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ASSESSMENT OF TURMERIC-BASED TREATMENT EFFICACY AGAINST HIGH FAT DIET-INDUCED DYSLIPIDAEMIA AND TESTICULAR DAMAGES IN WISTAR RATS

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ABSTRACT

The present study was carried out to evaluate the effect of turmeric and black pepper on high-fat-diet-related biochemical and histological disorders in WISTAR rats. Fifteen male rats were divided into 3 groups (n=5), G1: control that received 350g/d of standard diet; G2: 350g/d of High Fat Diet (HFD) and G3: that received 350g/d of HFD supplemented with turmeric and black pepper (TBP) for two months. Our results showed that HFD induced a significant increase in total cholesterol and triglycerides. However, the HDL level was decreased. In addition, a significant increase in weight gain values and a significant decrease in relative testicular weight were observed. Moreover, the HFD significantly increased testicular oxidative stress expressed as Thiobarbituric Acid Reactive Substance (TBARS) levels. The histological analysis showed a decrease in sperm cells number 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 the HFD biochemical and histopathological effects on testes and lipid profile. 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: fat, lipids, testicle, histology, turmeric.

INTRODUCTION

Infertility is one of the most public health concerns (Bach and Schlegel, 2019) that affect about 8-12% of couples worldwide in which male factors are involved in about 50% of cases (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 whose abnormalities in sperm count and morphology contribute to more than 90% of male infertility cases (Jiang et al. 2017). Indeed, the decrease in sperm quality has been related to several lifestyle factors such as cigarette, alcohol, Psychological stress, malnutrition, and obesity (Agarwal et al. 2020). In recent years, obesity has become a global epidemic. According to the World health organisation (WHO), the number of obesity cases has almost tripled since 1975. In fact, more than 1.9 billion adults are overweight, of which 650 million already reached the obesity stage (WHO, 2021). 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 leptin and insulin by inducing a decrease in their levels and a resistance to their actions, and a decrease in the suppression of ghrelin secretion following HFD (Hariri et al. 2010; Argente-Arizon et al. 2015).

On the other hand, medicinal plants offer a source of bioactive compounds that may be used to treat several pathologies. They have been used by a large proportion of the world population as alternatives to medicines (Fitzgerald et al. 2020). *Curcuma longa* L, or turmeric, is a spice with a strong yellow pigment commonly used in cooking and cosmetics (Vaughn et al. 2016). Its curcuminoids content makes this plant an excellent therapeutic agent against several pathologies, e.g. inflammation, adipogenesis diabetes, cardiovascular diseases, cancer and liver damage (Kocaadam and Şanlier, 2017; Mun et al. 2019).

However, curcumin, the main active compound of turmeric, has poor oral bioavailability. 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). Several approaches have been suggested to increase the bioavailability of curcumin (Zheng and McClements, 2020).The combination of turmeric and black pepper was among the strongly recommended approaches to improve the curcumin bioavailability. In fact, it is well thought that piperine, an important component of black pepper, can improve the oral bioavailability of curcumin by suppressing its rapid glucuronidation in the liver and intestine and then decreasing its urinary excretion (Kim and Clifton, 2018).

Herein we investigate the protective effect of turmeric and black pepper treatment against HFD induced obesity, lipid profile disturbance and testicular damage.

MATERIAL AND METHODS

Preparation of the high fat diet and the TBP treatment

The HFD consisted of 700g of powdered standard feed, 200g of lamb fat, and 100g of sunflower oil, representing70%, 20%, and 10% of the diet composition respectively. Preparations were renewed every three days and stored at 4°C.

Considering 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 *Curcuma longa* L rhizomes powder (turmeric) with black pepper to improve curcumin bioavailability. HFD was so supplemented with 20% of turmeric and 5% of black pepper.

Study design

This study was conducted on 15 adult male WISTAR rats weighing 242g ± 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 every week to assess the effect of each diet on body weight 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 revolutions per minute (rpm) for 5 minutes to separate the plasma. Total cholesterol (TC) (Naito, 1984) and triglycerides (TG) (Buccolo et al. 1973) 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 only 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 (g/L) = TC (g/L)-HDL-C (g/L)-[TG (g/L)/5)] (Krishnaveni and Gowda, 2015).

Testicular lipid peroxidation was assessed by the TBARS method as described by Botsoglou et al. 1994. Briefly, 1 gram of tissue was mixed with 8 mL of 5% TCA and 5 mL of 8% BHT prepared in hexane. The mixture was homogenized for 30 seconds and centrifuged at 3000g for 30 minutes. After that, the top hexane layer was eliminated and the rest was completed up to 10 mL with 5% TCA. Finally, 2.5mL of this solution was mixed with 1.5mL of 0.8% TBA prepared in distilled water and incubated at 100 °C for 30 min. The intensity of the red color formed was measured at 521 nm against the blank.

