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Biochemical, Histological and Ultrastructural Studies to Evaluate the Effect of Curcumin on the Testicular Injury Induced by Sofosbuvir in Immature Male Rats

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Abstract: The present study aims to evaluate the role of curcumin (Cur) against the adverse and carcinogenic effects induced by Sofosbuvir (Sofo) in the testicular tissues in immature male albino rats. Cur is effective substance extracted from turmeric (*Curcuma longa*) has active biological phenolic substances that possess anti-inflammatory, anti-oxidative and anti-carcinogenic activities. The experiment animals were four groups: 1: control animals (60- 70 g) were received olive oil; 2: rats were administrated with Cur (200 mg/kg/ b w); 3: rats were administrated with Sofo (14 mg/kg/ b w); 4: animals were administrated with Sofo (14 mg/kg/b w) and Cur (200 mg/kg/b w). All rats were orally received their respective treatments every other day for 4 weeks. The results illustrated that Sofo treated group exhibited a marked decrement of body and testis weights, while, Sofo and Cur group showed a cleared recovery in those parameters. Also, Sofo group exhibited a distinct decrement in epididymal sperm analysis, while, Sofo and Cur animals revealed an obvious elevation in epididymal sperm analysis. Concerning to biochemical data, Sofo rats group produced testosterone decrement, T3 and T4 elevation, MDA elevation, declined SOD activity and GSH level. However, Sofo with Cur group recorded an improvement testosterone level, decreased T3, T4 and MDA levels, increase SOD activity and GSH level. No marked change in PSA level. The present histological results of Sofo group, showed reduction of spermatogenic lineage cells, appeared of vacuolated cells, necrotic areas and almost giant spermatozoa. While Sofo with Cur group demonstrated improvements recovery in the spermatogenic cells. The ultra-structural observations of Sofo group exhibited some differences in comparison with control one as follows: thin basement membrane, shrinkage nuclei of some spermatogenic cells, appearance of most organelles with electron dense, few mitochondria, low secretory granules of Leydig cells. While Sofo and Cur group showed improvements in the basement membrane thickness, spermatogenic cells almost seemed as control with electron lucent, Leydig cells secretory granules increased. The present results suggest that, the exposure of immature rat's testis to sofosbuvir produces moderate alteration of spermatogenesis activity and the use of curcumin may decline the adverse effects of Sofo.

Keywords: Sofosbuvir, curcumin, immature rat testis, histology, ultrastructure, biochemical.

1. Introduction

Hepatitis C virus (HCV) is a most global disease causing chronic viral hepatitis which can progress to cirrhosis hepatitis and finally to hepatocellular carcinoma causing about 350,000 deaths people worldwide per year [1, 2,3]. Although the developing of HCV infection is slower in children than in adults, up to 5% of HCV infected children may suffer with severe hepatic fibrosis or cirrhosis by the time they reach adulthood [4].

Pegylated interferon (PEG-IFN) combined to ribavirin was the standard therapy against HCV up to 2011 that associated with poor response rates and tolerability. Recently, use of the direct acting antivirals (DAAs) has led to

improve the treatment of HCV infection and produce PEG-IFN free therapies [5].

Sofosbuvir (Sofo) is one of the most recent DAAs developed as an oral treatment for HCV infection in combination with other drugs and used since 2013. Sofo is one of special interest the DAAs under development, due to its high potency, low side effects, oral administration, reduced duration of therapy, and high barrier to resistance [6].

Sofo is uridine nucleotide analogue which acts directly by inhibiting NS5B polymerase which regulates the chronic HCV infection [7]. So, the therapeutic effects of Sofo on HCV have been well documented and was marketed since 2013, but up to our knowledge, there were limited studies to show its effect on the rat reproductive organs and its influence of male fertility while using this drug.

Curcumin (Cur) is a yellowish red phenolic dye extracted from the rhizome of turmeric (*Curcuma longa*) that belongs to the ginger (Zinigiberaceae) family. Turmeric is used as a spice and also has been used in medication for thousands of years in Asia. Cur is the main active component of turmeric and is responsible for its beneficial effects [8, 9].

Cur acquires its famous of biological and pharmacological activities including antioxidant, anti-inflammatory, antitumor, antimicrobial, anticarcinogenic, antidiabetic and immunomodulatory properties. Also, it exhibits a wide range of effects including neuroprotective, radioprotective, and antiangiogenic effects [10, 11]. Furthermore, it plays a role in the cholesterol level reduction, Alzheimer's disease protection, wound-healing properties and HIV replication inhibition [12].

2. Materials and Methods

2.1. Experimental animals

This study was carried out on immature male Wister albino rats with body weight ranged from (65 ± 5 g). They were obtained from Helwan Breeding Farm (Ministry of Health, Giza, Egypt) and were housed in stainless steel rodent cages at room temperature (25 ± 2 ºC) with 12 h dark/light cycle. Rats were permitted adequate standard diet and water was allowed *ad libitum*. Animal Ethics Committee of Mansoura University, Egypt was taken in consideration in care and use of the animals.

2.2. Drugs and agents

Sofo tablets were purchased from Marcyrl Pharmaceutical Industries (El Obour City, Egypt) and was dissolved in sterile distilled water to prepare a stock solution. Cur powder was provided by Sigma Company for Chemicals, Egypt and was dissolved in olive oil to prepare a stock solution.

