Research Article

SOHAG JOURNAL OF SCIENCES

https://doi.org/10.21608/sjsci.2025.341363.1239

The Ameliorative Effect of Curcumin and Curcumin Nanoparticles on Alteration of Hematological Profile and Testicular Damage in Male Albino Rats Feeding on Instant Noodles Overconsumption.

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Received: 6th December 2024, **Revised:** 1st January 2025, **Accepted:** 8th January 2025
Published online: 24th April 2025

Abstract: Overconsumption of instant noodles (IND) has recently received special attention, owing to its association with obesity and the cardiometabolic syndrome. Curcumin (CUR) exhibit cellular activity, supporting its numerous health advantages due to its anti-inflammatory and antioxidant effects. This study was performed to evaluate the ameliorative effect of oral administration of CUR or curcumin nanoparticles (CUR-NPs) in IND-fed rats. Forty male rats were separated into four groups of ten rats each, these groups were control (GTRL), IND, IND + CUR and IND + CUR-NPs groups. The IND- fed group showed a significant decrease in RBC count, HB, HCT, MCV and PLT with a significant increase in WBC count and neutrophils percentage. A significant reduction in the serum levels of total testosterone (Tt) and luteinizing hormone (LH) but a non- significant change in follicle-stimulating hormone (FSH) levels in IND- fed group. The testicular tissue from the IND-fed group exhibits remarkable degenerative changes in spermatogenic series as well as testicular atrophy as showen by reduction in diameter of seminiferous tubules. There was also a marked increase in the collagen fibers and TNF-α-immunostaining. Oral administration of CUR or CUR-NPs to the IND- fed group showed signs of improvement in the previous haematological and the alteration in the structure and function of testis compared to IND- fed group. There was no significant difference in the tested parameters between the CUR and CUR-NPs effect.

Key words: Curcumin, Curcumin Nanoparticles, Instant Noodles, Hematological Profile, Testis.

1. Introduction

Modern dietary intake is marked by high calories, high-energy carbohydrates, hydrolyzed fatty acids and processed foods as well as reducing fruit and vegetable, vitamins, and herbs [1]. The overconsumption of these diets may induce several health disorrdes, including metabolic syndrome, and increase and the risk of male infertility which is indicated by lower-quality sperm. In addition, the high-energy dietary consumption raises the incidence of asthenozoospermia, which may be mediated by elevated oxidative stress and insulin resistance induced through both type 2 diabetes and obesity [2]. Instant noodles (IND) is one of the most common convenience foods [3]. IND contains primary ingredients like wheat flour, iodized salt, vegetable oil, sodium carbonate, sodium polyphosphate, potassium carbonate, tartrazine and guar gum, while that of the seasoning powder is monosodium glutamate, iodized salt, hydrolyzed vegetable protein, soy powder, chili powder, garlic powder, and chicken flavour [4]. Morover, long-term, excessive consumption of IND could result in oxidative damage causing teratogenic or carcinogenic changes in rats [5]. However, blood has a high rate of regeneration, the hematopoietic system needs an enormous and organized supply of nutrients for formation and maturation of hematopoietic stem cells. Therefore, the hematopoietic system is one of the most important systems that can be affected by malnutrition resulting in disruptions in the formation of all blood cell lines [6].

Male infertility is one of the major male reproductive dysfunction that warrant public concern. It occurs when one or more parts of the male reproductive system aren't operating correctly and it can have a devastating impact on the person, leading to numerous secondary disorders [7]. The testes are an important primary organ in the male reproductive system, which is dependent on hormones. The testes produce testosterone which is the most potent male sex hormone. It plays important roles in reproductive function as well as male body composition and appearance. Testosterone is mainly produced by the testes' Leydig cells and is regulated by the axis of the hypothalamic-pituitary by luteinizing hormone (LH) as the major hormonal signal [8] and follicle-stimulating hormone (FSH) that regulates sperm maturation and production [9]. Moreover, testosterone helps in spermatogenesis stimulation and enhancing the development of immature spermatozoa [8].

Numerous lifestyle factors, such as smoking, alcohol drinking, being obese, using illegal substances, drinking caffeine, eating fast food, and diet, have been shown in several studies to have a detrimental impact on male fertility [10, 11]. It is important to note that nutrition has a strong impact on sperm quality, either positively or negatively [12, 13]. This impact is dependent on both qualitative and quantitative aspects of a diet, such as the particular nutrient types; protein, fatty acids and carbohydrates as well as the total calories of each nutrient. In this context, consumption of an unhealthy diet which contains high carbs, high fat and low vegetable and fruit may increase cholesterol and leptin levels, as well as lipid-soluble toxins, thus interfering with the hypothalamic-pituitary-gonadal axis, leading to hormonal disruption and reduced spermatogenesis [14].

Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and is commonly used as a spice and food-coloring agent [15]. It has a wide range of biological activities including antifertility, hypocholesteremic, antidiabetic, antioxidant, antifibrotic, anti-inflammatory, anticoagulant, anticarcinogenic, antimutagenic, antibacterial, antiprotozoal, antiviral, antifungal, antivenom, antiulcer, and hypotensive activities [16]. Despite that, the activities of CUR are not fully recognized due to its low stability, low water solubility and low bioavailability [17]. Researchers have found several approaches to address these CUR bioavailability, including pro-drug tactics and nanosystems, which have garnered a lot of interest [18].

