.05 significantly different from standard diet, [2]p<0.05 vs. HFD, [3]p<0.05 ITA vs. TKY, number of mice per treatment group =10. SD: standard diet HFD: High fat diet, TKY: Turkish, ITA: Italian					

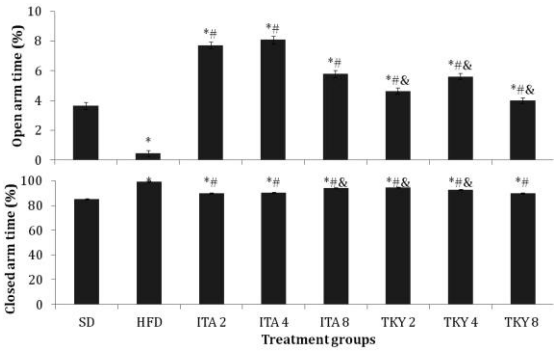

Figure 6. Effect of Italian and Turkish *Corylus avellana L*.varieties on time spent in the open arm (upper panel) and closed arm (lower panel) of the elevated plus maze. Each bar represents Mean ± S.E.M, *p<0.05 vs. SD, #p<0.05 vs. HFD, &p<0.05 ITA vs. TKY, number of mice per treatment group =10. SD: standard diet HFD: High fat diet, TKY: Turkish, ITA: Italian**.**

on blood glucose levels and serum lipid profile. There was a significant (F (7, 22) = 117.1, p<0.001) increase in blood glucose levels with HFD, ITA at 8%, and a significant decrease with ITA at 2 and 4% and TKY at 2, 4 and 8% compared to SD. Compared to HFD, glucose levels decreased with ITA and TKY at 2, 4 and 8% respectively. Dose for dose comparison revealed a significant decrease in glucose levels with TKY at 2, 4 and 8% compared to corresponding ITA groups.

Total cholesterol (TC) levels increased significantly (F (7, 22) = 117.1, p < 0.001) with HFD, ITA and TKY at 2, 4 and 8%, respectively compared to SD. Compared to HFD, TC also decreased significantly with ITA and TKY at 2, 4 and 8%. Dose for dose comparison revealed a significant decrease in TC levels with TKY at 2, 4 and 8% compared to corresponding ITA groups.

Triglyceride levels increased significantly (F (7, 22) = 117.1, p < 0.001) with HFD and decreased with ITA and TKY at 2, 4 and 8% compared to SD. Compared to HFD, triglyceride levels decreased with ITA and TKY at 2, 4 and 8%. Dose for dose comparison revealed a significant increase in triglyceride levels with TKY at 2, 4 and 8% compared to corresponding ITA groups.

High density lipoprotein (HDL) levels increased significantly (F (7, 22) = 117.1, p < 0.001) with HFD, ITA and TKY at 2, 4 and 8%, compared to SD. Compared to HFD, HDL increased significantly with ITA and TKY at 2, 4 and 8%. Dose for dose comparison revealed a significant increase in HDL levels with TKY at 2, 4 and 8% compared to corresponding ITA groups.

Low density lipoprotein (LDL) levels increased significantly (F (7, 22) = 117.1, p < 0.001) with HFD, ITA and TKY at 2, 4 and 8% compared to SD. Compared to HFD, LDL decreased significantly with ITA and TKY at 2, 4 and 8%. Dose for dose comparison revealed no significant difference in LDL levels with TKY compared to corresponding ITA groups.

Table 2. Effect of *Corylus avellana L*.on brain antioxidant status, MDA, Dopamine and acetylcholinesterase

Groups	MDA µM	SOD U/g	NO (µmol/g)	Dopamine ng/mg	ACHe nmol/mg	Caspase-3 (ng/mg)
SD	2.54±0.03	16.20±0.50	89.20±2.23	58.50±2.10	24.02±1.23	0.29±0.02
HFD	5.38±0.11[1]	28.24±1.04[1]	133.21±1.58[1]	43.21±1.61[1]	37.10±1.19[1]	0.46±0.10[1]
ITA 2	3.72±0.24[1,2]	19.10±1.13[1,2]	92.0±2.31[2]	72.30±2.40[1,2]	23.12±1.27[2]	0.23±0.02[1,2]
ITA 4	3.70±0.10[1,2]	18.20±1.10[1,2]	96.2±3.12[2]	59.10±2.01[2]	25.21±2.15[2]	0.26±0.01[2]
ITA 8	3.84±0.10[2]	19.30±1.18[1,2]	90 20±1.10[2]	56.21±1.50[1,2]	25.20±2.18[2]	0.28±0.01[2]
TKY 2	2.54±0.14[1,2,3]	16.05±0.13[1,2]	85.10±2.20[2]	53.18±1.61[2,3]	25.32±2.27[2]	0.27±0.02[2,3]
TKY 4	2.14±0.05[1,2,3]	15.08±0.10[1,2]	88.20±3.21[2]	59.50±1.05[2]	25.21±2.15[2]	0.25±0.03[1,2]
TKY 8	1.98±0.08[1,2,3]	16.10±0.05[1,2]	86.20±1.20[2]	68.26±1.07[1,2,3]	23.20±3.38	0.20±0.02[1,2,3]

