Original paper

Histological and biochemical study of turmeric-based treatment against HFD-induced dyslipidaemia and testicular damages in WISTAR rats.

Abstract

The aim of this study is to evaluate the effects of high fat diet (HFD) on lipid profile, testicular function in WISTAR rats, and to explore the effect of turmeric *(curcuma longa.L)* combined with black pepper. In this study, male rats were divided into 3 groups (n=5), G1: control, receiving 350g/d of standard diet; G2: receiving 350g/d of (HFD); G3: receiving 350g/d of HFD supplemented with turmeric and black pepper (TBP). Following two months of protocol experimentation, data showed that HFD induced a significant increase in total cholesterol (0.714±0.766 mg/dl) and triglycerides (0.588±0.164 mg/dl). However, the HDL level is decreased (0.390±0.547 mg/dl) .In addition a significant increase in weight gain (69.41±10.21 g) and a significant decrease in relative testicular weight were observed (0.43±0.005 %). Moreover, the HFD increased significantly the testicular TBARS (47.78±7.24 µmol/g). The histological analysis showed a decrease of sperm cells in the seminiferous tubules lumen , and reduced seminiferous epithelium thickness in rats treated with HFD. On the other hand, TBP treatment was able to prevent HFD effects on the studied parameters. In conclusion, our results indicate that TBP supplementation can effectively reduce the deleterious effects of HFD on the lipid profile and the testicular structure.

Keywords: HFD, testicles, *Curcuma Longa .L* , male rats, , TBP , TBARS , histology.

INTRODUCTION

Infertility is one of the most public health concerns. It is defined as the inability of a couple to conceive a child after one year on normal unprotected sexual activity (Bach and Schlegel, 2019). It is estimated that infertility affects about 8-12% of couples worldwide with male factors involved in about 50% of cases (Punab et al. 2016; Agarwal et al. 2020). Male infertility is a multifactorial condition that has received increasing attention in recent years due to the decline in male sperm quality (Benatta et al., 2020). Indeed, more than 90% of male infertility cases are due to abnormalities in sperm count or morphology (Jiang et al., 2017) and incriminate several lifestyle factors such as cigarette alcohol and caffeine consumption, malnutrition and obesity that can be involved in the development of male infertility(Sharma et al., 2013).

In recent years, obesity has become a global epidemic. According to the OMS, the number of obesity cases has almost tripled since 1975. Indeed, more than 1.9 billion adults are overweight, of which 650 million reached the obesity stage (WHO, 2021). Furthermore, obesity and overweight have been shown to have a negative impact on male reproduction. Alteration of the hypothalamic-pituitary-gonadal axis function, testicular steroidogenesis disturbance, accumulation of reactive oxygen species (ROS) in the reproductive organs, decreased sperm quality and increased scrotal temperature related to the body overweight have been reported in several studies (Katib, 2015; Liu and Ding, 2017; Leisegang et al. 2021; Oladele et al., 2022).

Excessive consumption of saturated fats is one of the underlying causes of obesity (Skoracka et al., 2020).Several studies have shown that HFD affects the regulating hormones of the energy balance, such as hyperleptinemia and hyperinsulinemia accompanied by leptin and insulin resistance, and a decrease in the suppression of ghrelin secretion following HFD(Dube et al., 2002; Hariri et al., 2010; Argente-ArizÃn et al., 2015).Furthermore, the low satiating effect of fat and the high efficiency of dietary fats to be stored in the body are all factors contributing to the induction of obesity (Hariri et al., 2010).

Similar to other areas of biomedical research, rodents constitute preclinical animal models to study the underlying mechanisms of obesity and to evaluate new therapeutic approaches due to the anatomical and physiological similarities between humans and rodents (Martins et al., 2022).Indeed, different animal models have been used whose male rodents remain the most widely used species in experimental studies to induce obesity with HFD(Fernandes et al., 2016).

On the other hand, the use of medicinal plants offers a source of healing since human civilisation. They are of contemporary importance to about 85% of the world's population as a primary health care model (Pant, 2014; Fitzgerald et al., 2020). *Curcuma longa* L, or turemeric, is a spice with a strong yellow pigmentcommonly used in cooking and cosmetics (Vaughn et al., 2016). Its richness in bioactive molecules such as curcumin gives it an advantage to interact with many molecular targets involved in inflammation, adipogenesis and in almost all phases of the development of many pathologies such as diabetes, cardiovascular diseases, cancer and liver damage (Kocaadam and Şanlier, 2017; Mun et al., 2019).