2.3. Experimental design

After the acclimation period, rats were divided into four groups (10 animals/each): group 1: animals were orally administrated with equivalent volumes of olive oil only as a vehicle; group 2: animals were orally administrated with Cur (200 mg/kg/b w) [13]; group 3: animals were orally administrated with Sofo (14 mg/kg/b w), a dose equivalent to that used in the human; group 4: animals were orally administrated with Sofo (14 mg/kg/b w) and Cur (200 mg/kg/b w). All animals were received their respective treatments every other day for 4 weeks.

2.4. Blood and tissue sampling

At the end of the experimental period, the animals were weighed and sacrificed by diethyl ether. Blood samples were collected, centrifuged at 3000 rpm for 15 minutes and separated sera were frozen for selected biochemical analysis. At the same time, the testes and epididymis were removed. The testes were weighed and used for histopathological and ultrastructural studies and the epididymis was used for semen analysis.

2.5. Body and testis weight

Body weights of all animals were recorded three times a week and at the end of the experiment. Testicles were accurately weighed (absolute testis weight) after sacrifice and the relative testis weight of each animal was then calculated.

2.6. Semen analysis

For epididymal sperm preparation, the cauda epididymis was dissected and placed in 3 ml normal saline solution. The sperms were released into the saline solution by mincing the cauda epididymis into tiny pieces. The semen samples were incubated at 37 °C for 15 min

before the analysis of sperm parameters was carried out [14] that determined using the Computer Assisted Semen Analysis (CASA) according to the methods proposed by the World Health Organization (WHO) in 2010 as described by [15].

An aliquot of sperm suspension was charged into the standard counting analysis chamber that placed on the heat plate of the microscope (37 °C) for 3 min before the analysis. Then, several fields of view were captured by a video camera which is connected to a computer and at least 400 spermatozoa were counted to determine spermatozoa count per ml (sperm concentration) and total sperm count. The sperm vitality was also determined using eosin/nigrosine stain. A total of 200 spermatozoa were counted within a few minutes after adding the dye. Evaluation of live (unstained) and dead (red stained) spermatozoa was carried out. Also, CASA provided the progressive motility and the total motility percentages at least 10 microscopic fields.

2.7. Assessment of biochemical parameters

2.7.1. Serum testosterone level

Serum testosterone level was evaluated using Calbiotech Mouse/Rat Testosterone ELISA Kit (Catalog number TE187S-100) as described by [16].

2.7.2. Serum triiodothyronine (T3) and thyroxin (T4) levels

Serum (T3) and (T4) levels were measured using Calbiotech Mouse/Rat ELISA Kits (Catalog numbers T3043T-100 and T4044T-100) for T3 and T4 respectively [17].

2.7.3. Biochemical markers of oxidative stress

Serum malondialdehyde (MDA) concentration was determined using Biodiagnostic Co. kit (Catalog number MD 25 29) [18]. Serum superoxide dismutase (SOD) activity was measured [19]. Reduced glutathione (GSH) content was measured using colorimetric method [20].

2.7.4. Prostate-Specific Antigen (PSA)

PSA concentration was determined by colorimetric method using Rat PSA ELISA Kit obtained from Cusabio Biotech Co. Ltd. [21].

2.8. Histological studies

The testes from all examined groups were immediately cut and fixed overnight in phosphate buffered 2 % glutaraldehyde (pH 7.4) at 4°C, followed by post-fixation in 1% osmium tetroxide for 1-2, dehydrated in ascending grades of ethyl alcohol, cleared in propylene oxide and finally embedded in epoxy resin. Semithin sections (0.6 – 0.7 µm thick) were cut with glass knives on ultra-microtome, mounted on glass slides, stained with toluidine blue and then examined and photographed using light microscope [22].

2.9. Ultrastructural studies

Ultrathin sections (50–80 nm) from selected areas of the trimmed blocks were cut using ultramicrotome with the diamond knife, collected on cleaned copper grids and stained with freshly prepared uranyl acetate and lead citrate [23]. The sections were examined and photographed in a JEOL JEM-210 transmission electron microscope in Electron Microscopy Unit, College of Agriculture, Mansoura University, Egypt.

2.10. Statistical analysis

All the grouped data were statistically evaluated with SPSS 25 software. Differences among groups were evaluated by one-way ANOVA (analysis of variance) test and post comparison was carried out with Tukey test. Data were expressed as means ± standard error (SE). The values of $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ were considered statistically significant, highly significant and very highly significant, respectively.

3. Results

3.1. Morphometric observations

As shown in figure (1) administration of Cur alone to normal rats produced a non-significant change ($P > 0.05$) in the percentage of change in the body weight gain, the final body weight and relative teses weight compared to normal rats. Administration of Sofo to normal rats produced a very high significant decrease ($P \leq 0.001$) in the percentage of change in the body weight gain, the final body weight and relative testis weight compared to control rats. The administration of Cur to Sofo group showed a very high significant increase ($P \leq 0.001$) in the percentage of change in the body weight gain and the final body weight and a significant

increase ($P \leq 0.05$) in relative testis weight comparing with either Sofo group or control group.

