MEDWIN PUBLISHERS
Committed to Create Value for Researchers

International Journal of Biochemistry & Physiology
ISSN: 2577-4360

Oxidative, Hepatoprotective and Ant-Inflammatory Responses to Perinatal Walnut (*Juglans regia* L.) Supplemented Diet in Offspring of Sprague-Dawley Rats

Igbayilola YD[1]*, Aina OS[1], Ogunkoya OO[2], Williams OD[2] and Olaoye FA[2]

[1]Department of Physiology, University of Lagos, Nigeria

[2]Department of Science Laboratory Technology, DS Adegbenro ICT Polytechnic Itori, Nigeria

***Corresponding author:** Igbayilola Yusuff, Department of Physiology, College of Medicine of the University of Lagos, Lagos, Nigeria, Tel: +23408161927278; Email: princeyai85@gmail.com

Research Article

Volume 7 Issue 1

Received Date: June 02, 2022

Published Date: June 27, 2022

DOI: 10.23880/ijbp-16000205

Abstract

Objective: The use of plants as source of remedies for the treatment of diseases can be traced back to the prehistoric times. This study investigated the role of perinatal walnut (*Juglans regia* L.) supplemented diet on oxidative and anti-inflammatory responses in male offspring of Sprague-Dawley rats.

Methods: Twenty-four pregnant female and 12 male Sprague-Dawley rats were used. They were fed either a normal diet or walnut supplemented (WS) diet. The dams were given WS diet up to parturition (in-utero group, IUWS), or from birth to post-natal day 21 (lactation group, LWS) or for a period covering both groups (combined group, CWS). Control (CONT) dams with CONT diet was run in parallel for comparison. On postnatal day 63, serum cholesterol (CHOL), triglyceride (TG), high density lipoprotein (HDL), hepatic lipase (HL), aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP). Superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), malonaldehyde (MDA) and tumor necrotic factor alpha (TNF-α) were assayed.

Results: The results revealed a significant decrease ($P = .05$) of AST in IUWS, LWS and CWS compared with CONT. ALT decreased in IUWS, LWS and CWS but not significant ($p>.05$) while AST significantly decreased ($P=.05$) only in CWS and non-significantly in IUWS and LWS ($p>.05$) compared with CONT. CHOL decreased significantly ($P = .05$) in LWS, CWS and non-significantly ($p>.05$) in IUWS. TG showed a significant decrease ($P = .05$) in IUWS and CWS compared with CONT while HL showed a significant increase ($P = .05$) in IUWS, LWS and CWS compared with CONT. Assay of antioxidant enzymes showed a significant increase ($P = .05$) of GSH, SOD and CAT with concomitant decrease of MDA in IUWS, LWS and CWS compared with CONT. TNF alpha significantly upregulated ($P = .05$) in IUWS, LWS and CWS compared with CONT.

Conclusion: In conclusion, the data described in this study suggest that perinatal walnut supplemented diet increased oxidative and anti-inflammatory responses and improved lipid and hepatic functions observed suggest an attestation to its use in folk medicine though the effects were dependent on the window of exposure.

Keywords: Antioxidant; Triglyceride; Intrauterine; Perinatal; Walnut

Abbreviations: WHO: World Health Organization; GSH: Reduced Glutathione Content; CAT: Catalase; MDA: Malonaldehyde; CHOL: Cholesterol; TG: Triglycerides; HDL: High Density Lipoprotein; HL: Hepatic Lipase; TNF-α: Tumor Necrotic Factor Alpha; CONT: Control; IUWS: In-Utero Walnut Supplementation; LWS: Lactational Walnut Supplementation; CWS: Combined Walnut Supplementation; SOD: Superoxide Dismutase Activity; AST: Aspartate

Amino Transferase; ALT: Alanine Amino Transferase; ALP: Alkaline Phosphatase; TBA: Thiobarbituric Acid; ELISA: Enzyme-Linked Immunoabsorbent Assay; SEM: Standard Error of Mean; ANOVA: One-Way Analysis of Variance; GOT: Glutamyl Oxaloacetic Transaminase; GPT: Glutamyl Pyruvic Transaminase; CCl_4: Carbon Tetrachloride; WP: Polyphenol-Rich Extract from Walnuts; TG: Triglycerides; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; LDL: Low-Density Lipoprotein.

Introduction

Foetal programming occurs during embryonic and foetal development, a critical period in which tissues and organs are created. Insufficient nutrition during this time results in permanent alterations to certain structural and physiological metabolic functions of the foetus. British epidemiologist Lucas first established the hypothesis, known as the "Lucas hypothesis," which states such programmed changes during this critical period predispose the fetus to certain postnatal diseases [1]. The critical period coincides with the timing of rapid cell differentiation. Essentially, programming refers to the process of sustaining or affecting a stimulus or impairment that occurs at a crucial point in its development [1]. The use of plants as remedies source for the treatment of diseases has been be traced back to the prehistoric times and medicinal herbs are being increasingly investigated by researchers in various field of medicine. Indian Ayurveda medicine used herbs as early as 1900 BC describing about 700 medicinal plants [2]. According to the World Health Organization (WHO) more than 80% of the world's populations, rely on traditional medicine for their primary health care, majority of which use plants or their active principles. Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants [2]. Medicinal plant also called medicinal herb have been discovered and used in traditional medicine practices since prehistoric times. These plants are the plants which have one or more of its part having substances that can be used for treatment of diseases.