Despite these attractive properties of curcumin, its poor oral bioavailability has been a major issue (Takahashi et al., 2013).The low bioavailability of curcumin can be explained by its low water solubility, as well as extensive systemic metabolism after oral delivery (Sasaki et al., 2011). However, several approaches have been suggested to increase the bioavailability of curcumin (Zheng and McClements, 2020). Adjuvants which can block the metabolic pathways of curcumin are being used to improve its bioavailability (Anand et al., 2007). In addition, the therapeutic combination of turmeric and black pepper has been developed on the basis that piperine, an important component of black pepper extract, can improve the oral bioavailability of curcumin (Volak et al., 2013). Adjuvant piperine when used with curcumin, increases free curcumin in tissues leading to enhance curcumin bioavailability by suppressing rapid glucuronidation of curcumin in liver and intestine and then decreasing its urinary excretion (Kim and Clifton, 2018).

Herein we investigate the effects of turmeric and black pepper treatment on HFD induced obesity and relative effects on lipid profile and testicular histology.

Materials and methods

Preparation of the high fat diet and the TBP treatment

The HFD consisted of 700g of powdered standard feed, 200g of mutton fat and 100g of sunflower oil, constituting 70%, 20% and 10% of the diet composition respectively. Preparations were renewed every three days and stored at 4°C.

The plant *Curcuma longa* L rhizomes were purchased from an herbalist then reduced into fine powder. Taking into account the fact that most of the ingested curcumin is metabolized and eliminated quickly and that piperine enhances the serum concentration of curcumin (Shoba et al., 1998), we mixed turmeric with black pepper to improve curcumin bioavailability. HFD was so supplemented with 20% of turmeric and 5% of black pepper in order to test the treatment efficacy against the HFD biochemical and histological effects.

Study design

This study was conducted on 15 adult male WISTAR rats weighting (242±48.92) for 2 months. Animals were housed in appropriate cages and had free access to food and water under stable housing conditions (temperature between 22 and 24°C, photoperiod: 12h/12h). Animals were divided into three groups (N=5) of homogeneous average body weight; Controls (C)(group 1) receiving 350 g/d of standard diet, Group 2 (HFD) fed a high-fat diet (350g/d), Group 3 (HFD+TBP) receiving 350 g/d of high-fat diet supplemented with TBP

(20% turmeric and 5% black pepper). Rats were weighed weekly to assess the effect of each diet on bodyweight evolution.

Specimens collection and analytical methods

After 12-hour of fasting prior to sacrifice, animals were anaesthetised with chloroform and sacrificed. Blood was collected by cardiac puncture in heparin tubes and centrifuged at 3000 rpm for 5 minutes to separate the plasma. Total cholesterol (TC) and triglycerides (TG) were measured by colorimetric enzyme assay according to the protocol described by the kits manufacturer (Spinreact ®).The determination of HDL-cholesterol (HDL-C) is carried out by the colorimetric enzymatic method. LDL, VLDL and chylomicrons in the sample are precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. The supernatant, after centrifugation, contains HDL-C. The HDL-C fraction is determined using the total cholesterol enzymatic reagent (Naito, 1984).

LDL-cholesterol (LDL-C) was finally calculated following the formula of Friedewald :

LDL-C (mg/dl) = TC (mg/dl)-HDL-C (mg/dl)-[TG (mg/dl)/5)].

Testicular lipid peroxidation was assessed by thiobarbituric acid reactive substances (TBARS) method according to Botsoglou et al (1994). Briefly, 1 gram of tissue was mixed with 8 mL of TCA (5%) and 5 mL BHT prepared in hexane (8%). The mixture was homogenized in the vortex for 30 seconds and was centrifuged at 3000g for 30 minutes. After that, the top hexane layer was eliminated and the rest was completed up to 10 mL with TCA (5%). Finally, 2.5mL of this solution was mixed with 1.5mL of TBA (0.8%) prepared in distilled water and incubated at 100 °C for 30 min. After cooling, absorbance has then measured at 521 nm against a blank containing all reagents except the samples.

The testes designated for the histological study were fixed in formalin 10% then prepared according to the standard histological techniques and then stained with haematoxylin-eosin to study the different histological structures.

Statistical analysis

Data of statistical analysis were carried out using IBM SPSS. 22. An analysis of variance (ANOVA) was performed to check the statistical significance of the results followed by a Post-Hoc Tukey (HSD) test for multiple comparisons between the different groups. A value of P<0.05 is considered significant for all tests.