Values are presented as Mean ± S.E.M, [1]p<0.05 significantly different from standard diet, [2]p<0.05 vs. HFD, [3]p<0.05 ITA vs. TKY, number of mice per treatment group =10. SD: standard diet HFD: High fat diet, TKY: Turkish, ITA: Italian

4.7. Effects of *Corylus avellana L.* on antioxidant, malondialdehyde, dopamine and acetylcholinesterase

Table 2 shows the effect of Italian (ITA) and Turkish (TKY) *Corylus avellana L* (hazelnut) varieties on brain levels of malondialdehyde, dopamine and nitric oxide as well as the activities of superoxide dismutase, and acetylcholinesterase. There was a significant ($F(7, 72) = 97.1$, $p < 0.001$) increase in brain levels of malondialdehyde (MDA) with HFD, ITA at 2, 4 and 8%, and a significant decrease with TKY at 2, 4 and 8% compared to SD. Compared to HFD, MDA levels decreased with ITA and TKY at 2, 4 and 8% respectively. Dose for dose comparison revealed a significant decrease in MDA levels with TKY at 2, 4 and 8% compared to corresponding ITA groups.

There was a significant ($F(7, 72) = 25.68$, $p < 0.001$) increase in brain superoxide dismutase (SOD) activity with HFD compared to SD. Compared to HFD, there was a significant decrease in SOD activity with ITA and TKY at 2, 4 and 8% Dose for dose comparison revealed no significant difference in SOD activity with TKY compared to corresponding ITA groups.

Brain nitric oxide levels increased significantly ($F(7, 72) = 31.26$, $p < 0.001$) with HFD compared to SD. Compared to HFD, there was a significant decrease in nitric oxide levels with ITA and TKY at 2, 4 and 8% Dose for dose comparison revealed no significant difference in nitric oxide levels with TKY compared to corresponding ITA groups.

Brain dopamine levels decreased significantly ($F(7, 72) = 121$, $p < 0.001$) with HFD, ITA at 8% and TKY at 2% and increased with ITS at 2% and TKY at 8% compared to SD. Compared to HFD, there was a significant increase in dopamine levels with ITA and TKY at 2, 4 and 8% respectively. Dose for dose comparison revealed a significant decrease in dopamine levels with TKY at 2% and an increase at 8% compared to corresponding ITA groups.

Brain acetylcholinesterase activity increased significantly ($F(7, 72) = 25.39$, $p < 0.001$) with HFD compared to SD. Compared to HFD, there was a significant decrease in acetylcholinesterase activity with ITA at 2, 4 and 8% and TKY at 2 and 4%. Dose for dose comparison revealed no significant difference in acetylcholinesterase activity with TKY compared to corresponding ITA groups.

Brain caspase-3 levels increased significantly ($F(7, 72) = 42.21$, $p < 0.001$) with HFD and decreased with ITA at 2% and TKY at 4 and 8% compared to SD. Compared to HFD, there was a significant decrease in brain levels of caspae-3 with ITA at 2, 4 and 8% and TKY at 2 and 4%. Dose for dose comparison revealed a significant decrease in caspase-3 levels with TKY at 2 and 8% compared to corresponding ITA groups.

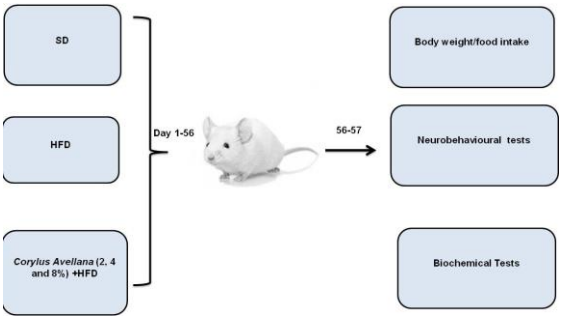

Figure 7. Graphical abstract showing the different methods employed in this study to evaluate the nutraceutical potential of *Corylus avellana L*.cultivars

5. DISCUSSION

In this study (Figure 7), it was observed that in mice fed HFD, dietary supplementation with *Corylus avellana L* (hazelnut) was associated with; a) a modulatory effect on weight gain with the Turkish hazelnut being associated with greater magnitude of reduction in weight gain; however, food intake only reduced significantly at the lowest concentration of Italian hazelnut supplement. b) general increase in locomotion and rearing, and a decrease in grooming behavior, c) improvement in working-memory in both Y and radial-arm maze, d) significant anxiolytic effect from both Italian and Turkish hazelnut, e) reduction in blood glucose with concomitant improvement in serum lipid profile, f) improved antioxidant status, increased dopamine level, reduced lipid peroxidation, caspase-3 mediated apoptosis and reduced acetylcholinesterase level.

In the present study, we observed the modulatory effects of hazelnut supplement on weight gain and food intake; with the Turkish hazelnut being associated with lesser weight gain compared to the Italian hazelnut, while the Italian hazelnut was associated with a greater magnitude of reduction in food intake. The results obtained here, corroborate those of a previous study in which dietary hazelnut can influence weight changes, probably through its metabolic effects on plasma lipid parameters and food intake (4). It also showed that hazelnut-supplemented diet can help to counteract HFD-induced weight gain.