Walnut (*Juglans regia* L.) is the most widespread tree nut in the world. The tree is commonly called as the Persian walnut, white walnut, English walnut or common walnut. It belongs to juglandaceae and has the scientific name *Juglans regia*. The walnut tree species is native to the old world. It is native in a region stretching from the Balkans eastward to the western Himalayan chain Godfrey KM, et al. [2] and was cultivated in Europe as early as 1000 BC. At present, walnut is cultivated commercially throughout southern Europe, northern Africa, eastern Asia, the USA and western South America. The walnut is a dietary plant with one of the highest levels of antioxidants Lopez JF, et al. [3] and it has the highest level of phenolic antioxidants among nut species [3,4].

Epidemiological and experimental studies reported a dose-dependent negative association between consumption of tree nuts and reduced risk diet-related disorders such as obesity and cardiovascular events. Whereas this benefit is usually attributed to the unsaturated fatty acid composition of nuts, there may be other relevant mechanisms involved including the modulating effect of bioactive nut components on oxidative damage. Walnuts are rich in the polyunsaturated fatty acids linoleic and α-linolenic at 52.4% and 12.5% of kcals respectively and thus potentially susceptible to oxidation. Walnuts have high amount of omega-6 and omega-3 PUFA, which are essential dietary fatty acids. Clinical studies suggest that omega-3 PUFA have significant role in prevention of coronary heart disease [5]. Oil rich in oleic acid displays greater oxidative stability therefore; it could be widely used as frying oil. According to an investigation conducted by several researchers, it was found that the average value for protein was 18.1% [6]. These lipids are naturally protected by tocopherols in the seed and phenolic compounds in the pellicle or seed coat [6]. Walnut phenolics are mostly of the nonflavonoid type belonging to the ellagitannin, or hydolyzable tannins, category.

Despite the above convincing evidences about medicinal significance of walnut, it is worthy to note that the nutritional significance of which has not been fully elucidated in respect to foetal programming. Precisely, there's death of information on antioxidant, anti-inflammatory and hepatic responses of perinatal walnut supplemented diet in offspring of Sprague-Dawley rats. In the present investigation, we assessed the offspring of female Sprague-Dawley rats exposed to perinatal protein walnut supplemented diet during different windows of early life exposure namely, in-utero, Lactational and "combined to evaluate its impact on markers of oxidative balance such as superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) and lipid peroxidation's index malonaldehyde (MDA), its impact on lipid parameters such as cholesterol (CHOL), triglycerides (TG), high density lipoprotein (HDL) and hepatic lipase (HL) as well as anti-inflammatory marker such as tumor necrotic factor alpha (TNF-α) and whether the effect is dependent on the window of exposure.

Materials and Methods

Animals

Twelve male and eighteen female Sprague-Dawley rats (n=30) weighing 120-150 grams were used. They were housed with plastic cages under standard lighting conditions 12 hrs. Light/dark cycles, and allowed free access to tap water and standard food and acclimatized for two weeks at the animal house of D. S. Adegbenro ICT Polytechnic, Eruku-Itori, Ewekoro, Ogun State. The experimental procedures

Copyright© Igbayilola YD, et al.

used were in accordance with the provisions of the Experimentation Ethics Committee on Animals Use of the College of Medicine of the University of Lagos, Lagos State and the United States National Academy of Sciences Guide for the Care and Use of Laboratory animals.

Walnut Collection

Walnut was purchased from a local seller in Gbangba market, Iyana Mortuary, Abeokuta, Ogun state, Nigeria.

Diet, Mating and Grouping

Approximately 850g of dried walnut were pelletized with 50kg of normal rat chow to from the walnut supplemented diet and another 50kg normal rat chow which constituted the control diet. Walnut diet supplemented with the following whole foods, typically as a dried ground powder: maize (W+M), sesame (W+SO), palm kernel cake (W+PKC), soya bean meal (W+SBM), bone meal (W+BM), limestone (W+LS), industrial salt (W+IS), grower premix (W+GP), lysine (W+L), methionine (W+M), alphatox (W+A), enzyme (W+E) and threonine (W+T). The diets provided a typical dietary intake of 1.5 servings of each food/d in a human diet, based on an energy content of a serving of food as set by the FDA for nutrition facts panels and a total daily diet intake of 2000 kcal [7]. Diets were macronutrient balanced such that every walnut supplemented diet contained the same total percentage of the normal rat diet.