Results

1. Evaluation of weight gain and relative testicular weight

Table 1 shows a highly significant increase in weight gain in the HFD group (P<0.001). In contrast, a highly significant loss of weight was found in the HFD+TBP group compared to controls (P<0.001). These results revealed the anti-obesity effect of the combined TBP treatment. On the other hand, HFD induced a highly significant decrease in relative testicular weight (testicular atrophy) (P<0.001) compared to control and TBP-treated rats.

Table 1. Effect of HFD and TBP on weight gain and testicular relative weight

Groups	Weight gain (g)	Relative weight (%)
C	31,8 ± 12,59	**0,74± 0,53**
HFD	69,41± 10,21***	**0,43 ± 0,005*** **
HFD+ TBP	**-7,2 ± 6,79*** **	**0,68 ± 0,081** **

C: *control,* ***HFD*** *: high-fat diet,* ***HFD+TBP*** *: high-fat diet supplemented with turmeric and black pepper.*

*Results are expressed as mean ± standard deviation; (n=5). * Significant difference (p<0.05), **very significant difference (p<0.01), ***highly significant difference (p<0.001)*

2. Effect of HFD and TBP on lipid profile

In table 2 results indicate a significant ($p < 0.05$) and a highly significant ($p < 0.001$) increase of total cholesterol in the HFD and HFD+TBP groups respectively.

Compared to all other studied groups a significantly elevated level of HDL-C in HFD+TBP group ($p < 0.01$) was detected. In other terms, TBP treatment combined with the high-fat diet rather improves the HDL-c/total cholesterol ratios indicating an anti-obesity and a possible cardio-protective effect of the suggested treatment. However, was noted a non-significant increase in LDL-C level in the HFD group compared to the other groups. In addition, our results also showed a highly significant increase in TG levels in the HFD-treated rats compared to controls and HFD+TBP ($p<0.001$), while no significant difference in TG levels was observed between the HFD+TBP group and controls.

Table 2: Effect of HFD and TBP on lipid profile

Lipid profile				
Groups	**Total cholesterol (mg/dl)**	**HDL-c (mg/dl)**	**LDL-c (mg/dl)**	**Triglycerides (mg/dl)**
C	0,4280 ± 0,109	0,406 ± 0,054	0,031 ± 0,159	0,282 ± 0,985
HFD	0,714 ± 0,766 *	0,390 ± 0,547**	0,208 ± 0,164	0,588 ± 0,164***
HFD+TBP	0,886 ± 0,766***	0,642 ± 0,120**	0,158 ± 0,164	0,412 ± 0,438*

***C :** control, **HFD** : high-fat diet, **HFD+TBP** : high-fat diet supplemented with turmeric and black pepper. Results are expressed as mean ± standard deviation; (n=5). * Significant difference (p<0.05), **very significant difference (p<0.01), ***highly significant difference (p<0.001).*

5. Effect of HFD and TBP on lipid peroxidation

The results of the present study showed a non-significant increase in testicular TBARS in the HFD group compared to controls (figure.1). While, a highly significant decrease in TBARS was found in the TBP-treated group ($p<0.001$) compared to the HFD group and controls indicating an important antioxidant effect of TBP treatment.