Many of the earlier studies have shown that antioxidants protect spermatozoa from ROS-induced production of abnormal spermatozoa, so the current work was designed to assess the ameliorative role of CUR or CUR-NPs on haematological profile as well as testicular damage through histological studies with concurrent observation on the hormonal status when applying the long-term consumption of IND in male rats.

2. Materials and methods

2.1. Chemicals and diets

Cartons of one mostly consumed variety of instant noodles (normal size consists of 70 g, containing 7 g seasoning) were obtained from local Market, Sohag, Egypt. Curcumin (70% purity) was acquired from Sigma-Aldrich. Polyvinylpyrrolid-one (PVP; 40 000 g gmol−1, Sigma-Aldrich, 99% purity), PVP/CUR-NPs were prepared via the simple nanoprecipitation method as described previously [19-22] at Faculty of Science, Sohag University, Sohag governorate.

2.2. Animals

A total of 40 apparently healthy adult male Wistar albino rats of 7-9 weeks old and weighing approximately 180–200 gm were purchased from the Animal Experimental Research Unit (EAU), Faculty of medicine, Sohag University, Sohag, Egypt, for this experiment. All animals were housed in filter-top polycarbonate cages in a room free from any source of chemical contamination, artificially illuminated (12 h dark/light cycle) and thermally controlled (25 ± 1◦C). The rats were fed on constant supplies of standard pellet diet, fresh and clean drinking water were supplied *ad-libitum*. Approval of protocol for animal experimentation (SOHAG -22- 2023 -03) was obtained for all experiments. All animals received human care in compliance with the guidelines of the Animal Care and Use Committee of the Faculty of Medicine, Sohag University.

2.3. Experimental design

After two weeks' period of acclimatization, the animals were randomly assigned into four (4) groups (10 animals /group), as follows: The 1st group is the control group (CTRL), which received standard rat diet. In the second, group rats received 100% uncooked IND [4] mixed with seasoning according to Zailani et al. [19]. The third group: were supplied with 100% of uncooked IND spiced with the seasoning and then rats treated orally with CUR (100 mg/kg b. wt.). The fourth group were supplied with 100% of uncooked IND spiced with the seasoning and then rats treated orally with CUR-NPs (25 mg/kg b. wt.). The dose selection for IND (20 g of IND for each 200g) according to Curfs et al. [20], while the dose selection of CUR and CUR-NPs according to De Almeida et al. [21]. Rats were orally administered their respective doses every day for eight weeks.

The experimental period lasted eight weeks for each group. The rats were scarified 24 hours following the final treatments at the conclusion of the trial. The whole blood samples were collected in two different types of tubes; the first included EDTA, an anticoagulant, for the complete blood count (CBC) test. Use the second tube, without anticoagulant, to extract the sera. The sera were stored at -20°C until needed after centrifugation at 3,000 x g for 10 minutes at room temperature.The animals' testis tissues were removed for histological, histochemical and immunochemical analysis.

2.4. Haematological assays

A complete blood count (CBC) test was performed using cell counter HA-Vet Automatic Hematology Analyzer, Belgium; S/N HA3DM004 to detect the changes in the blood constituents of the different study groups. The hematological analysis included white blood cells (WBC), lymphocytes, monocytes, granulocytes, red blood cells (RBC), hemoglobin (HB), hematocrits (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT).

2.5. Biochemical analysis

Immune analysis for male sex hormones LH, FSH and Testosterone were detected according to Uotila et al. [22], Rebar et al. [23], and Sauer et al. [24], respectively using Enzyme-linked Immune Sorbent Assay (ELISA) TOSOH; AIA-600. ELISA kits a purchased from Biocheck, Inc Company, Foster city, U.S.A

2.6. Histological and histochemical examinations

The testis specimens were excised, washed with normal saline, fixed immediately in 10% neutral buffered formalin, dehydrated in graded series of ethyl alcohol, cleared, and was then embedded in paraffin wax blocks. 5µm-thick sections were cut using Microtome (Leica), stained with Harris's haematoxylin and eosin. Then, sections were dehydrated in ascending grade of ethanol, cleared in xylene, and mounted with DPX, according to Bancroft and Gamble [25]. Other slides were stained with Mallory's trichrome stain for collagen fibers differentiation as described previously [26].

2.7. Immunohistochemical Examination

Tumor necrosis factor alpha (TNF-α) antibody was used following the instructions of kits (ABclonal Co., Catalog No., A11534, USA) for immunohistochemistry to differentiate and localize the TNF-α antigen in the testis tissues. The intensity of staining of testis was evaluated as previously described by Saxlin et al. [27].

Morphometric analysis

The histopathological score of testis was evaluated with Johnsen's Testicular Biopsy Score (JTBS) from 1 to 10 as described previously [28]. The testicular seminiferous tubules (ST) of all animals were examined under 40x magnification. The tubules were graded according to the presence or absence of various germ cell types, including spermatogonia, germ cells, spermatocytes, spermatids, and spermatozoa. Each tubule was assigned a score ranging from 1 to 10. Well spermatogenesis is indicated by a higher Johnsen's score, whereas more severe dysfunction is indicated by a lower score. The measurement of the diameter of the seminiferous tubules is employed under 10X magnification, at least over 10 seminiferous tubules in randomly selected different areas of each animal, and at least over totally 100 tubules for each group, for each tubule, it is calculated through the averages of the lengths of the short edge and the long edge (two diameters perpendicular to each other) measured in µm [29]. Morphometric measurements were analyzed using FIJI ImageJ. Also, Image analysis software (FIJI ImageJ) was used to quantify the mean area percent of collagen fiber and TNF-α positive reactions in 10 non-overlapping high-power fields (40) of paraffin sections of testis in each group. The results were then statistically evaluated.