The animals were mated overnight with proven male breeders, one male to two females and maintained in their respective diet throughout gestation. They were maintained under room temperature 25±1^{0}C with 12 h light/dark cycle. The day on which spermatozoa was present on a vaginal smear which was washed with normal saline NaCl 0.9% was designated as day of conception i.e. pregnancy day 0. The pregnant rats were allocated to one of four groups to be fed either a control diet or walnut supplemented diet. Food and water were available for all animals and grouped thus (six animals per group) [8].

- **Group A:** Control-**CONT** (fed with control diet throughout the experiment).
- **Group B:** In-utero walnut supplementation–**IUWS** (fed with walnut supplemented diet only during gestation).
- **Group C:** Lactational walnut supplementation-**LWS** (fed with walnut supplemented diet only during lactation).
- **Group D:** Combined walnut supplementation-**CWS** (fed with walnut supplemented diet during both windows). Litters were reduced to 8 – 10 pups on postnatal day (PND) 1 (birth, day 0). They were weaned on PND 21 and housed in groups of three or four male rats per cage. All male pups were transitioned to control diet, except control group, until the end of the experiment (PND 63). For consistency, only male offspring were used for the study because early-life programming is known to occur in a sexually dimorphic manner Igbayilola YD, et al. [9,10] which was outside the focus of this study.

Sample Preparation and Assessment of Proximate, Vitamins and Mineral Compositions

Sample Preparation: The nuts collected were dehulled and washed with de-ionized water and placed in a tray for the drying. Different processing treatments of cooking, roasting, and toasting were used. The walnut was further divided into three portions. The three portions of sample were cooked at 100°C for 30min, 40min and 50min respectively after which the different portions were dehulled for the second time for exposure of the walnut seed. The individual portions were milled using attrition mill. The purpose of milling was to expose more of the surface area of the nut for easy drying. After milling, each portion was dried in an oven at a temperature of 60°C for 15min to constant weight; the process was closely monitored to avoid charring. After oven drying, the samples were further milled so as to further reduce the particle size. With the help of a 0.3mm sieve, the different samples were sieved gently and the walnut flour for analyses was obtained while the fibre was discarded. Airtight container was used to package the walnut flour which was obtained in preparation for analysis

Proximate Analysis: Prior to palletization, walnut was analyzed for moisture, protein, fat, ash, fiber, and nitrogen free extract composition by the methods [11] and conducted in the laboratory of the Technology Incubation Centre, Federal Ministry of Science and Technology, Abeokuta, Ogun state.

Determination of Vitamin compositions: All vitamins were determined Using Atomic Absorption Spectrophotometer.

Determination of Minerals composition: Acid Digestion: The samples for the determination of the minerals elements of interest were subjected to acid digestion by weighing out 0.2g of the sample into a dried crucible. It was then ashed on a furnace until colorless, then, 5mls of Conc. Hydrochloric acid (HCL) was used to digest it. When cooled, distilled water was added and then filtered into 50ml volumetric flask with no 41 filter Whattman paper. The crucible was rinsed with 0.1N HCl and water to make up to mark. It was then filtered to get a clear solution free of particles. Subsequently, the different elements were determined Using Atomic Absorption Spectrophotometer.

Collection of Blood Sample

Five (5ml) of blood sample was taken by retro-orbital puncture. Blood was allowed to clot for 1 hour at 4°c. Samples were centrifuged at 3,000 rpm for 10 minutes and the serum samples were kept at -20°c until analyses [12].

Copyright© Igbayilola YD, et al.

Tissue isolation: At postnatal day 42 the rats were sacrificed using cervical dislocation. The animals were dissected and liver, skeletal, intestinal and pancreatic tissues were removed and washed in an ice cold and rinsed with 1.15 % KCl, blotted after which all the tissues were weighed [8]. Briefly, all tissues obtained were homogenized with 0.5 M phosphate buffer and centrifuged at 3000 rpm for 10 minutes; the supernatant was decanted and stored at – 4°C until analyses.

Oxidative Stress Study

Superoxide Dismutase (SOD) activity: Briefly; SOD activity was measured by the inhibition autoxidative capacity of pyrogallol. The SOD activity was evaluated using a spectrophotometer at 420 nm. A calibration curve was constructed using SOD as standard. A 50 % inhibition of autoxidation of pyrogallol was defined as one SOD unit [13].

Catalase activity: Briefly, sample (1ml) was mixed with 49 ml of distilled water to give a 1 in 50 dilution of the sample. The assay mixture contained 4ml of H_2O_2 solution (800 μmoles) and 5ml of Phosphate buffer in a 10ml flat bottom flask. One (1 ml) of properly diluted enzymes preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1ml portion of the reaction mixture was blown into 2ml of dichromate acetic acid reagent at 60s intervals. Catalase (CAT) activity was determined by measuring the exponential disappearance of H_2O_2 at 240 nm and expressed in units/mg of protein [14].