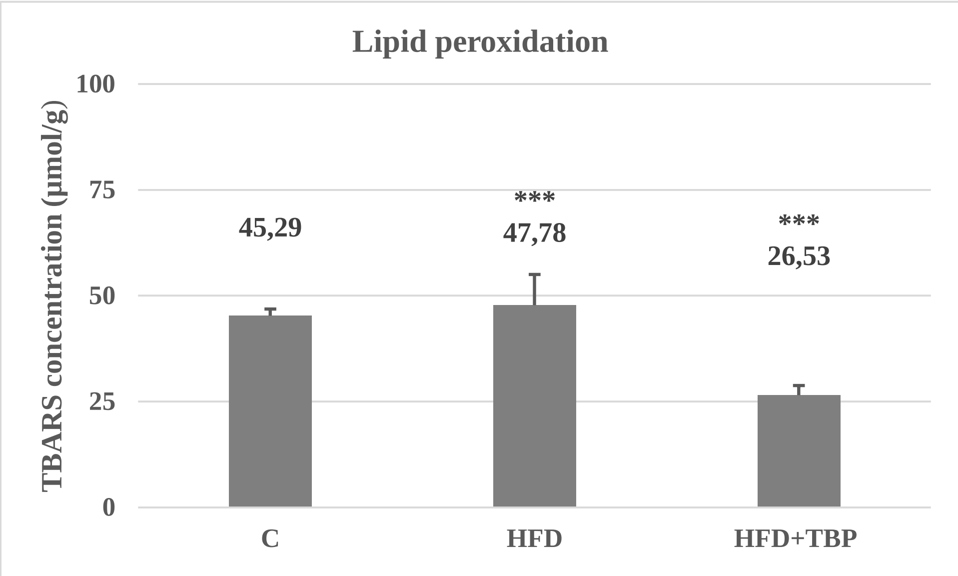


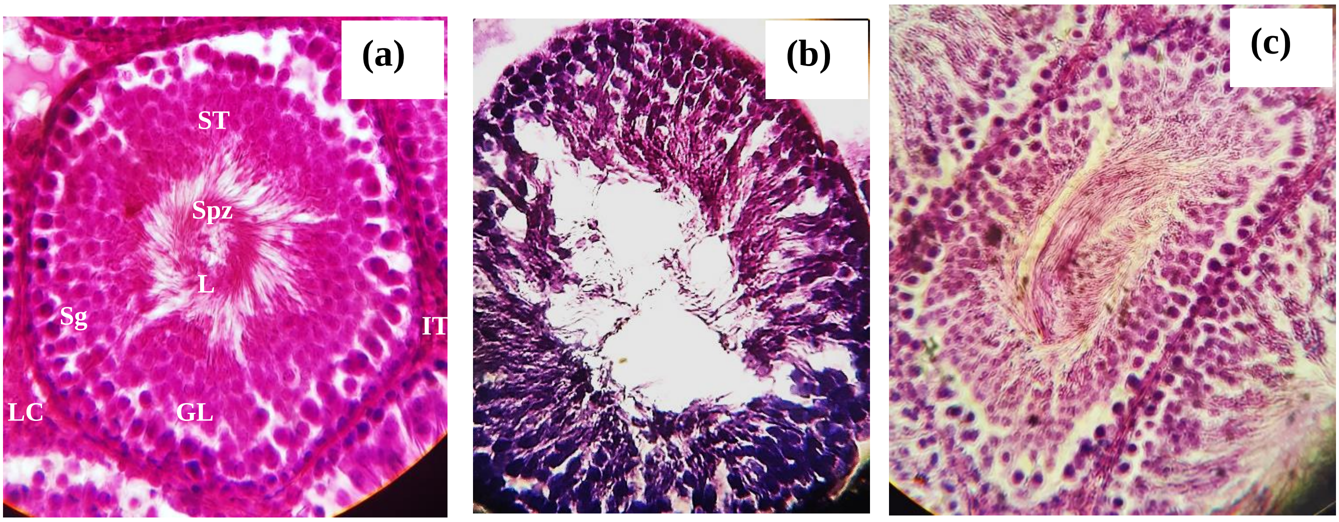
Figure.1: Effect of HFD and TBP on testicular TBARS concentration

C: *control,* ***HFD*** *: high-fat diet,* ***HFD+TBP*** *: high-fat diet supplemented with turmeric and black pepper. Results are expressed as mean ± standard deviation; (n=5). * Significant difference (p<0.05), **very significant difference (p<0.01), ***highly significant difference (p<0.001).*

6. Histological study

Testicular histology analysis showed an intact testicular structure with a well formed interstitial tissue surrounding seminiferous tubules of normal diameter and containing numerous Leydig cells (Figure 2-A). The presence of all the germ cell line indicated a normal spermatogenesis process. (Figure 2-B) shows the HFD-induced alteration of the histological structure of the testes reflected by a spacing of the seminiferous tubules, a decrease in the number of spermatozoa in the lumen as well as a decrease in the seminiferous epithelium thickness with reduced germ cells number. On the other hand, the TBP treatment was able to preserve a normal histological structure compared to controls (Figure 2-C).

Figure.2: Microscopic observation of testicular sections in wistar rat groups (Gr x40) (H&E).



(a): The histological structure of the testes of controls; Seminiferous tubules (ST); Interstitial tissue (IT); Leydig cells (LC). Tube lumens (L); Spermatozoa (Spz); Germ layer (GL); Spermatogonia; (Sg). (b): Testicular histological sections of HFD group (c): TBP+HFD histological sections.