Organs designated for the histological study were fixed in formalin 10%. Samples were then prepared according to the standard histological techniques and stained with haematoxylin-eosin for the histological analysis.

Statistical analysis

Statistical analysis was carried out using IBM SPSS. 22. An analysis of variance (ANOVA) test was performed to check the statistical significance of the results, followed by a Post-Hoc Tukey test for multiple comparisons between the different groups. A value of $P<0.05$ is considered significant for all the applied 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 TBP treatment. On the other hand, HFD induced a highly significant decrease in relative testicular weight ($P<0.001$) compared to controls and TBP-treated rats.

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

Effect of HFD and TBP on lipid profile

Results shown in table 2 indicate a significant ($p < 0.05$) and a highly significant ($p < 0.001$) increase in total cholesterol in the HFD and HFD+TBP groups respectively. In addition, we noted a significant increase in HDL-C level in the HFD+TBP group ($p < 0.01$) and a non-significant increase in LDL-C level in the HFD compared to controls. In other terms, TBP treatment combined with the high-fat diet rather improves the HDL-c/total cholesterol ratio indicating a possible cardio-protective effect of the suggested treatment. On the other hand, our results showed a highly significant increase in TG levels in the HFD-treated rats compared to controls and the HFD+TBP group ($p<0.001$).

Table 2: Effect of HFD and TBP on lipid profile

Groups	Lipid profile Total cholesterol (g/L)	HDL-c (g/L)	LDL-c (g/L)	Triglycerides (g/L)
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).*