As shown in figure (2) Cur administration resulted in non-significant changes ($P > 0.05$) in all tested epididymal sperm parameters compared to control group. Sofo group exhibited a very high significant decrease ($P \leq 0.001$) in epididymal sperms concentration, count, vitality and progressive and total motility compared to control group. However, administration of Cur to Sofo group resulted in a significant increase ($P \leq 0.05$) in epididymal sperms concentration, count and progressive motility and a very high significant increase ($P \leq 0.001$) in epididymal sperms vitality and total motility compared to Sofo treated group. While the same group revealed a non-significant decrease ($P > 0.05$) in epididymal sperms concentration and count and a very high significant decrease ($P \leq 0.001$) in epididymal sperms vitality and progressive and total motility respective to control group.

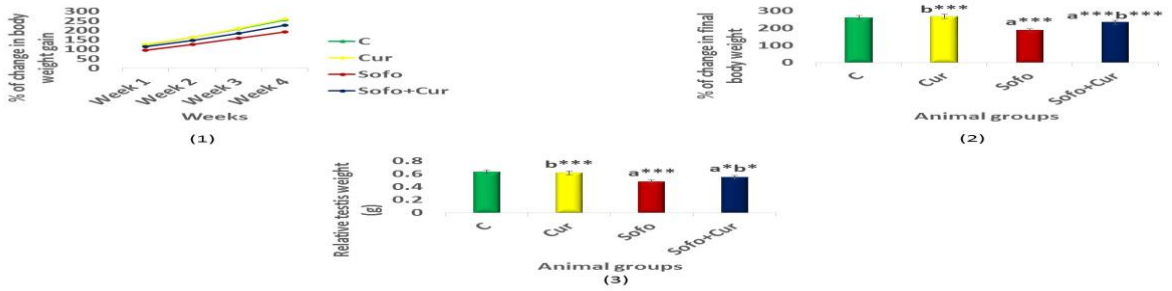

Fig. (1): The percentage of change in the body weight gain (1), the percentage of change in the final body weight (2) and relative testis weight (g) (3) in control and different rats' groups; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group. (a) Significant as compared with control group, (b) Significant as compared with sofosbuvir group, * Significant ($P \leq 0.05$), *** Very high significant ($P \leq 0.001$).

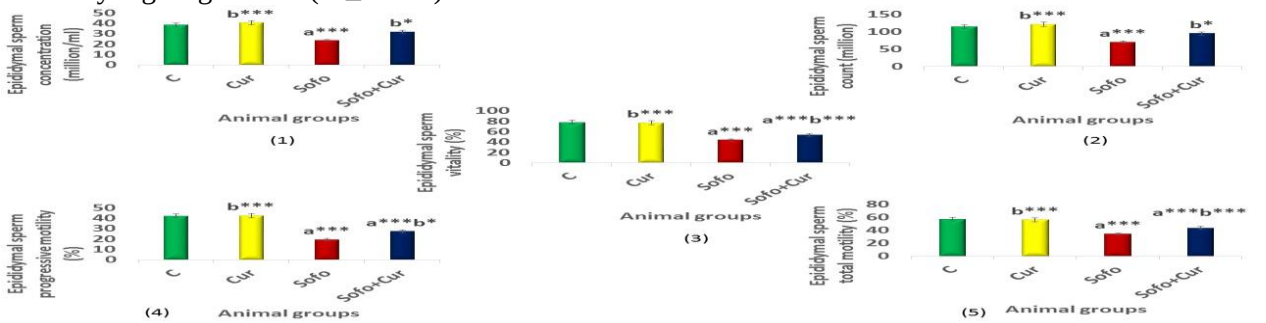

Fig. (2): The epididymal sperm concentration (million/ml) (1), count (million) (2), vitality (3), progressive motility (4) and total motility (5) in control and different rats' groups; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group. (a) Significant as compared with control group, (b) Significant as compared with sofosbuvir group, * Significant ($P \leq 0.05$), *** Very high significant ($P \leq 0.001$).

3.2. Biochemical observations

As shown in figure (3) administration of Cur alone produced a non-significant change ($P > 0.05$) in the testosterone level compared to control group. Sofo administration produced a very high significant decrease ($P \leq 0.001$) in the testosterone level compared to control group. Administration of Cur to Sofo rats group produced a very high significant increase ($P \leq 0.001$) in the testosterone level in comparison with Sofo rats and a very high significant decrease ($P \leq 0.001$) compared to control group.

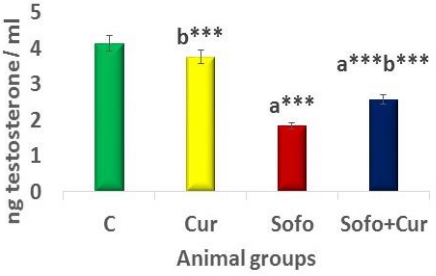

Fig. (3): Serum testosterone level (ng testosterone/ml) in all groups; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group. (a) Significant as compared

with control group, (b) Significant as compared with sofosbuvir group, *** Very high significant ($P \leq 0.001$).

As shown in figure (4) Cur administration produced a non-significant change ($P > 0.05$) in T3 and T4 levels. Sofo rats group exhibited a very high significant increase ($P \leq 0.001$) in T3 and T4 levels compared to control rats. Administration of Cur to Sofo group resulted in a very high significant decrease ($P \leq 0.001$) in T3 and T4 levels compared to Sofo group. While the same group revealed a very high significant increase ($P \leq 0.001$) in T3 level and a high significant increase ($P \leq 0.01$) in T4 level compared to control group.