Discussion

We assessed the protective effect of a combined treatment of turmeric and black pepper on HFD-induced biochemical and histological side effects on the lipid profile and the testicular structure. The study results showed that the high-fat diet induced an increase in mean body weight by increasing the weight gain rapidly. These results agree with those of Armitage et al (2005) and Laissouf et al (2014) who reported an important increase in body weight associated with increased adipose tissue weight confirming the obesogenic properties of high-fat diets. Studies on animals and humans have shown that polyunsaturated fatty acids (PUFAs) are more easily used as energetic substrates, whereas saturated fatty acids (SFAs) are more likely to be accumulated in adipose tissue (Hariri et al., 2010). In addition, the analysis of the chemical composition of the mutton fat reveals that it contains more than 50% of SFAs (Wertelecki and Bodarski, 2003; Ahmad Nizar et al., 2013), of which palmitic acid represents about 27% (Marikkar et al., 2021).This finding justifies the use of sheep fat, which is rich in saturated fatty acids in the diet we used. Bodyweight gain values were successfully reduced when the HFD was supplemented with turmeric and black pepper. These results are supported by several studies which demonstrated the anti-obesity effect of turmeric and curcumin and their attenuating effects on lipogenesis and adipogenesis (Alappat and Awad, 2010; Lecerf, 2012). We suggest that these effects may be ameliorated by using black pepper as a curcumin Bioavailability enhancer.

We noted that the HFD-induced body weight increase was associated with a significant increase in total cholesterol and triglyceride levels. Similar results were reported in numerous studies (Arafa, 2005; Bajerska et al. 2015; Bouderbala et al. 2016). In addition, a highly significant decrease in HDL levels was observed in rats fed the HFD only or supplemented with TBP. Bouderbala et al (2016) also found a decrease in HDL levels in a rat model fed with a HFD. On the other hand, the turmeric and black pepper treatment induced a hypolipidemic effect by decreasing plasma triglyceride (TG) and LDL-C levels and increasing the HDL-C compared to the HFD group. Similarly, several studies have reported that turmeric ameliorates the dyslipidaemic state associated with HFD by reducing plasma (TG) and free fatty acid levels in the blood and in cells (Arafa, 2005; Manjunatha and Srinivasan, 2006; Feng et al. 2010; Lecerf, 2012; Hadi et al., 2016). However, we noted that the levels of total cholesterol (TC) were significantly increased in the TBP+HFD group, which is not consistent with the results obtained in those studies. This observation may be due to higher doses of the supplements in the diet, which may result in non-expected adverse effects. Even though, the proportionality higher of HDL-C levels indicates a favourable HDL-C/TC ratio which can be associated with a protective effect against the HFD side effects. Furthermore, Jang et al. (2008) reported in a study on hamsters that curcumin at a dose of 0.05g/100g diet increased HDL-C and improved the HDL-C/TC ratio. Additionally, HDL is known for its central role in reverse cholesterol transport, in which HDL absorbs excess cholesterol from peripheral cells and transports it to the liver for catabolism and excretion in bile(Ganjali et al., 2017; Nicholls and Nelson, 2019). Moreover, HFD significantly decreased the relative weight of male gonads compared to controls and those treated with turmeric combined with black pepper. This result is similar to that found in the study of Mu et al. (2016) reporting a significant decrease in relative testicular weight in rats fed HFD. On the other hand, turmeric treatment combined with black pepper was able to prevent the testicular relative weight decrease, which is consistent with the study that was conducted by Jensen et al. (2004).

Furthermore, HFD induced a significant increase in tissue TBARS in the testes. These results can be explained by lipid auto-oxidation, which is probably enhanced by obesity. In addition, the sperm cells membrane contains a large amount of PUFAs and makes them more sensitive to oxidative stress that can affects their quality and increase TBARS levels. Indeed, many studies reported a link between increased oxidative stress and the fertility disorders pathogenesis (Saka et al., 2011; Carillon et al., 2013; Johnson et al., 2016; Gil-Cardoso et al., 2017). TBP significantly decreased the testicular TBARS levels in the second group. This decrease may be due the turmeric main compound, curcumin, which can enhance of antioxidant enzymes activity e.g. superoxide dismutase, catalase and glutathione peroxidase;and increase antioxidant vitamin levels(Wu et al., 2006; Alappat and Awad, 2010).

The histological study revealed an impairment of the testicular structure in rats subjected to HFD. We observed an alteration of the seminiferous epithelium, a decrease in the number of sperm cells in the lumen, a decrease in germ cells associated with a decreased thickness of the seminiferous epithelium were noted. These results are in agreement with those of Erdemir et al.(2012) and Mu et al.(2016). On the other hand, the turmeric and black pepper treatment was able to preserve a normal histological structure comparable to that of the controls, indicating the safety and efficacy of the treatment against HFD-induced testicular damages. This turmeric benefit is supported by the study of Mu et al (2016) who reported that curcumin may prevent the alterations testis histological structure and the spermatogenesis induced by the HFD.