Reduced glutathione (GSH) content: The protein content of the samples was initially precipitated by metaphosphoric acid (MPA) at the ratio of 1:1 (homogenate/MPA). The samples were centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and mixed with sodium phosphate buffer (0.1M, pH 7.4), containing EDTA (5mM) and ortho-phthaldialdehyde (1 mg/mL in methanol). The mixture was incubated in the dark at room temperature for 15 min and fluorescence was measured at 350 nm (excitation) and 420 nm (emission). A standard curve of GSH (0.001–0.1 mM) was used for linear regression [13].

Malondialdehyde (MDA): Briefly, the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems, MDA was estimated with the method of Uchiyama and Mihara which is based on its interaction with thiobarbituric acid (TBA) to form pink complex with absorption at 535 nm. Absorbance was read using Microlab 300 recording spectrophotometer (UV 160) in all measurements [15].

Assay of Tumor Necrotic Factor (TNF-A)

The enzyme–linked immunoabsorbent assay (ELISA) test was employed in the determination of serum tumor necrotic factor (TNF-α) level with the aid of rat TNF-α ELISA kit (Elabscience, Wuhan, China). Briefly; 50 μl of the samples were pipetted into an eppendorf tube. A 100 μl of the conjugate was added to the samples labeled accordingly. 50 μl of the standard were also pipetted into another eppendorf tube, 100 μl of the conjugate was added. Another was created as blank and was incubated alongside with the standard and sample at room temperature (22 – 28 ^{0}C) for 2 hours and the absorbance was read at 450 nm against blank.

Liver Functions Assay

Albumin, alkaline phosphatase (ALP), alkaline amino transferase (ALT) and aspartate amino transferase (AST) were determined using liver homogenate samples by an automated analyzer (Mindray BS-120, Chema Diagnostica, Italy).

Determination of Lipid Profile

Cholesterol, triglyceride, high density and low density lipoproteins levels were carried out from the serum and liver homogenate samples with the aid of an automated Analyzer (*Mindray BS-120, Chema Diagnostica, Italy*).

Determination of Hepatic Lipase (HL)

Hepatic lipase (HL) activity was determined in liver tissue homogenate thus: The assay system (final volume 1 ml) contained 0.1 ml of glyceride emulsion, 0.2 ml of serum albumin, 0.6 ml of 0.1 M phosphate buffer (pH 7.4). 0.1 ml of enzyme approximately 200 pg of lipase protein was dissolved in glass-distilled water and the incubations lasted for 60 min at 37 0C in a shaking water bath.

Statistical Analysis

The results are presented as the mean standard error of mean (SEM). Graph Pad Prism Software (GraphPad, Inc., La Jolla, CA, USA) was used for statistical analyses. One-way analysis of variance (ANOVA) with post hoc Tukey's multiple comparison test was used. The level of significance was set at (*P*=.05).

Results

Proximate Composition of Walnut Powder

Table 1 showed proximate compositions of dried walnut which revealed the presence of moisture, fat, ash, crude fibre, crude protein and carbohydrate and the respective values are presented in (Table 1).

Copyright© Igbayilola YD, et al.

Parameters	Values (%)
Moisture Content	16.57
Fat Content	52.68
Ash Content	1.56
Crude Fibre	5.98
Crude Protein	12.87
Carbohydrate	10.34

Table 1: Proximate composition of the dried walnut.

Vitamin Composition of the Walnut Powder

Table 2 displayed vitamin compositions of dried walnut which revealed the presence of fat and water soluble vitamins A, B1, B6, B7, B12, C, D and E the respective values are presented in (Table 2).

Vitamins	Result	Unit
Vitamin A	6.121	(ug/100g)
Vitamin B1	0.328	(mg/100g)
Vitamin B6	1.104	(mg/100g)
Vitamin B7	0.788	(ug/100g)
Vitamin B12	0.056	(ug/100g)
Vitamin C	1.283	(ug/100g)
Vitamin D	0.008	(ug/100g)
Vitamin E	0.718	(mg/100g)

Table 2: Vitamin composition of the dried walnut.

Mineral Composition of the Walnut Powder

Table 3 displayed minerals compositions of dried walnut which revealed the presence of Na, K, Ca, Fe, Zn, Mg, Pb and Mn the respective values are presented in (Table 3).

Minerals	Result (mg/100g)
Sodium (Na)	2.456
Potassium (K)	4.281
Calcium (Ca)	106.372
Iron (Fe)	3.678
Zinc (Zn)	2.986
Magnesium (Mg)	154.732
Lead (Pb)	0.035
Manganese (Mn)	3.402

Table 3: Mineral composition of the dried walnut.