As shown in figure (5) Cur administration resulted in a non-significant change ($P > 0.05$) in MDA level, SOD activity and GSH level. Sofo rats group resulted in a very high significant increase ($P \leq 0.001$) in MDA level and a very high significant decrease ($P \leq 0.001$) in SOD activity and GSH level compared to control group. Administration of Cur to Sofo group produced a very high significant decrease ($P \leq 0.001$) in MDA level and a very high significant increase ($P \leq 0.001$) in SOD activity and GSH level compared to Sofo group. While the same group revealed a high significant increase ($P \leq 0.01$) in MDA level, a very high significant decrease ($P \leq 0.001$) in SOD activity and a high significant decrease ($P \leq 0.01$) in GSH level compared to control group.

Fig. (4): Serum triiodothyronine level (ng T3/dL) (1) and thyroxin (T4) level (µg T4/dL) (2) in all groups; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group, (a) Significant as compared with control group, (b) Significant as compared with sofosbuvir group, ** High significant ($P \leq 0.01$), *** Very high significant ($P \leq 0.001$).

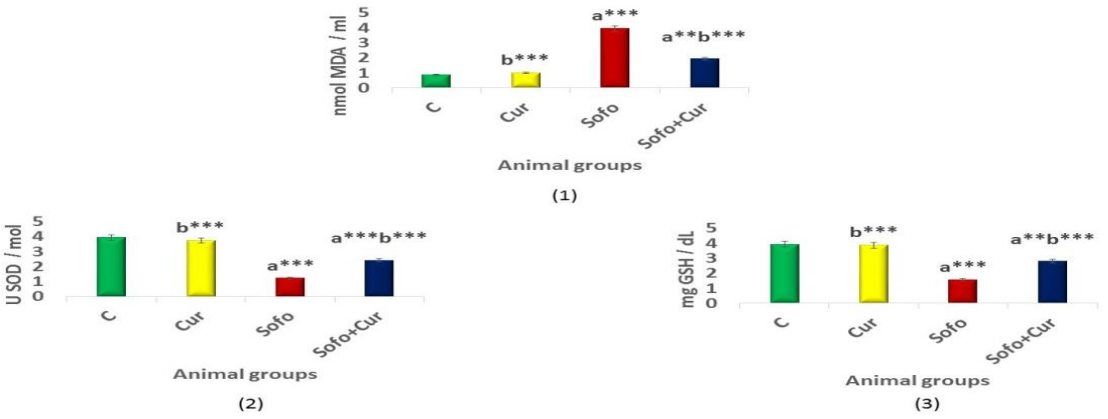

Fig. (5): Malondialdehyde (MDA) level (nmol MDA/ml) (1), superoxide dismutase activity (U SOD/mol) (2) and reduced glutathione (GSH) level (mg GSH/dL) (3) in all groups; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group, (a) Significant as compared with control group, (b) Significant as compared with sofosbuvir group, ** High significant ($P \leq 0.01$), *** Very high significant ($P \leq 0.001$).

As shown in figure (6) administration of Cur resulted in a non-significant change ($P > 0.05$) in PSA level. Sofo group exhibited a non-significant increase ($P > 0.05$) in PSA level compared to normal value. Administration of Cur to Sofo group produced a non-significant decrease ($P > 0.05$) in PSA level compared to Sofo group and a non-significant change compared to control group.

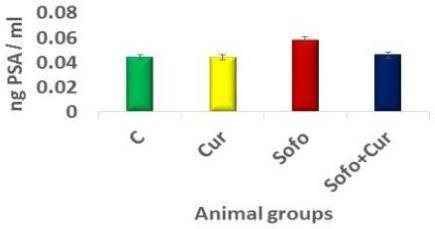

Fig. (6): Prostate-specific antigen level (ng PSA/ml) in all groups; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group.

3.3. Histological observations

The semi-thin sections of seminiferous tubules of control group showed normal histological structure (Figs 7 & 8 (C)). The testicular tissue of Cur semi-thin sections rats showed almost the same seminiferous tubule structure as control one (Figs 7 & 8 (Cur)). In Sofo group, the testicular tissue showed reduction of spermatogonia type A and primary spermatocytes, appear of vacuolated cells, necrotic areas and almost giant spermatozoa (Figs 7 & 8 (Sofo)). On the other hand, the testicular tissue of Sofo with Cur group demonstrated improvements recovery in the number of spermatogonia type A and primary spermatocytes (Figs 7 & 8 (Sofo + Cur))