Conclusion

High fat diet consumption results in several significant health problems due to metabolic and biochemical homeostasis disturbance. We noted that HFD induced an obesity condition associated with dyslipedemia characterised by hypertriglyceridemia, hypercholesterolemia and low HDL levels. These metabolic disturbances affects the testicular tissue by perturbing the spermatogenesis process and inducing an oxidative stress in the testes. We attempt to prevent these HFD-induced biochemical and histological negative effects by using a combined treatment of turmeric and black pepper supposed to be more effective in terms of curcumin bioavailability. In conclusion, our data indicate a significant protective effect of turmeric and black pepper-based treatment against HFD side effects. Further experimental study is needed to elucidate the protective effect mechanisms and the pharmacological properties of the proposed treatment.

Conflicts of interest

Authors declare that they have no competing interests.

References

Agarwal A , Baskaran S , Parekh N, Cho CL , Henkel R , Vij S, Arafa M , Panner Selvam M K, Shah R .(2020) . Male infertility. The Lancet.

Ahmad Nizar NN , Nazrim Marikkar JM , Hashim DM.(2013). Differentiation of Lard, Chicken Fat Beef Fat and Mutton Fat by GCMS and EA-IRMS Techniques. J Oleo Sci. 62:459–464.

Alappat L, Awad A.B. (2010). Curcumin and obesity: evidence and mechanisms. Nutr Rev 68 :729–738.

Anand P, Kunnumakkara AB , Newman RA , Aggarwal BB.(2007). Bioavailability of curcumin: problems and promises. Mol Pharm 4: 807–818.

Arafa HM. (2005). Curcumin attenuates diet-induced hypercholesterolemia in rats. Medical science monitor: international medical journal of experimental and clinical research 11: BR228-234.

Argente ArizÃn P , FreireRegatillo A , Argente J, Chowen JA .(2015). Role of Non-Neuronal Cells in Body Weight and Appetite Control. Front. Endocrinol 6:42.

Armitage JA, Taylor PD, Poston L. (2005). Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. J Physiol .565:3–8.

Bach PV , Schlegel PN. (2019). Male Infertility. In Yen and Jaffe's Reproductive Endocrinology Elsevier. (pp. 582-593).

Bajerska J, Chmurzynska A, Mildner Szkudlarz S , Drzymała Czyż S .(2015). Effect of rye bread enriched with tomato pomace on fat absorption and lipid metabolism in rats fed a highfat diet. J Sci Food Agric 95:1918–1924.

Bakos HW, Mitchell M, Setchell BP, Lane M.(2011). The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model. Int J Androl 34: 402–410.

Benatta M , Kettache R , Buchholz N, Trinchieri A.(2020). The impact of nutrition and lifestyle on male fertility. Arch Ital Urol Androl vol.92.

Botsoglou NA, Fletouris DJ, Papageorgiou GE,Vassilopoulos VN, Mantis AJ, Trakatellis AG.(1994). Rapid Sensitive and Specific Thiobarbituric Acid Method for Measuring Lipid Peroxidation in Animal Tissue Food and Feedstuff Samples. J. Agricultural and Food Chemistry. 42: 1931–1937.

Bouderbala H, Kaddouri H, Kheroua O, Saidi D. (2016). Effet anti-obésogène du vinaigre de cidre de pomme chez le rat soumis à un régime hyperlipidique. In Annales de Cardiologie et d'Angéiologie Elsevier Masson. Vol. 65.3 pp. 208-213.

Carillon J, Rouanet JM , Cristol, JP, Brion R..(2013). Superoxide dismutase administration, a potential therapy against oxidative stress related diseases: several routes of supplementation and proposal of an original mechanism of action.J Pharmaceutical research30: 2718–2728.

Dussault , johanne.(2009). Le spermogramme, sommaire scientifique .vol.16 : pp 1-9.

Erdemir F, Atilgan D, Markoc F, Boztepe O, Suha Parlaktas B, Sahin S(2012). The effect of diet induced obesity on testicular tissue and serum oxidative stress parameters. JActas Urológicas Españolas English Edition .36: 153–159.

Feng D , Ohlsson L , Duan RD.(2010). Curcumin inhibits cholesterol uptake in Caco-2 cells by down-regulation of NPC1L1 expression. Lipids in health and disease. 9 : 40.

Fernandes MR , Lima NV de Rezende KS , Santos ICM, Silva IS, Guimarães R. de C. A.(2016). Animal models of obesity in rodents. An integrative review. Acta Cirurgica Brasileira, vol .31 pp 840–844.