2.8. Statistical analysis

The organization and analysis of the data was done using GraphPad Prism® version 5.01 (GraphPad Software Inc., San Diego, CA, USA). The means ±SE are used to express the results. A one-way analysis of variance (ANOVA) was used to statistically analysis the results followed by Bonferroni's multiple comparison test. Significant (P values) were defined as those ≤ 0.05.

3. Results:

3.1. Haematological Results:

According to the current study's findings, IND feeding for eight weeks significantly reduced RBC count ($P < 0.001$), HB, HCT, and MCV ($P < 0.01$), but MCH and MCHC decreased non-significantly ($P > 0.05$). When IND-fed groups were administered with CUR or CUR-NPs orally, the values of previous parameters did not restore to normal values and still record a significant ($P < 0.01$) decrease in HB and HCT% and a non-significant ($P > 0.05$) decrease in RBC count compared to the normal control rats. Oral administration of CUR or CUR-NPs to the IND-fed group produced a somewhat better outcome than the IND-fed group, with a significant ($P < 0.05$) rise RBC count and a non-significant ($P >0.05$) increase in HB and HCT compared to the IND-fed group. On the other hand, there was non-significant decrease ($P > 0.05$) for MCH and MCHC across all experimental groups (Table 1). The IND-fed group showed a very highly significant ($P < 0.001$) increase in the WBCs and highly significant ($P < 0.01$) increase in the percentage of neutrophils with a non-significant ($P > 0.05$) change in the percentage of monocytes and lymphocytes compared to the control group. Whereas oral administration of CUR or CUR-NPs to the IND-fed group showed a significant ($P < 0.05$) change in the WBCs count and the percentage of neutrophils lymphocytes and monocytes, compared to IND-fed groups. There is no significant difference between the administrations of CUR or CUR-NPs groups to IND-fed group (Table 1).

3.2. Biochemical Results:

The data obtained in Figure 1. showed that, the animals fed with IND revealed a very highly significant decrease ($P<0.001$) in serum LH level and serum Tt compared to control group. likewise, the administration of CUR or CUR-NPs along with IND-fed group showed also a very highly significant decrease ($P < 0.001$) in the serum levels of LH and Tt compared to the control group. On the other hand, the administration of CUR and CUR-NPs to the IND-fed group increase LH and Tt level with a significant change ($P < 0.05$) when compared to the IND-fed group. While the serum level of FSH showed a non-significant decrease ($P>0.05$) in all experimental groups.

3.3. Histological Results:

Following eight weeks of administration, the histological investigations of testis sections in all experimental animal groups are provided in Figure 2. The CTRL testis tissue showing, normal structures of seminiferous tubules at different spermatogenic stages in their lumina with normal appearance of Leydig cells in the interstitial spaces between the tubules (Fig. 2 A). Complete spermatogenic series includes spermatogonia, primary and secondary spermatocytes and spermatids as well as numerous spermatozoa all were observed in the most seminiferous tubules lumina (Fig. 2 B). The testicular tissue from the IND-fed group exhibits remarkable degenerative changes in spermatogenic series, with a few or absence of spermatozoa in most seminiferous tubules. Moreover, the number of Leydig cells declined, and the interstitial spaces between the tubules enlarged with edema (Fig. 2 C&D). The group that consumed IND and CUR (Fig. 2 E) or those that got IND along with CUR-NPs (Fig. 2 F) showed relatively normal structures in many seminiferous tubules at different spermatogenic stages with a relatively normal appearance of Leydig cells in the interstitial spaces between the tubules compared to those observed in the IND-treated group.

Table 1: Effect of oral administration of CUR or CUR-NPs on the hematological parameters in IND-treated male rats.

Parameter	Control	IND	IND +CUR	IND+CUR-NPs
RBCs [10^6/mm^3]	6.91±0.14	6.04±0.1^{a**}	6.46±0.15^{b}	6.43 ±0.09^{b}
HB [g/dl]	14.77±0.14	12.59±0.16^{a*}	12.73±0.12 ^{a*}	13.03 ±0.18 ^{a*}
HCT[%]	35.7±0.9	28.88±0.5 ^{a*}	30.76±0.8 ^{a*}	29.99±0.68 ^{a*}
MCV [fl]	51.6±0.58	48.02±0.6^{a*}	48.2±0.36 ^{a*}	47.33±0.42 ^{a*}
MCH [pg]	21.27±0.26	20.75±0.38	19.83±1.04	20.59±079
MCHC [g/dl]	41.26±0.79	40.17±053	41.17±0.27	41.54±0.81
PLT [10^3/µL]	802.7±16.3	727±52.7^{a}	823±67.3^{ab}	793.5.5±30.4^{b}
WBCs [10^3/µL]	13.4±0.62	20.1±0.55^{a**}	15.57±0.87^{b}	16.74±0.8^{b}
Neutrophils[%]	13.81±0.5	17.82±0.9^{a*}	15.65±1.2^{b}	15.17±0.8^{b}
Lymphocytes[%]	75.23±1.7	76.50±1.1	74.79±1.9	75.27±3.04
Monocytes[%]	8.425±0.4	7.963±0.35	7.686±037	8.100±0.14

Significance (a): relative to the control group, significance (b): relative to the IND group. Significance: P < 0.05, (*): P < 0.01, (**): P < 0.001.