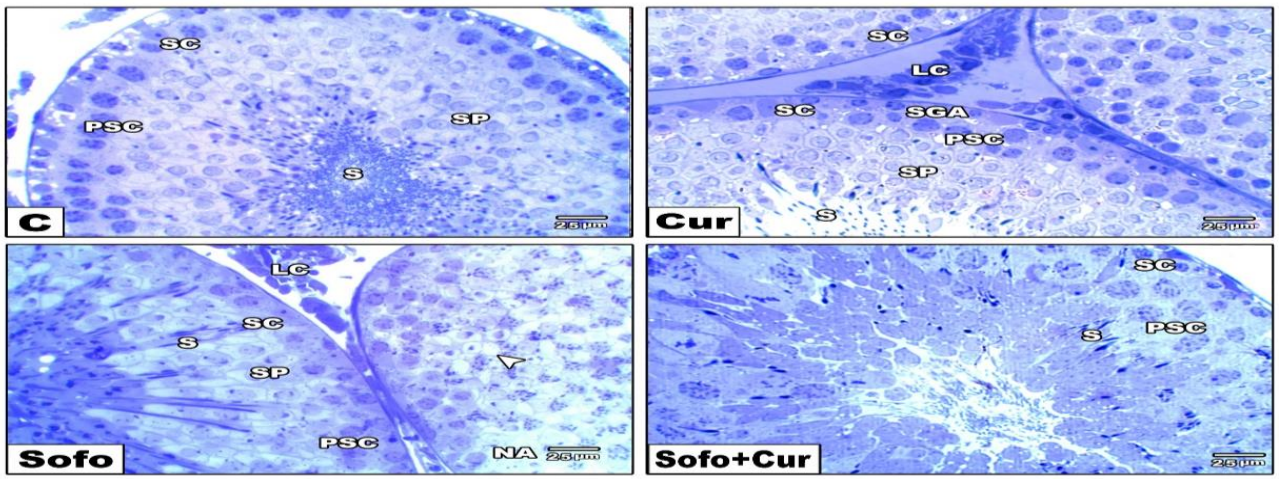

Fig. (7): Photomicrograph of semithin sections of testicular tissues stained with toluidine blue of all groups which; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group. Abbreviations: SGA, spermatogonia type A; PSC, primary spermatocyte; SP, spermatid; S, spermatozoa; SC, Sertoli cell; LC, Leydig cells; NA, necrotic area; arrow head, vacuolated cell.

3.4. Ultrastructural observations

At the ultrastructural level, by using TEM the testicular tissue of control group revealed normal spermatogonia type A with a recognizable oval nucleus, mostly euchromatin, less heterochromatin, numerous mitochondria in their cytoplasm, all with electron lucent and clear plasma membrane (Fig 9 (C)). The primary spermatocyte revealed with normal large spherical nucleus with most euchromatin and less heterochromatin, and the appearance of the cytoplasm with normal mitochondria and an obvious plasma membrane, all the structures exhibited electron lucent (Fig 10 (C)). Normal structure of Sertoli cells with basal nuclei could exhibited fine granules of chromatin and small compact nucleolus, electron lucent cytoplasm with mitochondria and lipid droplets were arrested on normal basement membrane (Fig 11 (C)). The typical structure of Leydig cells was seen, in which both cytoplasm and nucleus appeared with electron lucent, the dense cytoplasm demonstrated with large number of secretory granules and the large nucleus almost revealed euchromatin and little heterochromatin (Fig 12 (C)). The testicular tissue of rats given Cur showed almost the same spermatogonium, primary spermatocyte, Sertoli and Leydig cells structure as control one (Figs 9, 10, 11 &12 (Cur)).

In Sofo group, the testicular tissue showed the spermatogonium type A with shrinkage nucleus and few mitochondria, both cytoplasm and nucleus revealed electron dense (Fig 9 (Sofo)). The primary spermatocyte demonstrated nucleus with electron dense; the cytoplasm with mitochondria and clear plasma membrane (Fig 10 (Sofo)). Sertoli cell showed

the basal nucleus with electron dense and the cytoplasm with electron lucent, possessed some mitochondria with electron dense. The basement membrane was thinner than the control one (Fig 11 (Sofo)). The Leydig cells showed both cytoplasm and nucleus with electron dense and the cytoplasm appeared with fewer number of secretory granules in comparison with the control cell (Fig 12 (Sofo)). While the testicular tissue of Sofo with Cur group showed spermatogonium type A seemed more or less similar to control one with electron lucent (Fig 9 (Sofo + Cur)). The nucleus of the primary spermatocyte exhibited with electron lucent in comparison with the Sofo treated one (Fig 10 (Sofo + Cur)). Sertoli cell showed the structure of basal nucleus with nucleolus and the cytoplasm revealed large lipid droplets and few mitochondria (Fig 11 (Sofo + Cur)). This group showed a degree of improvement in the structure of Leydig cells with the number and size increase of secretory granules (Fig12 (Sofo + Cur))