Effect of Perinatal Walnut Supplemented Diet on Hepatic Functions in CONT and Treated Rats

The results in (Table 4) revealed a significant decrease (P = .05) in AST level in IUWS, LWS and CWS while ALT showed a non-significant increase (P=.05) in IUWS, LWS and CWS with a significant decrease in ALP in CWS compared with CONT. ALP significantly (P = .05) increased in IUWS and LWS compared with CONT.

Parameters (u/l)	CONT	IUWS	LWS	CWS
AST	3136±18.87	2746±38.16$^{\#}$	287.4±48.44$^{\#}$	2808±57.65$^{\#}$
ALT	1506±27.31	1572±18.55	1510±26.83	1486±20.25
ALP	60.60±1.91	59.20±3.51	54.00±2.98	38.20±1.99$^{\#a1}$

Table 4: Effect of perinatal walnut supplemented diet on AST, ALT and ALP levels in CONT and treated rats.
Values represent Mean ± SEM; Significant ($^{\#}$(P=.05) vs. CONT, ^{a}(P=.05) vs IUWS, ^{1}(P=.05) vs. LWS). **KEY: CONT:** Control; **IUWS:** In-utero walnut supplementation; **LWS:** Lactation walnut supplementation; **CWS:** Combined walnut supplementation. **AST:** Aspartate amino transferase, **ALT:** Alanine amino transferase, **ALP**: Alkaline phosphatase

Effect of Perinatal Walnut Supplemented Diet on Lipid Parameters in CONT and Treated Rats

There was increase (P=.05) in CHOL in IUWS and a significant increase (P = .05) in LWS with a significant decrease (P = .05) in IUWS compared with CONT. CHOL significantly decreased (P = .05) in IUWS and LWS compared with CWS. Table 5. TG significantly increased (P = .05) in IUWS, increased but not significant (P=.05) in LWS and significantly increased (P = .05) in CWS compared with CONT. A significant decreased (P = .05) was observed in CWS compared with IUWS and LWS Table 5. HL showed a significant increase (P = .05) in IUWS, LWS and CWS compared with CONT (Table 5).

Copyright© Igbayilola YD, et al.

Parameters (u/l)	CONT	IUWS	LWS	CWS
CHOL (mmol/l)	0.64±0.02	0.74±0.02	0.84±0.04[#]	0.28±0.05[#a1]
TG (mmol/l)	2.48±0.08	3.00±0.03[#]	2.66±0.04[a]	0.82±0.02[#a1]
HL (unit/mg/min)	60.00±2.23	84.00±2.45[#]	84.00±2.45[#]	86.00±2.45[#]

Table 5: Effect of perinatal walnut supplemented diet on lipid parameters (CHOL, TG and HL) in CONT and treated rats. Values represent Mean ± SEM; Significant ([#](P=.05) vs. CONT, [a](P=.05) vs IUWS, [1](P=.05) vs. LWS). **KEY: CONT:** Control; **IUWS:** In-utero walnut supplementation; **LWS:** Lactation walnut supplementation**; CWS:** Combined walnut supplementation, **CHOL:** Cholesterol, **TG:** Triglycerides, **HDL:** High density lipoprotein, **LDL:** Low density lipoprotein, **HL**: Hepatic Lipase.

Effect of Perinatal Walnut Supplemented Diet on Oxidative Status in CONT and Treated Rats

Assay of antioxidant enzymes showed a significant decrease (*P* = .05) in GSH activity in IUWS and CWS and increase (*P*=.05) in LWS compared with CONT. GSH increased significantly (*P* = .05) in LWS compared with IUWS and CWS (Figure 1). There was a significant increase (P = .05) in SOD in IUWS, LWS and CWS compared with CONT and a significant decrease (*P* = .05) in CWS compared with LWS and CWS. CAT increased significantly (*P* = .05) in IUWS, LWS and CWS compared with CONT (Figures 2&3). MDA in figure 4 significantly decreased (*P* = .05) in LWS and CWS compared with CONT (Figures 4&5).

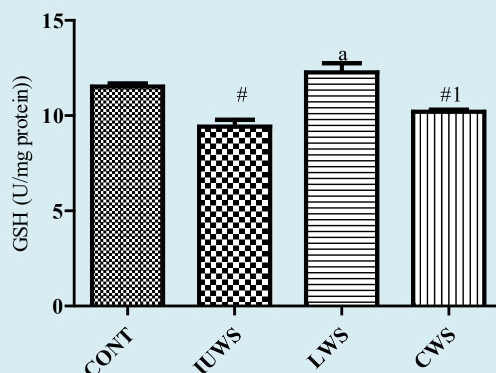

Figure 1: Effect of walnut supplemented diet on GSH activity in CONT and treated rats. Values represent Mean ± SEM; n=6. Significant levels ([#] (P=.05) vs CONT, [a](P=.05) vs IUWS, [1](P=.05) vs LWS). **CONT**: Control; **IUWS**: In-utero walnut supplementation; **LWS**: Lactational walnut supplementation; **CWS**: Combined walnut supplementation. GSH: Reduced gluthiatione.