3.4. Histological Results:

Following eight weeks of administration, the histological investigations of testis sections in all experimental animal groups are provided in Figure 2. The CTRL testis tissue showing, normal structures of seminiferous tubules at different spermatogenic stages in their lumina with normal appearance of Leydig cells in the interstitial spaces between the tubules (Fig. 2 A). Complete spermatogenic series includes spermatogonia, primary and secondary spermatocytes and spermatids as well as numerous spermatozoa all were observed in the most seminiferous tubules lumina (Fig. 2 B).

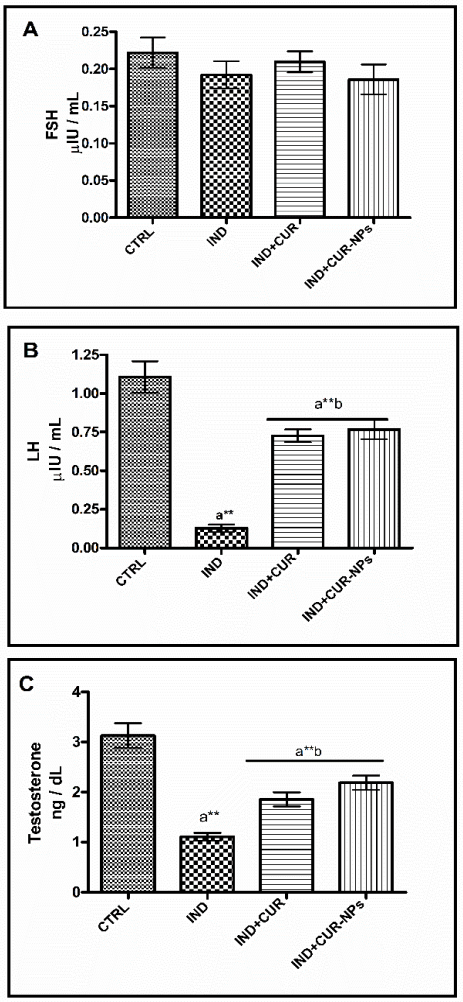

Figure 1. Effect of CUR or CUR-NPs administration on the serum level of FSH (A), LH (B) and Tt (C) of rats fed with IND. Significance (a): relative to the control group, significance (b): relative to the IND group. Significance: P < 0.05, (*): P < 0.01, (**): P < 0.001.

The testicular tissue from the IND-fed group exhibits remarkable degenerative changes in spermatogenic series, with a few or absence of spermatozoa in most seminiferous tubules. Moreover, the number of Leydig cells declined, and the interstitial spaces between the tubules enlarged with edema (Fig. 2 C&D). The group that consumed IND and CUR (Fig. 2 E) or those that got IND along with CUR-NPs (Fig. 2 F) showed relatively normal structures in many seminiferous tubules at different spermatogenic stages with a relatively normal appearance of Leydig cells in the interstitial spaces between the tubules compared to those observed in the IND-treated group.

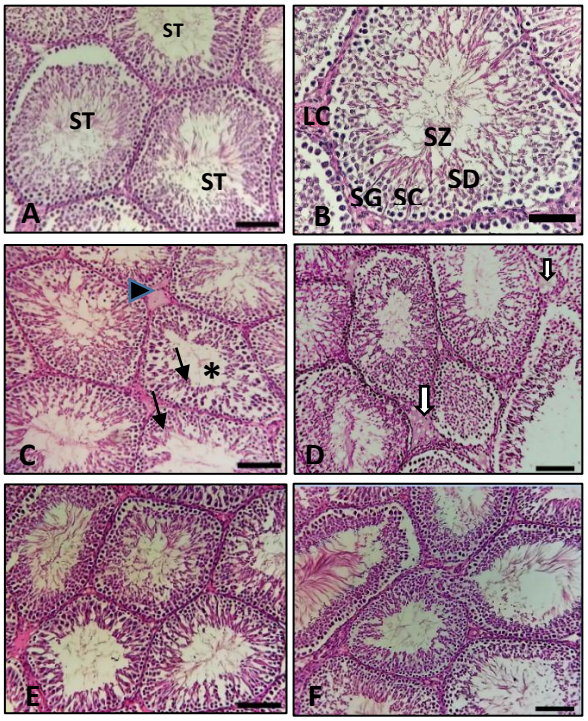

Figure 2. Photomicrographs stained with hematoxylin–eosin from testes of (A&B) control male rats showing, normal structures of seminiferous tubules (ST) at different spermatogenic stages including; spermatogonia (SG), primary and secondary spermatocytes (SC), and spermatids (SD) as well as numerous spermatozoa (SZ) with normal appearance of Leydig cells (LC) between the tubules. (C&D) IND-fed rats showing, remarkable degenerative changes in spermatogenic series (black arrow) with few or absence spermatozoa (*) in most seminiferous tubules and degenerative Leydig cells (arrow head), enlarged interstitial spaces with edema (white arrow). (E&F) CUR and CUR-NPs administration to the IND-fed rats showing, sings of improvement in many seminiferous tubules at different spermatogenic stages. Scale bar 50 µm.