Effect of HFD and TBP on testicular oxidative stress

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

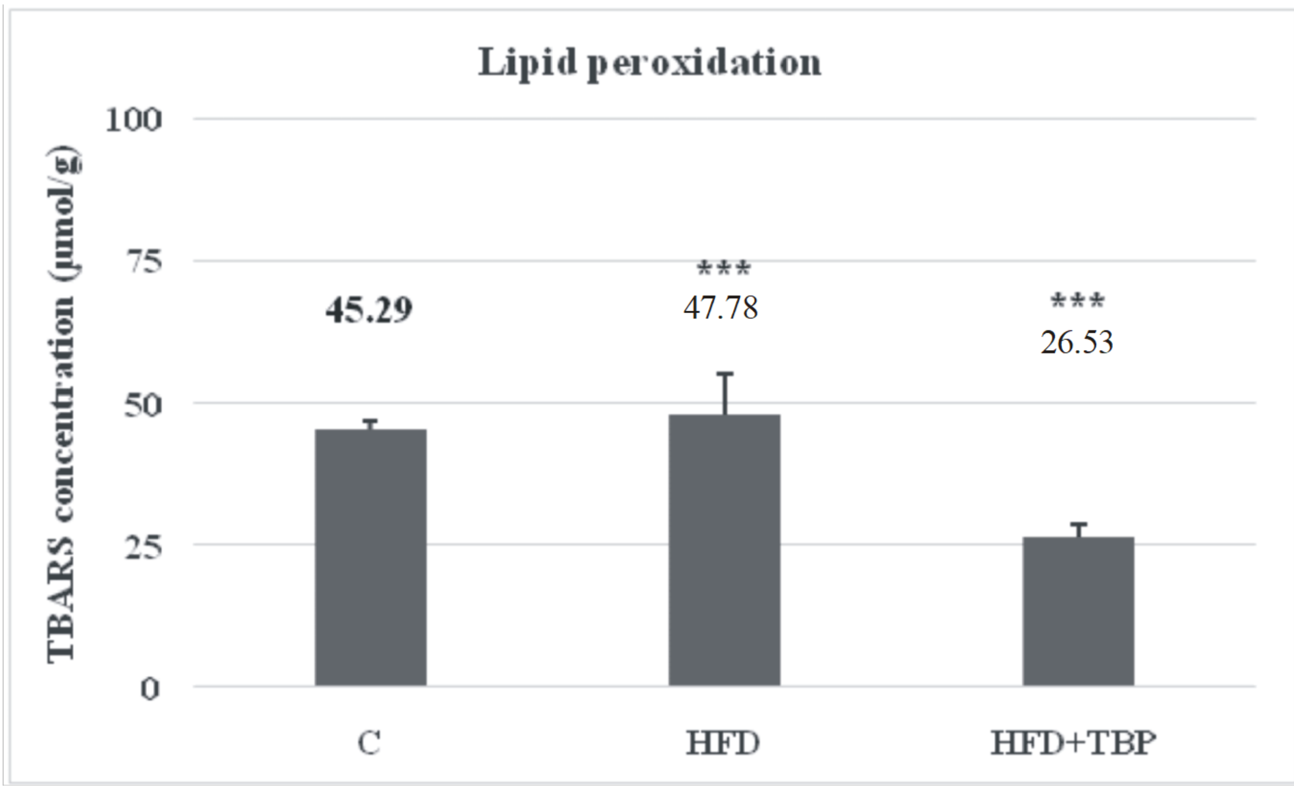

Figure.1:Effect of HFD and TBP on testicular TBARS concentration

*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).*

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 cells line indicated a normal spermatogenesis process. (Figure. 2-b) shows that HFD damaged the histological structure of the testes by inducing a decrease in the number of sperm cells, as well as a reduced thickness of seminiferous epithelium with reduced number of germ cells. On the other hand, the TBP treatment was able to preserve a normal histological structure in rats of group 3 compared to controls (Figure. 2-c).

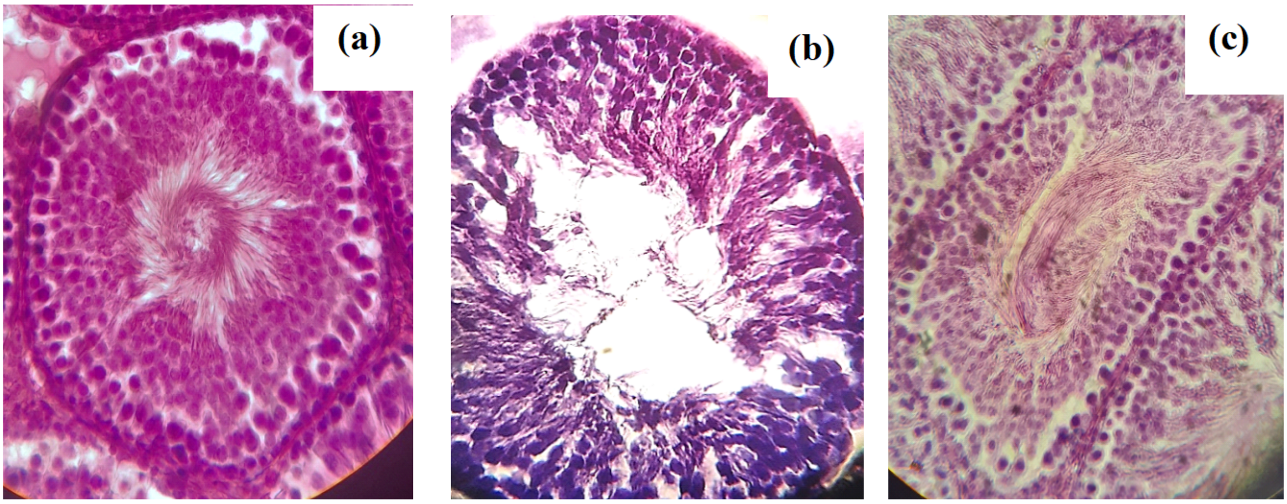

Figure.2: Microscopic observation of testicular histological sections from the study groups (Gr x 40) (H & E).

(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 other studies that reported an important increase in body weight associated with increased adipose tissue weight confirming the obesogenic properties of high-fat diets (Armitage et al. 2005; Laissouf et al. 2014). 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 (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. Body weight 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 even though the poor bioavailability of curcumin (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 (Bajerska et al. 2015; Bouderbala et al. 2016). In addition, a highly significant decrease in HDL level was observed in rats fed the HFD only. Bouderbala et al. 2016 also found a decrease in HDL levels in a rat model fed with an 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, the study conducted by Lecerf, 2012 reported that turmeric ameliorates the dyslipidaemic state associated with HFD by reducing plasma (TG) and free fatty acid levels in the blood and cells. 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 higher proportionality of HDL-C

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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 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 Biliary excretion (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 relative testicular 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 cell membrane contains a large amount of PUFAs that makes them more sensitive to oxidative stress. Indeed, many studies reported a link between increased oxidative stress and fertility disorder's pathogenesis (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 to the curcumin antioxidant effect and ability to improve the activity of antioxidant enzymes e.g. superoxide dismutase, catalase and glutathione peroxidase (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, and a decrease in germ cells associated with a reduced thickness of the seminiferous epithelium. 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 compared to controls, indicating the safety and efficacy of the treatment against HFD-induced testicular damages.

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 hypertrigly ceridemia, hypercholesterolemia and low HDL levels. These metabolic disturbances affect the testicular tissue by perturbing the spermatogenesis process and inducing oxidative stress in the testes. On the other hand, the turmeric and black pepper combined treatment we proposed was able to prevent the HFD biochemical and histological side effects. Further experiments are 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.

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