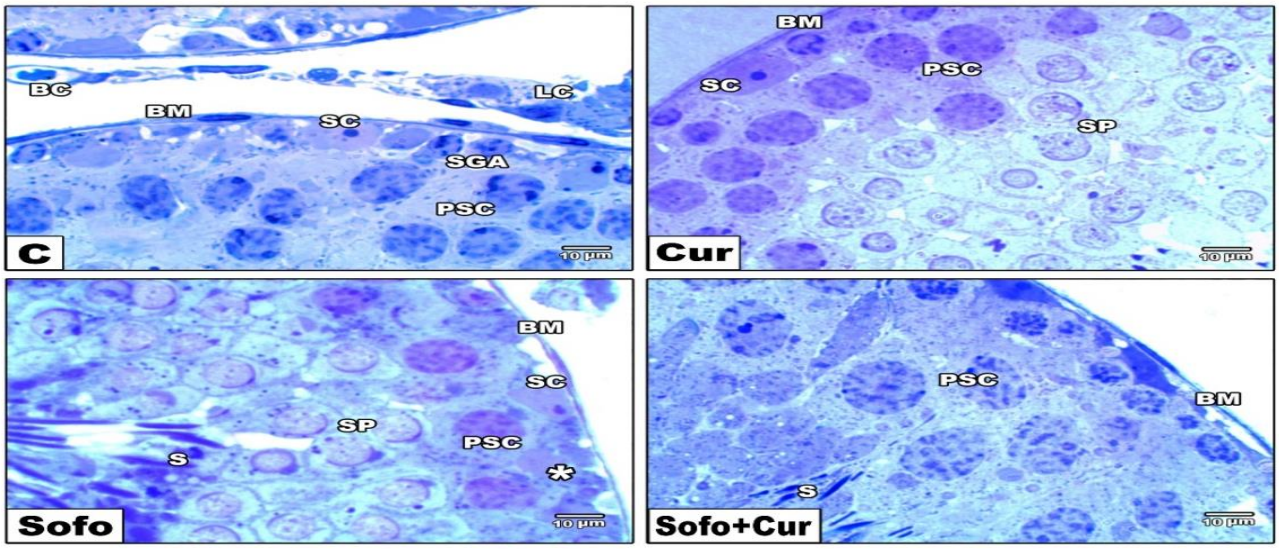

Fig. (8): Photomicrograph of semithin sections of testicular tissue stained with toluidine blue of all groups which; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group. Abbreviations: SGA, spermatogonia type A; PSC, primary spermatocyte; SP, spermatid; S, spermatozoa; SC, Sertoli cell; BM, basement membrane; LC, Leydig cells; BC, blood capillary; star, degenerated spermatogonia.

4. Discussion

Our study aims to evaluate the adverse effects on testicular tissue due to Sofo treatment and the ameliorating effect of Cur against the testicular toxicity in immature albino rats.

The present study reveals that, the administration of Sofo results in diverse toxic effects on the testicular tissue. The morphometric observations of Sofo treated rats exhibited a significant decrease of body weight gain and relative testis weight comparing to control animals. This histological study exhibited degeneration in the spermatogenic linage with an obvious decrement of both spermatogonia type A and primary spermatocytes, existence of many vacuolated cells and necrotic areas as well as an injury in the structure of Sertoli cells. These findings were confirmed by many authors [24, 25].

The ultrastructural observations of the present work supported the histology results. The cytotoxic effects of Sofo treatment on the testicular tissue induced shrinkage nuclei and few mitochondria of spermatogonia type A. The nuclei and cytoplasm of the primary spermatocytes, Sertoli cells and Leydig cells appeared with electron dense. Moreover, there are decrement of Leydig cell's number and secretory granules. These results are in agreement with [24] who reported that, Sofo treatment increased apoptosis in both spermatogenic and Leydig cells. Also, they described a fine collagen deposition in peritubular and tunica albuginea increased in Sofo treated group. These findings of degenerative spermatogenic cells and the increment of connective tissue may be leads to decline the testis weight.

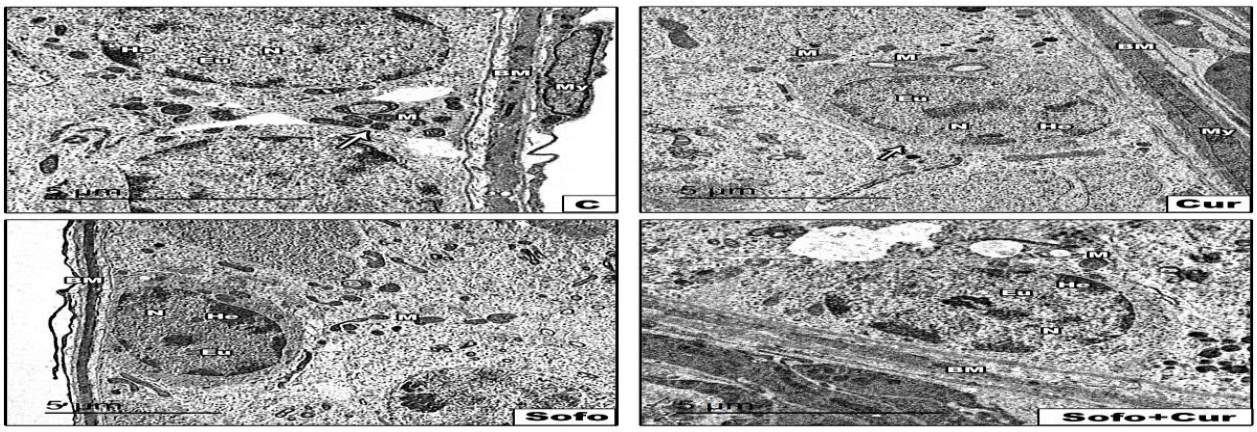

Fig. (9): Ultrastructural micrograph through the seminiferous tubules of the different rat groups which, (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group. Abbreviations: N, nucleus; Eu, euchromatin; He, heterochromatin; M, mitochondria; arrow, plasma membrane; BM, basement membrane; My, myoid cell.