3.5. Morphometric results

A semi-quantitative method, Johnsen method is used for determining the degree of the damage on the testicular seminiferous tubules. The testicular tissue from the IND-fed group showed a significant ($P < 0.001$) decrease in Johnsen's score compared to controls. The group that consumed IND and CUR or those that got IND along with CUR-NPs showed relatively normal structures in many seminiferous tubules at different spermatogenic stages with a significant change to ($P < 0.001$) in Johnsen's score compared ND-fed rats (**Fig. 3** A). The morphometric measurements, showed that rats fed IND for 8 weeks developed testicular atrophy, as evidenced by by histomorphometric analysis of the seminiferous tubules, where the diameter of the seminiferous tubules was significantly reduced (111±8.5 µm) in IND-fed animals than in control rats (155.6±10.5 µm). Compared with IND-fed animals, the administrated IND-fed animals with CUR or CUR-NPs showed an improvement in the mean tubular diameter at values 135.8±2.852 and 143.8 ±3.529, respectively (**Fig. 3** B).

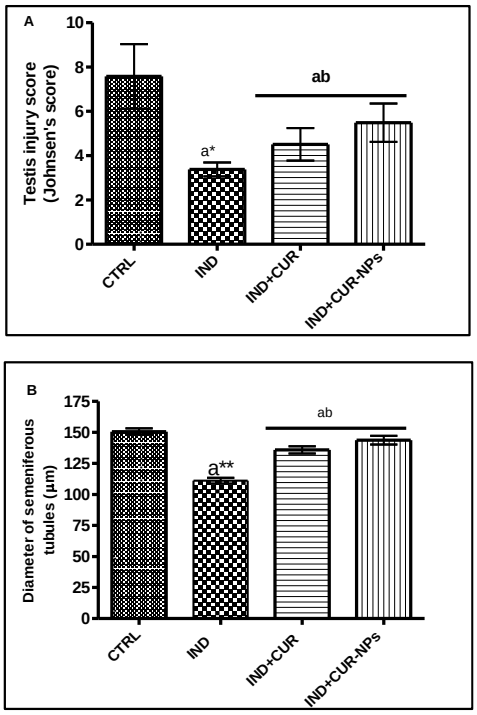

Figure 3. Testicular injury score through Johnsen's score (A) and changes in the diameter of the seminiferous tubules (B). (a): Significance relative to the control group, (b): Significance relative to the IND group. Significance: $P < 0.05$, (*): $P < 0.01$, (**): $P < 0.00$

3.6. Histochemical Results

Using Mallory's trichrome stain, the amount of collagen fibers in testicular tissues from all groups was determined (**Figure 4)**. Normal distribution of the collagen fibers located in the capsule and the interstitial cells connective tissue surrounding the seminiferous tubules (**Fig. 4**A). In IND-fed rats, testicular sections demonstrated a marked increase in the collagenous content in the testicular capsule with a congested blood vessel in it, and around seminiferous tubules (**Fig. 4** B). The collagen fibers in the testicular tissues of IND combined with CUR (**Fig. 4** C) or CUR-NPs (**Fig. 4** D) showing somewhat moderate a decrease in the collagen fibers distribution in comparison to the IND-fed group. The area percent of collagen fibers in the IND–fed group was substantially higher ($P< 0.001$) than in the control group. When compared to the IND–fed group, CUR or CUR-NPs plus IND fed rats had a significantly lower collagen fiber area percent ($P < 0.01$) (**Fig. 6** A).

3.7. Immunohistochemical Results

IND- fed rats had more TNF-α immunoreactivity in their testicular tissue than the rats in the CTRL group (Figure **5**). TNF-α showed intensive and widespread reaction for positive immunoreactivity for anti- TNF-α indicated by dense brown staining in the cells in the interstitial connective tissue surrounding the seminiferous tubules in IND-fed group (**Fig. 5**

B). In contrast to the IND-fed rats adminestrated with CUR or CUR-NPs also showed a decrease in TNF-α expression, as seen by a less brown stain (**Fig. 5** C&D). In comparison to the control group, there was a substantial ($P < 0.001$) increase in the area percent of TNF-α expression. Oral administration of IND- fed rats with CUR or CUR-NPs showed a considerably lower TNF-α expression area percent ($P < 0.001$) than the IND- fed rats (**Fig. 6** B).

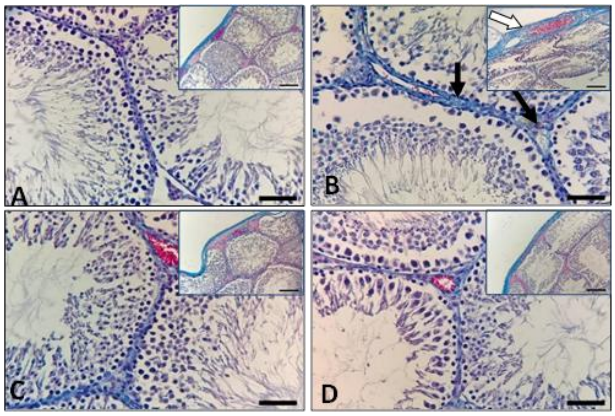

Figure 4. Photomicrographs stained with Mallory's trichrome stain collected from control rats (A) showing the normal distribution of the collagen fibers located in the capsule and also in the interstitial cells connective tissue surrounding the seminiferous tubules. (B) IND- fed rats showing marked increase in the collagenous fibes content, in the testicular capsule with a congested blood vessel (white arrow) and around seminiferous tubules (Black arrows). (C and D) testicular tissue of IND + CUR and IND + CUR –NPs groups showing somewhat moderate a decrease in the collagen fibers distribution in comparison to the IND-fed group. Scale bar 50 µm.