Fitzgerald M , Heinrich M , Booker A.(2020). Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques.. 10: 1480.

Ganjali S , Blesso CN, Banach M , Pirro M , Majeed M , Sahebkar A.(2017). Effects of curcumin on HDL functionality. J Pharmacological Research 119 : 208–218.

Ghanayem BI, Bai R, Kissling GE, Travlos G, Hoffler, U.(2010). Diet-Induced Obesity in Male Mice Is Associated with Reduced Fertility and Potentiation of Acrylamide-Induced Reproductive Toxicity. J Biology of Reproduction 82 : 96–104.

Gil Cardoso K , Ginés I, Pinent, M, Ardévol A, Terra X , Blay M.(2017). A cafeteria diet triggers intestinal inflammation and oxidative stress in obese rats. British Journal of Nutrition .117: 218–229.

Hadi M , Hameedi E, Kadhum N, Zeki D , Al-Saadi A , Zaidan H.(2016). Ameliorative Effect of Curcuma longa L. Rhizomes against Biochemical Toxicity Induced by Dichlorvos in Female Albino Rats. Journal of Chemical and Pharmaceutical Sciences. 9 : 1098–1106.

Hariri N, Gougeon R , Thibault L .(2010). A highly saturated fat-rich diet is more obesogenic than diets with lower saturated fat content. Nutrition research .30 : 632–643.

Jang EM, Choi MS, JungUJ, Kim MJ, Kim HJ, Jeon SM , Shin SK , Seong CN, Lee MK.(2008). Beneficial effects of curcumin on hyperlipidemia and insulin resistance in high-fat–fed hamsters. Metabolism .57:1576–1583.

Jensen TK , Andersson AM , Jørgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE.(2004). Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. Fertility and Sterility.82:863–870.

Jiang D, Coscione A, Li L , Zeng BY.(2017). Effect of Chinese Herbal Medicine on Male Infertility, in: International Review of Neurobiology. Elsevier, vol 135 pp 297–311.

Johnson AR , Wilkerson MD , Sampey BP, Troester MA , Hayes DN, Makowski L.(2016.) Cafeteria Diet-induced Obesity Causes Oxidative Damage in White Adipose. Biochemical and biophysical research communications. 473: 545–550.

Katib A (2015). Mechanisms linking obesity with male infertility. Central European journal of urology 68:79

Kim Y, Clifton P. (2018). Curcumin, Cardiometabolic Health and Dementia. International journal of environmental research and public health .15: 2093.

Kocaadam B, Şanlier N. (2017). Curcumin, an active component of turmeric (*Curcuma longa*), and its effects on health. Critical Reviews in Food Science and Nutrition 57: 2889–2895.

Komninos D. (2022). High fat diet-induced obesity prolongs critical stages of the spermatogenic cycle in a Ldlr-/-.Leiden mouse model. Scientific Reports .12:1-11.

Kort HI (2006). Impact of Body Mass Index Values on Sperm Quantity and Quality. Journal of Andrology 27: 450–452.

Laissouf A, Soulimane NAM , Merzouk H .(2014). L'effet thérapeutique de l'huile de lin « linumusitatissimum » sur l'hypertriglycéridémie et l'hypercholestérolémie chez des rats obèses âgés. Afrique Science: Revue Internationale des Sciences et Technologie . Vol.10.

Lecerf JM.(2012). Effets métaboliques du Curcumin (obésité, lipides circulants, insulinorésistance, diabète et athérosclérose). J Phytothérapie 10: 100–104.

Leisegang K, Sengupta P, Agarwal A, Henkel R.(2021). Obesity and male infertility: Mechanisms and management. Andrologia 53.

LiuY , Ding Z.(2017). Obesity, a serious etiologic factor for male subfertility in modern society. Reproduction 154:R123–R131.

Manjunatha H, Srinivasan, K.(2006). Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats. The FEBS journal.273: 4528–4537.

Marikkar N, Alinovi M , Chiavaro E.(2021). Analytical approaches for discriminating native lard from other animal fats. Italien.Journal of Food Science .33: 106–115.

Martins T, Ferreira T, Nascimento-Gonçalves E, Castro-Ribeiro C, Lemos S, Rosa E, Oliveira PA. (2022). Obesity Rodent Models Applied to Research with Food Products and Natural Compounds. Obesities, 2: 171-204.

Mu Y, Yan WJ, Yin TL, Yang J.(2016). Curcumin ameliorates high‑fat diet‑induced spermatogenesis dysfunction.Molecular medicine reports14: 3588–3594.