While the animal group received Cur with Sofo, in the present experiment, exhibited significant increase in body and testis weight comparing to both control and Sofo groups, that possessed an improvement in the number and vitality of the spermatogenic cells, and Leydig cell's number and secretion. Similar results are demonstrated by other authors [26, 27] who reported that Cur is a potent inducer of detoxifying enzymes and ameliorates body weight loss.

The present biochemical results and testis activities recorded that the administration of Sofo to immature male rats produced a significant decrease in the testosterone level, epididymal sperm concentration, count, vitality and progressive and total motility compared to control group. These results are parallel to the results of other authors [24, 28] who explained that, the reduction in serum testosterone level may be due to an atrophy of Leydig cells. These observations were also reported [29] that described the spermatogenesis, sperm maturation and testis function are supported by testosterone hormone. So, any disorder in testosterone synthesis could influence the male fertility. Moreover, the present work explore that the rats received Cur with Sofo showed a very high significant increase in the rats testosterone level in comparison with Sofo treated

The cytotoxic effects of Sofo treatment also are proved in the present work by the results of some markers of oxidative stress determination which recorded very high significant elevation of DMA level and very high significant decrement of both SOD activity and GSH level.

Additionally, the present observations recorded a very high significant increase of the T3 and T4 levels in Sofo group comparing to c

ntrol group

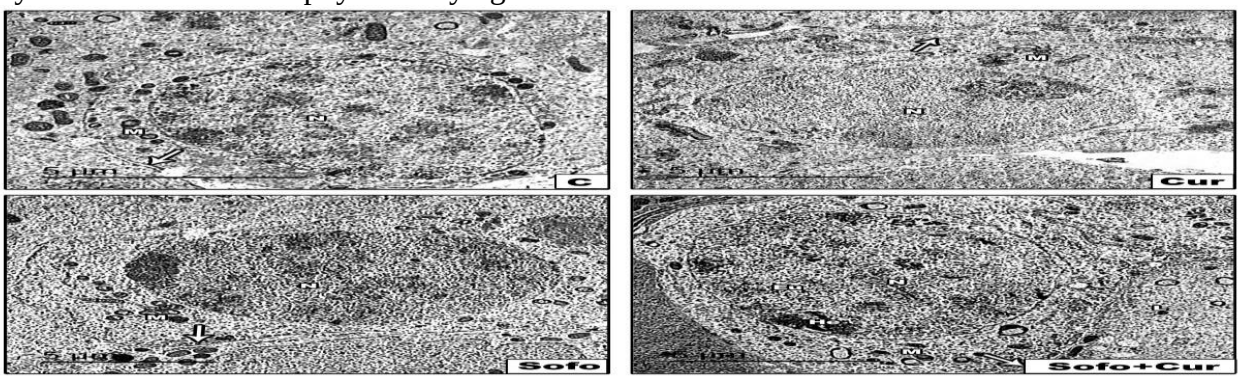

Fig. (10): Ultrastructural micrograph through the seminiferous tubules of the different rat groups which; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group. Abbreviations: N, nucleus; M, mitochondria; arrow, plasma membrane; L, lipid droplets; Eu, euchromatin; He, heterochromatin

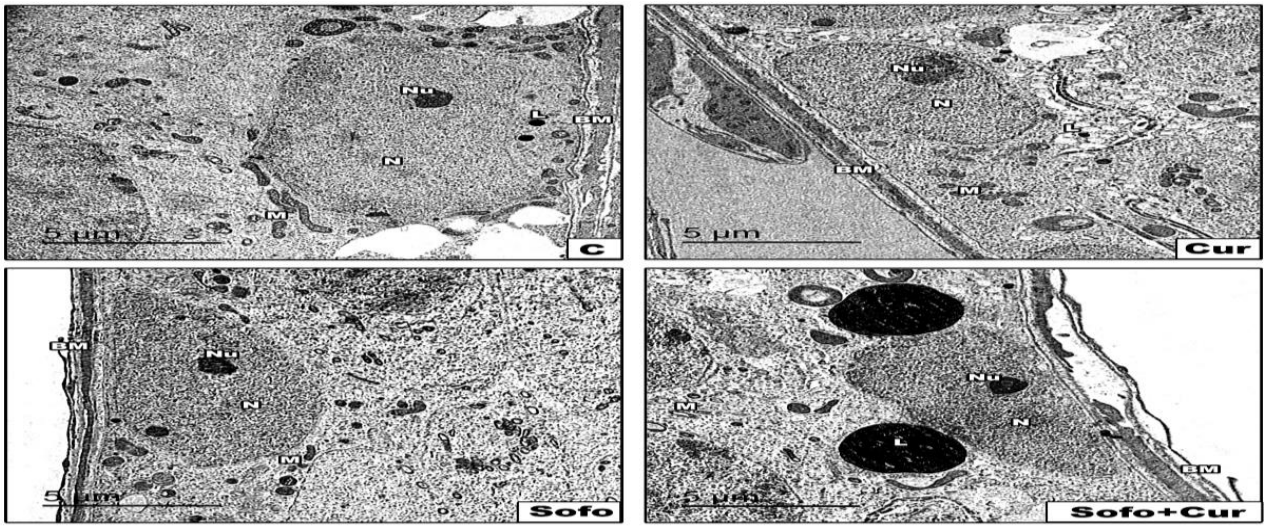

Fig. (11): Ultrastructural micrograph through the seminiferous tubules of the different rat groups which; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group and (Sofo + Cur) sofosbuvir and curcumin group. Abbreviations: N, nucleus; Nu, nucleolus; M, mitochondria; L, lipid droplets; BM, basement membrane.