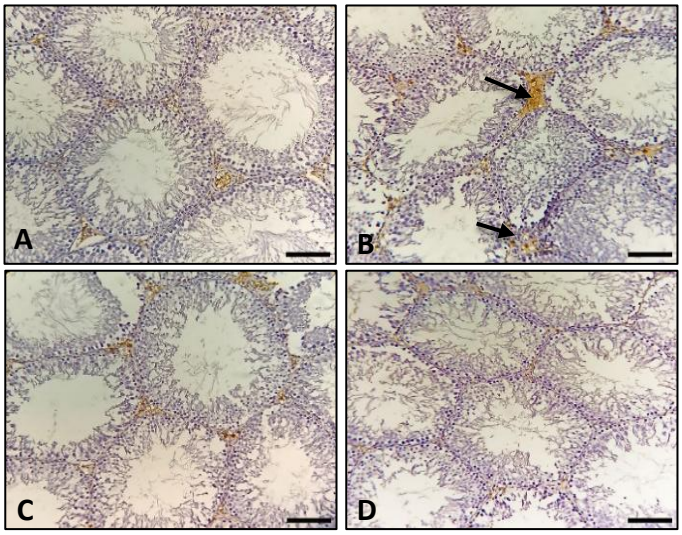

Figure 5. Photomicrographs of testis tissue sections of male rats stained with TNF-α-immunostaining (A) control group showing no-detectable levels of TNF-α expression (B) IND-fed group showed intensive and widespread reaction for positive immunoreactivity for anti- TNF-α in the interstitial connective tissue surrounding the seminiferous tubules (arrow). (C and D) IND + CUR and IND + CUR -NPs groups showing a marked decrease in TNF-α expression compared to IND-fed group alone. Scale bar: 50µm.

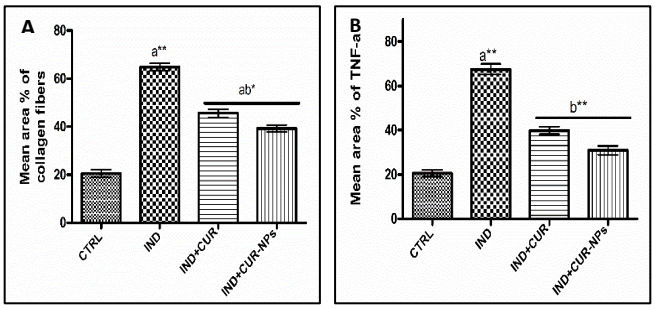

Figure 6. The mean area percent of collagen fiber (A) and TNF-α positive reactions (B). Data presented as mean ± SE of ten animals per group. (a): Significance relative to the control group, (b): Significance relative to the IND group. Significance: $P < 0.05$, (*): $P < 0.01$, (**): $P < 0.001$

4. Discussion

In the present study, detectable changes in the testis tissue as well as the hormones induced by the effect of IND-feeding, these changes were rebalanced with CUR and CUR-NPs administration. According to the most of current haematological data, giving IND to male rats causes haematological abnormalities as evidenced by a marked decrease in RBC count, HB, HCT, and MCV. These findings align with the research of Khudhur et al. **[30]** who found a significant decrease in most of the hematological parameters of rats fed at 30, 50, 70 and 100% of IND suggesting that the decreased manufacture of haeme in the bone marrow is likely to blame for the decrease in haemoglobin in the corpuscles. Similarly, weanling albino rats that were fed IND at levels of 25, 50, 75, and 100% along with pellets had low levels of PCV and HB. This may be because those rats didn't eat enough protein **[31]**. Contrary, Eteng et al. Eteng, Bassey, Nelson and Udosen **[32]** observed a non-significant decrease in RBC count, HB, and platelets with a non-significant increase in WBC count and lymphocytes in the group fed with 80% instant noodles prepared with noodle seasoning compared to control and the group fed with 80% noodles prepared without noodle seasoning. Furthermore, the dietary treatments had no influence on total leucocyte count, demonstrating that the addition of instant noodles in the diets had no pathogenic effect **[33]**.

Because of the high carbohydrate content of instant noodles, it has been shown that feeding the rats a high fat, high carbohydrate diet (HFHCD) for 12 weeks increased the number of leukocytes, primarily granulocytes, and increased the number of PLT and their collagen-induced aggregation as well **[34]**. Feeding rats, a high fat diet (HFD) increased the number of neutrophils, but the diet had no effect on the blood

count [35]. Additionally, for groups of rats fed cholesterol, there was a substantial rise in WBCs and PLT and a decrease in total RBCs, HB levels, and HCT [36]. According to several authors, rats who were inebriated with MSG had the lowest levels of WBC, HB, HCT, and RBCs [37, 38]. Likewise, the different TAZ treatments may cause the haematological abnormalities as evidenced by significantly reduced HCT and HB [39] as well as RBCs count, HB content, PCV, total WBCs count, lymphocytic, monocytic, and granulocytic counts [40]. The haematological alterations may be due the oxidative stress induced by TAZ in RBCs leading to increase in MDA level and decrease in GSH level of RBCs, which may shorten their lifespan by oxidizing the phospholipids in their membranes [40].