Mun J , Kim S , Yoon HG , You Y, Kim OK, Choi KC , Lee YH, Lee J, Park J, Jun W (2019). Water Extract of Curcuma longa L. Ameliorates Non-Alcoholic Fatty Liver Disease. Nutrients 11: 2536.

Naito HK. (1984). HDL Cholesterol. Kaplan A et al.Clin Chem. The CV. Mosby Co., St Louis Toronto Princeton 437: 1207-1213.

Nicholls SJ, Nelson AJ. (2019). HDL and cardiovascular disease. Pathology.51: 142–147.

Oladele CA, Akintayo CO, Badejogbin OC, Oniyide AA, Omoaghe AO, Agunbiade TB, Olaniyi KS, (2022). Melatonin ameliorates endocrine dysfunction and defective sperm integrity associated with high-fat diet-induced obesity in male Wistar rats. Andrologia 54: 4242.

Pant B.(2014). Application of Plant Cell and Tissue Culture for the Production of Phytochemicals in Medicinal Plants in Infectious Diseases and Nanomedicine II Advances in Experimental Medicine and Biology Springer India New Delhi. (pp. 25–39).

Punab M , Poolamets O, Paju P, Vihljajev V, Pomm K, Ladva R, Korrovits P, Laan M (2016). Causes of male infertility a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Hum. Reprod. Human reproduction .32:18-31.

Saka S, Bahi A, Aouacheri W. (2011). L'effet du stress oxydant induit par l'acétate de plomb sur le système enzymatique du glutathion chez les rats. In Annales de Toxicologie Analytique .EDP sciences . 23:139–145.

Sasaki H, Sunagawa Y, Takahashi K, Imaizumi A, Fukuda H, Hashimoto T, Wada H Katanasaka Y, Kakeya H, Fujita M, Hasegawa, K, Morimoto .(2011). Innovative Preparation of Curcumin for Improved Oral Bioavailability. Biological Pharmaceutical Bulletin.34: 660–665.

Sharma R, Biedenharn KR, Fedor JM, Agarwal A. (2013). Lifestyle factors and reproductive health. taking control of your fertility. Reproductive Biology and Endocrinology 11:66.

Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med 64 :353–356.

Skoracka K. Eder P, Łykowska-Szuber L, Dobrowolska A, Krela-Kaźmierczak I. (2020). Diet and Nutritional Factors in Male.In Infertility Underestimated Factors. Journal of Clinical Medicine.9 : 1400.

Takahashi M, Suzuki K, Kim H, Otsuka Y, Imaizumi A, Miyashita M, Sakamoto S. (2013). Effects of Curcumin Supplementation on Exercise-Induced Oxidative Stress in Humans. Int J Sports Med 35: 469–475.

Tvrdá E, Tušimová E, Kováčik A, Paál D, Greifová H, Abdramanov A, Lukáč,N (2016). Curcumin has protective and antioxidant properties on bull spermatozoa subjected to induced oxidative stress. Animal Reproduction Science.172:10–20.

Vaughn AR, Branum A, Sivamani RK.(2016). Effects of Turmeric (*Curcuma longa*) on Skin Health: A Systematic Review of the Clinical Evidence: Effects of *Curcuma longa* on Skin Health. Phytotherapy Research.30: 1243–1264.

Volak LP, Hanley MJ, Masse G, Hazarika S, Harmatz JS, Badmaev V, Majeed M, Greenblatt DJ, Court MH. (2013). Effect of a herbal extract containing curcumin and piperine on midazolam flurbiprofen and paracetamol (acetaminophen) pharmacokinetics in healthy volunteers Curcuminoid/piperine pharmacokinetic interactions. British Journal of Clinical Pharmacology. 75: 450–462

Wang Y, (2002). Epididymal Sperm Count. Current Protocols in Toxicology.14:16-6

Wertelecki TJ, Bodarski RK. (2003). FATS Production of Animal Fats, in: Encyclopedia of Food Sciences and Nutrition. Elsevier, (pp. 2261–2270).

Wu A, Ying Z, Gomez Pinilla F. (2006). Dietary curcumin counteracts the outcome of traumatic brain injury on oxidative stress, synaptic plasticity and cognition. Experimental Neurology.197: 309–317.

Zhang L, Diao RY, Duan YG ,Yi TH, Cai ZM.(2017). In vitro antioxidant effect of curcumin on human sperm quality in leucocytospermia. Andrologia 49:10

Zheng B, McClements DJ, (2020). Formulation of More Efficacious Curcumin Delivery Systems Using Colloid Science. Enhanced Solubility Stability and Bioavailability Molecules 25: 2791.