The present histological and ultrastructural observations exhibited a degree of improvement of the spermatogenic linage cells after given Cur. These findings are combatable with [30] who mentioned that Cur has a protective effect on testes, able to maintained the structural and functional activities of seminiferous tubules. On the other hand, the present histological study showed the testicular tissue almost with giant heads of spermatozoa in Sofo treated rats. Since, thyroid hormones have critical effects on testis development, the efficiency of spermatogenesis, reflected by daily sperm production in adulthood, correlates to the total number of functional Sertoli cells established during pre-pubertal hood [31]. Changes in thyroid hormones during early testis development affect testicular maturation and reproduction later during life.

Most patients with thyroid hormone disorders possess some kind of sexual dysfunction [32, 33], and also, T3 hormone regulates both maturation and growth of the testis, controlling Sertoli cells and Leydig cells proliferation and differentiation during testicular development in rats [34]. In addition, T3 is necessary to initiate differentiation of mesenchymal cells into Leydig cells progenitor cells, and it works in concert with LH and IGF-1 to promote Leydig cells development [35]. So, the excess of T3 level during the pre-pubertal hood may be leads to producing abnormal spermatozoa and affecting the male fertility as the present investigation demonstrated that, the testis activities in rats treated with Sofo revealed significant decrease in epididymal sperm concentration, count, vitality and progressive and total motility compared to control rats. This opinion is parallel to [36] who reported that the uses of Sofo treatment caused cytogenetic abnormalities resulted in structural chromosomal aberration and abnormal sperm head. And also, the hyperthyroidism plays a role in oxidative stress induction due to increasing mitochondrial activity that concurrent electrons releasing from mitochondrial electron transport chain due to increased production of thyroxine [37]. In addition, the decrease in GSH level may be due to increased utilization of GSH for detoxification of excessive free radicals generated [38].

The elevation in the antioxidant enzymes level in the Sofo rats treated with Cur recorded an increment of GSH level, increasing the activity of SOD and decline MDA level. These results are agreed with the results of other authors [13 & 39] that Cur has an ability to scavenge reactive oxygen and nitrogen free radicals or by modulating cellular defenses which themselves exert antioxidant effects.

The determination of prostate- specific antigen level recorded non-significant alteration of Sofo group which indicates that the uses of Sofo treatment at the present dose and duration does not promote testicular cancer cells.

In conclusion, the current study showed that, treating immature rats with Sofo leads to a decrement in testosterone and an increasing in thyroid hormones, which negatively affected the productivity of the testis and increased the chance of forming abnormal patterns of sperm, thus affecting male fertility, however, administration of Cur to Sofo group reduced symptoms pathogenicity and toxic effects of Sofo as a result of containing antioxidant and anti-inflammatory phenols. Therefore, the study recommends using Cur for young patients who take Sofo to improve the condition of testes, and the study may be promising for them in the future not to deform the spermatozoa, so that their reproductive health is not affected after pubert

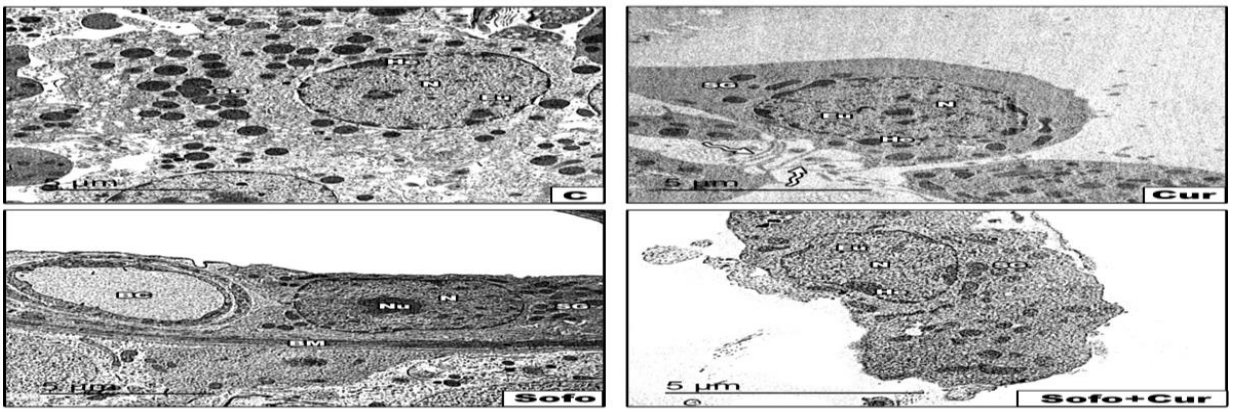

Fig. (12): Ultrastructural micrograph through the seminiferous tubules of the different rat groups which; (C) control group, (Cur) curcumin group, (Sofo) sofosbuvir group, (Sofo + Cur) sofosbuvir and curcumin group. Abbreviations: N, nucleus; Nu, nucleolus; Eu, euchromatin; He, heterochromatin; SG, secretory granules; wavy arrow, intracytoplasmic bands; BC, blood capillary; BM, basement membrane.

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