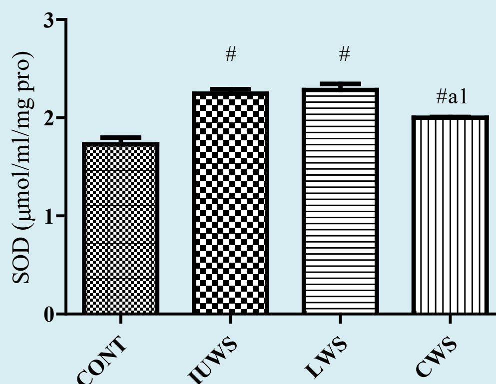

Figure 2: Effect of walnut supplemented diet on SOD activity in CONT and treated rats. Values represent Mean ± SEM; n=6. Significant levels ([#] (P=.05) vs CONT, [a](P=.05) vs IUWS, [1](P=.05) vs LWS). **CONT**: Control; **IUWS**: In-utero walnut supplementation; **LWS**: Lactational walnut supplementation; **CWS**: Combined walnut supplementation. SOD: Superoxide dismutase.

Copyright© Igbayilola YD, et al.

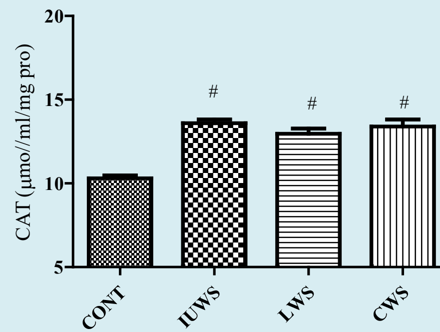

Figure 3: Effect of walnut supplemented diet on CAT activity in CONT and treated rats. Values represent Mean ± SEM; n=6. Significant levels (#(P=.05) vs CONT). **CONT**: Control; **IUWS**: In-utero walnut supplementation; **LWS**: Lactational walnut supplementation; **CWS**: Combined walnut supplementation. CAT: Catalase.

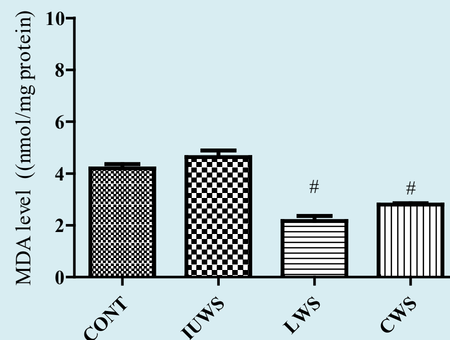

Figure 4: Effect of walnut supplemented diet on MDA level in CONT and treated rats. Values represent Mean ± SEM; n=6. Significant levels (#(P=.05) vs CONT). **CONT**: Control; **IUWS**: In-utero walnut supplementation; **LWS**: Lactational walnut supplementation; **CWS**: Combined walnut supplementation. MDA: Malonaldehyde.

Effect of Perinatal Walnut Supplemented Diet on Inflammatory Response

There was a significant increase *(P* = .05) in TNF-alpha in IUWS and LWS compared with CONT while LWS and CWS significantly decreased *(*P = *.05)* compared with IUWS and significantly decreased (P = .05) in CWS compared with LWS.

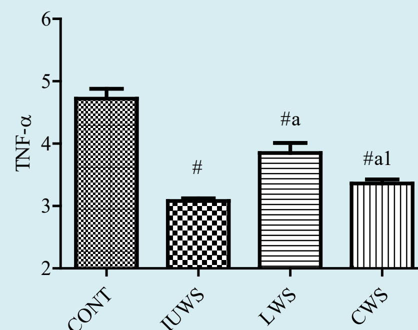

Figure 5: Effect of walnut supplemented diet on TNF alpha level in CONT and treated rats. Values represent Mean ± SEM; n=6. Significant levels #(P=.05) vs CONT, a*(P=.05)* vs IUWS, 1(P=.05) vs LWS). **CONT**: Control; **IUWS**: In-utero walnut supplementation; **LWS**: Lactational walnut supplementation; **CWS**: Combined walnut supplementation. TNF: Tumor necrotic factor.

Copyright© Igbayilola YD, et al.

Discussion

Findings from the present study revealed significant presence of vitamins including mineral elements which are required for body growth and metabolism. Walnut has globally been used in human nutrition since ancient times, the high protein and fat contents of the kernels of *Juglans regia* L. (Juglandacea) make it highly indispensable for human consumption. Therefore, the walnut is categorized as a strategic species for human nutrition which is included in the FAO list of priority plants [16]. The seed part of the fruit (kernel) is consumed fresh, toasted and can be supplemented with other foods. In the Middle East walnuts are added alone or along with almonds, date, and raisin as a special pastry preparation called Ma'moul. Walnuts are nutrient-rich food due to high contents of fats, proteins, vitamins and minerals [17].