Furthermore, in this study, dietary supplementation with hazelnut was associated with improvement in indices of open-field exploration such as horizontal locomotion and rearing; and significant reduction in grooming behavior in HFD mice; in essence, counteracting the deleterious effects of HFD on open-field parameters. The deleterious effects of HFD on open-field behaviours, anxiety related behaviors and memory observed are in agreement with the observation previously reported by other authors (8, 9, 17). Gainey *et al.* (8) showed that the administration of HFD to rodents was associated with increased anxiety and impaired cognition. While Hassan *et al.* (9) reported impaired open-field exploratory activities such as horizontal locomotion and rearing in mice fed HFD. In this study we observed a reversal of these deleterious effects with the dietary supplementation with hazelnut. Both cultivars of hazelnut were also associated with improvement in anxiety-related behavior such as time spent in the open-arm of the elevated plus maze, and improvement in working memory in the Y-maze and the radial arm maze. The results obtained highlight the ability of hazelnut supplement to significantly alter behaviors in rodents, corroborating the findings of the study by Bahaeddin *et al.* (6) that associated hazelnut-rich diet in rodents with improvement in memory, and anxiolysis.

The neurochemical correlate of improved open-field locomotor activity and improved performance in the anxiety paradigm is an increase in brain dopamine level that was associated with hazelnut supplementation. It is generally known that dopamine is a neurotransmitter that plays important role in rodent exploratory activities; and apart from this, dopamine also plays modulatory roles in anxiety-related behaviors (25). The improvement in exploratory activities seen in the study may be attributable to increase in brain dopamine level.

In this study, hazelnut supplementation was associated with decreased acetylcholinesterase activity in mice that were fed HFD, when compared to HFD controls that had increased acetylcholinesterase activity. Increased brain cholinesterase activity had been documented to be associated with HFD (26). An increased rate of degradation of brain acetylcholine is a plausible

explanation for the impaired cognition that is associated with HFD. Hazelnut supplementation of the diet led to a reduction in HFD-induced increase in acetylcholinesterase activity, which in turn led to improved performance in the working-memory tests as seen in this study.

The beneficial effects of hazelnut-enriched diet on blood glucose and serum lipid levels have been reported in both humans and rodents (4, 27, 28). The results of this study showing the reversal of HFD-induced alteration in glucose and lipid levels not only corroborates these other studies but also buttresses the results of a previous study carried out in our laboratory (4), that showed slight differences in the effects observed wit h the two hazelnut varieties.

In this study, mice treated with HFD had increased caspase-3 levels, compared to mice fed SD. Rodents fed diets that are high in fat have been reported to show increased caspase-3 mediated apoptosis (29). However, hazelnut enriched diet had been associated with decreased caspase-3 mediated apoptosis (6). In this study, both hazelnut varieties decreased caspase-3 levels in whole brain homogenates, suggesting preservation of neuronal integrity, and an ability to prevent neuronal apoptosis; while also corroborating the neuroprotective ability of hazelnut in mice fed HFD as previously reported (6)

Finally, dietary supplementation with hazelnut was also associated with a reversal of HFD-induced lipid peroxidation and oxidative stress as evident by decreased MDA levels, decreased SOD activity and nitric oxide level. Hazelnut's ability to mitigate oxidative stress and lipid peroxidation as observed in this present study corroborates the results of previous studies that had associated hazelnut with a decreased susceptibility of lipids to peroxidation (30,31), largely due to its high polyphenol content.

6. CONCLUSION

In conclusion, the study shows that hazelnut-supplemented diet can be beneficial in mitigating the deleterious consequences of high-fat diet in the mouse brain through its ability to modulate changes in open field behaviours, and reduce high-fat diet induced anxiogenesis and reduction in working-memory. There was also improvement in the metabolic indices, lipid peroxidation status, and antioxidant status that were deranged by high-fat diet. A reduction in brain acetylcholinesterase activity, improvement in dopamine levels, and reduction in caspase-3 that accompany hazelnut-supplemented diet are believed to contribute significantly to the behavioural modulation, through improved neurotransmitter balance and preservation of neuronal integrity. Based on these observations, hazelnut could be of value in managing similar conditions in humans, and may be employed for the developing of nutraceutical formulations.

7. ACKNOWLEDGMENTS

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Abbreviations: HDL-High: density lipoprotein, HFD: High-fat diet, LDL: Low density lipoprotein, MDA: Malondialdehyde, SD: Standard diet, SOD: Superoxide dismutase, TBARs: Thiobarbituric acid-reactive species, TC: Total cholesterol, TG: Triglyceride

Key Words: Antioxidant; Brain; *Corylus avellana*; Functional food; Nutraceutical; Neurobehaviour; Metabolic

Send correspondence to: Olakunle J. Onaolapo, Behavioral Neuroscience and Neuropharmacology Unit, Department of Pharmacology, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria, Tel: 2347031986101, E-mail: olakunleonaolapo@yahoo.co.uk