In this study, the IND-fed group that received oral delivery of CUR or CUR-NPs, revealed some significant improvements in the previous haematological tests when compared to IND-fed group. Similar previous reports demonstrated that CUR has the potential to be used to protect against hematological toxicities in rats exposed to carbofuran [41] or metallic mixture [42]. Similarly, [43] found that giving mice CUR in their diets improved all haematological parameters following gasoline-induced depression, and this may be due to the potent inhibitory effect of CUR on the key enzyme in benzene hematotoxicity, myeloperoxidase activity. Additionally, CUR is powerful antioxidants capable of preventing ROS formation which caused oxidative hemolysis. Moreover, the hematoprotective effect with the highest percentages of improvement in the hematological parameters has been recorded after the administration of CUR in combination with other substances such as cerium oxide nanoparticles in carboplatin-treated rats [44] and cinnamon in lead acetate-treated rats Emam, Farouk, Aljazzar, Abdelhameed, Eldeeb and Gad [45]. In rats given cisplatin, the addition of CUR or CUR-NPs tended to standardize haematological abnormalities and bring several of the haematological parameters back to normal in the treated ovarian cancer rats [46].

In the present study, the 8-week feeding on IND altered the serum levels of LH and total testosterone (Tt) with no change in FSH levels and caused a marked alteration in the histopathological examination of testes as evidenced by disrupted spermatogenesis. In agreement with earlier studies related to feeding with IND, the present findings corroborate those of Khudhur et al. [30] who postulated that a notable reduction in testosterone's influence on spermatogenesis in male rats was responsible for the markedly detrimental effects of graded concentrations of IND on reproductive health. Due to the lack of studies on the relationship between eating IND and reproduction, it would have been preferable to cite studies on the components included in IND. However, Chiu et al. found that consuming carbs was associated with reduced sperm motility in healthy young males [47]. Also, there may be a connection between dietary carbs and decreased sperm motility due to the rise in insulin resistance, which is related to sperm's ineffective use of glucose [48]. In addition, the sperm mitochondria are commonly targeted by oxidative stress and testosterone levels, which lowers their effectiveness. Obesity induced by a high fat diet has an impact on sperm quality because it induces germ cells apoptosis, alters testicular histology, causes epithelial cell atrophy reduces seminiferous tubule diameter [49]. Similarly, food additives have many concerns that must be taken into consideration, such as overuse, abuse, and even potentially dangerous additions [50]. The use of dietary additives has been shown in multiple studies to have a deleterious effect on the health of the reproductive system; this is also the case with IND seasoning, which contains a number of hazardous food additives such as MSG and TAZ. Many studies found a link between the presence of MSG in IND seasoning and alteration in testicular structure and function. However, MSG administration adversely affected testosterone production, hormonal levels, and sperm parameters, marked testicular degeneration, elevated level of MDA and decrease in SOD, CAT antioxidant enzymes [51, 52]. Not only MSG but also the TAZ included in IND may cause the disturbance in the structure and function of testis. Furthermore, significant alterations in free testosterone and sperm parameters as well as DNA damage and histological changes in testicular tissues were seen following a 60-day course of treatment with TAZ at dosages of 10 and 20 mg/kg [53]. Additionally, Waly et al. [54] reported that metabolic byproducts of TAZ stimulate oxidative stress, leading to cytological reactions that cause lipid peroxidation and subsequent histological deterioration in the testis of rats exposed to TAZ.

In the present study, the administration of CUR or CUR-NPs to IND-fed rats showed signs of improvements in alterations of histopathological examination as shown by normal spermatogenesis in many seminiferous tubules as well as an improvement in altered serum levels of LH and total testosterone (Tt) compared to IND-fed rats. Previous research has demonstrated that consuming CUR improves testis in long- and short-term models of testicular tissue damage and toxicity, suggesting a possible application for CUR in the management of testicular problems. However, CUR (100 mg/kg/day) could prevent testicular toxicity of testicles as it significantly improved the histopathological structural impairments in rat's testis when it was induced by lead acetate [55] or doxorubicin [56]. In addition, pretreatment with CUR reduced testicular weight, ameliorated morphometric parameters, increased Johnsen's scoring, elevated testosterone levels, and increased histological criteria. CUR also improved sperm parameters, and percentage of abnormality significantly increased FSH and testosterone levels [57]. According to Bayramova et al. [58] who came to the conclusion that CUR's anti-inflammatory and

antioxidant qualities shield testicular tissue from harm brought on by CCl4. In this case, testicular morphology and biochemical markers improved along with a decrease in the expression of TNF-α. and caspase-3 after CUR treatment. In another study, CUR may be able to improve spermatogenesis and sperm function in low-carbohydrate-fed animals, as well as reverse oxidative stress, reduce inflammation, and decrease apoptosis [59]. What's more, studies of the CUR-NPs application have also proven effective in the improvement of testis structure and function. CUR nanomicelle administration could enhance sperm quality, including total sperm count, concentration, and motility, as well as improve plasma levels of MDA, total antioxidant capacity, TNF-α and C-reactive protein [60]. Also, the treatment with CUR and CUR-NPs exhibited remarkable restorative effects on semen quality, sex hormone levels, antioxidant capacity, and mRNA expression of the targeted genes [61]. Similarly, CUR-NPs administration has significantly enhanced the sperm quality in aluminum phosphide intoxicated rats [62].