Perinatal walnut supplemented diet produced decreased liver enzymes' activities of ALT, AST and ALP in offspring of rats and this is suggestive of heptoprotective effect. Previous study reported that orally fed Walnut polyphenols prepared from the kernel pellicle demonstrated a dose dependent lowering effect in glutamyl oxaloacetic transaminase (GOT) and glutamyl pyruvic transaminase (GPT) in carbon tetrachloride (CCl_4) induced liver damage in mice, an indication that walnut is heptoprotective in nature [18].

Increased cholesterol and triglycerides in rats exposed to perinatal walnut supplemented diet may be due to the high presence of fat components as revealed from the proximate compositions of the sample of the blended dried walnut. Previous investigation revealed that oral administration of a polyphenol-rich extract (WP) from walnuts in high fat diet fed mice significantly reduced liver weight and serum triglycerides (TG). A polyphenol-rich extract was found to possess hypotriglyceridemic activity via enhancement of peroxisomal fatty acid β-oxidation in the liver which suggested that tellimagrandin I is involved in the hypotriglyceridemic mechanism [19].

This study showed that perinatal walnut supplementation improved oxidative balance in offspring of rats by increasing the activities of antioxidant enzymes with a concomitant decrease in lipid peroxidation. Previous study revealed that the antioxidant potential of ethyl acetate, butanol, methanol, ether and aqueous methanol extract of walnut kernels, husks and leaves were measured by different methods such as reducing power, scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and lipid oxidation inhibition by β-carotene linoleate system. All the extracts showed strong antioxidant activity Shimoda H, et al. [20-25] reported a decrease in the antioxidant burden observed in enzymatic and non-enzymatic antioxidant systems after the consumption of a whole-walnut or a walnut-skin diet in C57BL/6 mice. The same author also reported that consumption of walnuts and walnut skins have no deleterious effect on low-density lipoprotein (LDL) oxidizing capability, despite their higher contents of omega-6 PUFAs. Several phenolic compounds isolated from *J. regia* such as pyrogallol, p-hydroxybenzoic acid, vanillic acid, ethyl gallate, protocatechuic acid, gallic acid, 3,4,8,9,10-pentahydroxydibenzo pyran-6-one, tannins, glansrins, adenosine, adenine could provide a chemical basis for some of the health benefits claimed for *J. regia* in foods and folk medicine [26].

Tumor necrosis factor alfa is naturally produced by activated macrophages and monocytes and it has been reported to have a pleiotropic effect on normal and malignant cells. Findings from the present investigation revealed anti-inflammatory potential of perinatal walnut supplemented diet in offspring of rats with the significant decrease of the anti-inflammatory marker, TNF-alpha. In agreement with the previous study by Oliveira I, et al. [27], the ethanolic extracts of *J. regia* leaves exhibited potent anti-inflammatory activity. Fukuda T, et al. [28] stated that the alcohol extract of walnut leaves in dose of 1.5 mg/kg caused a significant nociception decrease in acute phase of formalin test whereas the aqueous (2.87 and 1.64 g/kg) and ethanolic (2.044 and 1.17 g/kg) extracts of leaves showed anti-nociceptive activity in hotplate test suggesting a promising analgesic and anti-inflammatory agents against diseases such as rheumatoid arthritis. On the basis of the result by Shimoda H, et al. [20], a protective role of methanolic *J. regia* extract against CSE-induced acute lung toxicity in Wistar rats was suggested

Conclusion

In conclusion, the data described in the present investigation suggest that perinatal walnut supplemented diet improved oxidative and anti-inflammatory responses and the improved lipid and hepatic functions observed is an attestation to the therapeutic significance of walnut in folk medicine though the effects seen in this study were dependent on the windows of exposure.

Conflict of Interests

The authors wish to declare no personal or financial conflict of interest

Author's Contributions

Author IYD' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author AOS' and 'Author OOO' managed the analyses of the study. 'Authors OFA, WOD' managed the literature searches…… All authors read and approved the

Copyright© Igbayilola YD, et al.

final manuscript."

Ethical Approval

Ethical approval was sought and given by the institution's animal care use and research committee.

Funding Statement

We did not receive any specific grant for this research from any funding agencies either the public, commercial or not-for-profit sectors.

References

1. Lucas A (1998) Programming by early nutrition: an experimental approach. J Nutr 128(2S): 401S-406S.
2. Godfrey KM, Barker DJ (2001) Fetal programming and adult health. Public Health Nutr 4(2B): 611-624.
3. Lopez JF, Aleta N, Alias R (2000) Forest Genetic Resources Conservation of *Juglans regia* L. IPGRI Publishers, Rome, Italy, pp: 20-25.
4. Espin JC, Conesa MTG, Barberan FAT (2007) Nutraceuticals: Facts and fiction. Phytochemistry 68(22-24): 2986-3008.
5. Vinson JA, Cai Y (2012) Nuts, especially walnuts, have both antioxidant quantity and efficacy and exhibit significant potential health benefits. Food Funct 3(2): 134-140.
6. Kaileh MB, Berghea WV, Boonec E, Essawib T, Haegemana G (2007) Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. J Ethnopharmacol 113(3): 510-516.
7. US Food and Drug Administration (2014) A food labeling guide, guidance for industry.
8. Muradoglu FH, Oguz I, Yildiz K, Yilmaz H (2010) Some chemical composition of walnut (*Juglans regia* L.) selections from Eastern Turkey. Afr J Agric Res 5(17): 2379-2385.
9. Igbayilola YD, Morakinyo AO, Iranloye BO (2021) Adverse effects of perinatal protein restriction on glucose homeostasis in offspring of Sprague-Dawley rats. Scientific African 14: 1-11.
10. Gabory A, Attig L, Junien C (2009) Sexual dimorphism in environmental epigenetic programming. Mol Cell Endocrinol 304(1-2): 8-18.
11. Chisari AN, Podaza E, Echarte EM (2015) A low maternal protein diet during pregnancy and lactation induce liver offspring dysfunction, in the rat. Am Nutr Disord &Ther 2(1): 10-20.
12. AOAC (2003) Official methods of analysis of the association of official's analytical chemists. In: 17th (Edn.), Association of official analytical chemists, Arlington, Virginia.
13. Morakinyo AO, Iranloye BO, Ogunsola OA (2018) Glucometabolic effects of single and repeated exposure to forced-swimming stressor in Sprague-Dawley rats. Endocr Regul 52(2): 85-92.
14. Vilela DD, Peixoto LG, Teixeira RR, Baptista NB, Caixeta DC, et al. (2016) The Role of Metformin in Controlling Oxidative Stress in Muscle of Diabetic Rats. Oxid Med Cell Longev.
15. Aebi H (1974) Catalase in vitro. Methods Enzymology 105: 121-126.
16. Uchiyama M, Mihara M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituris acid test. Anal Biochem 86(1): 271-278.
17. Gandev S (2007) Budding and grafting of the walnut (*Juglans regia* L.) and their effectiveness in Bulgaria (Review). Bulgar J Agri Sci 13: 683-689.
18. Nael AT, Mohammed AA (2021) Significance and use of walnut, *Juglans regia* Linn: A review. Advance J Micrb Resr 15(1): 1-10.
19. Hiroshi S, Tanaka J, Kikuchi M, Fukuda T, Ito H, et al. (2008) Walnut polyphenols prevent liver damage induced by carbon tetrachloride and d-galactosamine: heptoprotective hydrolyzable tannins in the kernel pellicles of walnut. J Agric Food Chem 56(12): 4444-4449.
20. Shimoda H, Tanaka J, Kikuchi M, Fukuda T, Ito H, et al. (2009) Effect of Polyphenol rich extract from walnut on diet-induced hypertriglyceridemia in mice via enhancement of fatty acid oxidation in the liver. J Agric Food Chem 57(5): 1786-1792.
21. Qamar W, Sultana S (2011) Polyphenols from *Juglans regia* L. (Walnut) kernel modulate Cigarette smoke extract induced acute inflammation, oxidative stress and lung injury in Wistar rats. Hum Exp Toxicol 30(6): 499-506.
22. Carvalho M, Ferreira PJ, Mendes VS, Silva R, Pereira JA, et al. (2010) Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. Food Chem

Copyright© Igbayilola YD, et al.

Toxicol 48(1): 441-447.

23. Abbasi MA, Raza A, Riaz T, Shahzadi T, Rehman AU, et al. (2010) Investigation on the volatile constituents of *Juglans regia* and their *in vitro* antioxidant potential. Pakistan Acad Sci 47(3): 137-141.

24. Rahimipanah M, Hamedi M, Mirzapour M (2010) Antioxidant activity and phenolic contents of Persian walnut (*Juglans regia* L.) green husk extract. Afr J Food Sci Technol 1: 105-111.

25. Almeida IF, Fernandes E, Lima JLFC, Costa PC, Bahia MF (2008) Walnut (*Juglans regia*) leaf extracts are strong scavengers of pro oxidant reactive species. Food Chem 106(3): 1014-1020.

26. Bullo M, Nogues MR, Uriarte PL, Salvado JS, Romeu M (2010) Effect of whole walnuts and walnut-skin extracts on oxidant status in mice. J Nutr 26(7-8): 823-828.

27. Oliveira I, Sousa A, Ferreira ICFR, Bento A, Stevinhol LE, et al. (2008) Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. Food Chem Toxicol 46(7): 2326-2331.

28. Fukuda T, Ito H, Yoshida Y (2003) Antioxidative polyphenols from walnuts (*Juglans regia* L.) Phytochem 63(7): 795-801.

Copyright© Igbayilola YD, et al.