The interstitial fibrosis of testis causes destruction of the spermatogenic environment of testis and impairment of testosterone secretion and spermatogenesis leading to male infertility and sexual dysfunction [63]. Consequently, preventing testicular fibrosis has become an important way to cure testicular damage, where testicular interstitial fibrosis causes Leydig cell apoptosis, decreasing testosterone production and hence the amount and activity of germ cells [64]. In the present study, testicular tissue sections in IND-fed rats revealed a substantial deposition of collagen fibers in the interstitial cells and in the capsule. The observed collagen fibers may be due to the replacement of testicular parenchyma by collagen and fibroblasts after inflammation and necrosis [65]. This collagen deposition prevents the proper vascularity of testicular cells, increasing the chance of damage and atrophy [66]. The connection between consuming IND and testicular fibrosis has not been studied but similar testicular fibrosis was documented after Wistar rats were fed high-fat diets [68]. In addition, Abdelhamid et al. [67] found a considerable increase in collagen fibre deposition in the liver, kidney, and testis in rats treated with MSG. In a recent study, the testicular tissue staining with sirius red confirmed fibrosis in the interstitial and perivascular spaces in the TAZ-treated rats [55]. In this study, the administration of CUR or CUR-NPs to IND-fed rats showed a significant decrease in collagen fibre deposition compared to IND-fed rats. The present results are in agreement with Abdelhamid et al. [68] who found CUR ameliorated the deleterious effects of MSG on the percentage of fibrosis in some organs including testis. Also, the administration of either curcumin or selenium after Pb exposure dramatically alleviated all testicular abnormalities due to its antiapoptotic, antifibrotic, and antioxidant effects [69]. Also, CUR or CUR-NPs decreased both the amount of collagen fiber and percentage of fibrosis in acrylamide–treated male mice [70].

Furthermore, the current heamatological results, which showed a significant increase of WBC and neutrophils were confirmed by the immunohistochemical results of TNF-α in the IND-fed group. However, testicular tissue sections in IND-fed rats revealed a substantial increase in TNF-α immunoreaction. There is no research on the link between consuming IND and inflammation, but as components found in IND. It has been reported that there is proof that inflammatory cytokines disrupt the function of the hypothalamic-pituitary (HP) axis, indicating a clear connection between inflammation caused by obesity and low testosterone [71]. Also, Liu et al. [72] recorded an increase in TNF-α protein expression in male mice with high-fat diet-induced obesity. Furthermore, MSG oral administration-induced testicular toxicity in rats significantly increased levels of serum TNF-α [73] and upregulated the pro-inflammatory cytokine TNF-α gene expression in testicular tissue. Similarly, Abd-Elhakim et al. [74] found marked upregulation of inflammatory markers (IL-1β, IL-6, and IL-10) on long-term exposure to TAZ in rats. In this study, the administration of CUR or CUR-NPs to IND-fed rats showed a significant decrease in TNF-α protein expression compared to IND-fed rats. It has been reported that curcumins, flavonoids, and tannins act as anti-inflammatory and antioxidant agents to inhibit proinflammatory enzymes [75]. In confirmation with our current results of leucocyte profile which are in agrrement with Rinkunaite et al. [76] who found liposomal CUR reduced the levels of immune cells (neutrophils and WBC) with a reduction in IL-6, IL-1, and TNF-α levels near to healthy control values. Recently, CUR has anti-inflammatory effect as evidence by a adecrease in TNF-α expression on testicular tissue of rats treated with CCl4 [59].

5.Conclusion

Overall data obtained in this study have shown that 8 weeks feeding of rats with IND may exert some adverse effects, where long-term consumption of IND altered the haematological parameters as well as testicular structure and function. the serum levels of sex hormones. The administration of CUR or CUR-NPs to IND-fed rats showed signs of improvements in haematological abnormalities and alterations of histopathological examination as well as an improvement in altered serum levels of LH and total testosterone (Tt). It must be the consumers understand the health hazards linked to consuming IND and the food additives it contains. The current investigation provides additional support for the use of CUR or CUR-NPs in reducing the negative effects associated with long-term IND consumption. Additional research is recommended in this regard to determine the optimal ameliorative concentration.

CRediT authorship contribution statement:

Conceptualization, H.H., H.S. and H.O.; methodology, R.M.; software, R.H.; validation, H.O. and R.H. and H.S.; formal analysis, R.H.; investigation, H.O. and R.H..; resources, R.M.; data curation, R.H. and H.O.; writing—original draft preparation, H.O.; writing—review and editing, H.H., H.S, H.O.and R.H.; visualization, H.O. and H.S.; supervision, H.H.; All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data presented in this study are available upon request from the corresponding author.

Declaration of competing interest

The authors declare no conflicts of interest.

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