DOI: 10.4081/aiua.2023.11593

ORIGINAL PAPER

Effects of testosterone replacement on lipid profile, hepatotoxicity, oxidative stress, and cognitive performance in castrated wistar rats

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Summary ***Objective: Androgen deficiency is associated with multiple biochemical and behavioral disorders. This study investigated the effects of testosterone replacement and Spirulina Platensis association on testosterone deficiency-induced metabolic disorders and memory impairment.***
Methods: Adult male rats were randomly and equally divided into four groups and received the following treatments for 20 consecutive days. Control group: non-castrated rats received distilled water. Castrated group received distilled water. Testosterone treated group: castrated rats received 0.20 mg of testosterone dissolved in corn oil by subcutaneous injection (i.p.). Spirulina co-treated group: castrated rats received 0.20 mg of testosterone (i.p.) dissolved in corn oil followed by 1000 mg/kg of Spirulina per os.
Results: Data showed that castration induced an increase in plasma ALT, AST, alkaline phosphatase (PAL), cholesterol, and triglycerides level. Castrated rats showed a great elevation in SOD and CAT activities and MDA and H_2O_2 levels in the prostate, seminal vesicles, and brain. Testosterone deficiency was also associated with alteration of the spatial memory and exploratory behaviour. Testosterone replacement either alone or with Spirulina combination efficiently improved most of these biochemical parameters and ameliorated cognitive abilities in castrated rats.
Conclusions: Testosterone replacement either alone or in combination with Spirulina improved castration-induced metabolic, oxidative, and cognitive alterations.

KEY WORDS: Castration; Testosterone; Spirulina Platensis; Cognition; Oxidative stress.

Submitted 18 July 2023; Accepted 30 July 2023

INTRODUCTION

With aging men can develop several cognitive and metabolic impairments due to the reduction in endogenous testosterone production (1). Androgen deficiency is referred to as hypogonadism, it is a health issue that can occur within men aged from 40 to 80 years old and includes fatigue, cognitive and mood disorders as clinical symptoms (2). It is also associated with common medical conditions such as hypertension, diabetes, and obesity (3). In fact, a very common consequence of testosterone deficiency in men is a decline in some forms of memory such as episodic and working memory (4, 5).

Numerous animal studies including models of castrated rodents were useful to mimic the hypogonadism medical condition and evaluate the effects of androgen depletion on the cognitive functions (6). Multiple tasks were used to assess the spatial learning and working memory abilities of castrated rodents varying from navigating toward hidden platform in a pool of water in the Morris water maze test (7), to look for displaced objects in the novel object recognition and location tests (8). Although, the mechanism by which testosterone influences the working of spatial memory is poorly understood, many of the animal studies showed a positive correlation between high/optimum testosterone level and better cognitive abilities in males including improved spatial memory (9, 10). Moreover, on a physiological level it has been reported that testosterone deficiency caused metabolic disorders including changes in body composition, fat distribution (11) and promoted oxidative stress and inflammation (12).

The main treatment for hypogonadism in men is *testosterone replacement therapy* (TRT). Therapeutic options for TRT varied from oral and injectable testosterone administration to patches and testosterone gels. Innovations and advances in TRT during the years have enhanced the role and safety of the use of testosterone as a metabolic hormone and had beneficial effects on obesity, cardiovascular and hepatic diseases (13). Many research data nonetheless consolidated the long-term side effects of the TRT. In fact, long-term use of TRT has been associated with elevated oncologic risks mainly in the prostate (14) as well as the likelihood of developing obstructive sleep apnea and erythrocytosis (15).

Therefore, potential alternatives, mainly plants and their derived natural substances, are being studied to replace and/or minimize the TRT side effects. For instance, onion supplementation was positively correlated to an increase in *Luteinizing Hormone* (LH) production and has been proven to reduce testis oxidative stress (16). Ginger supplementation effect on intoxicated rats was also hypothesized to reduce oxygen species production and lipid peroxidation in the gonads thus improving testosterone level (17).

Arthrospira platensis also commercialized under the name of *Spirulina* is a cyanobacterium which captured the scientists and food industry's attention during the last few decades for its high nutritional as well as potential thera-

peutic values (18-20). The huge interest in *Spirulina* is essentially due to its high protein level and the protein quality as it contains essential amino acids as well as the availability of vitamins and minerals notably vitamin B12, iron and calcium (21). Many human and animal studies on the effects of *Spirulina* intakes have been reported, yet their results varied regarding the duration of administration, the doses and target groups. In fact, evidence from animal studies were in favour of a potential reproprotective effect of *Spirulina* particularly by enhancing antioxidant enzymes activities, hence restoring the production of testosterone in bifenthrin-intoxicated mice (22),and mitigating pro-inflammatory cytokines in furan exposed rats (23). Additionally, a previous study showed that *Spirulina* supplementation could prevent the memory impairment in senescence-accelerated mice through counteracting oxidative stress damages (24) which calls attention to the possible beneficial effect of *Spirulina* in mitigating memory and metabolic impairment induced by testosterone deficiency in castrated group.

The present study was assigned to analyse the effects of testosterone replacement with or without *Spirulina* combination on castration-induced metabolic, oxidative stress and cognitive alterations in adult male Wistar rats.

Materials and methods

Animals

Male Wistar rats weighing 155-250 gr at the beginning of the experiment, purchased from Pasteur Institute, Tunisia, were housed in separate cages under controlled conditions of temperature (25°C) and a 12:12 light/dark cycle. All animals were provided with water and food ad libitum. All rats were acclimatized 10 days prior to the beginning of the experiment. Animals were cared for in compliance with the *Institutional Ethics Committee* code of practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the *Ethics Committee of Faculty of Sciences, Bizerta, Tunisia*.

Castration surgery

The castration surgery was performed under ether anaesthesia. All rats were bilaterally castrated, each testis was excised through a small incision at the posterior end of the scrotum and then ligated. The testis was exposed by performing a transverse resection on both scrota in the supine position, and the spermatic cord and blood vessels were ligated and resected (25).

Experimental design

Ten days after surgery, rats were randomly assigned in 4 groups, five animals per group, and treated for 20 consecutive days as follows: Group 1 (Control group): non-castrated rats received distilled water orally (10 ml/kg). Group 2: *Castrated group* (CT) was given distilled water orally after castration (10 ml/kg). Group 3: *Testosterone treated group* (TT) castrated rats received 5 mg/kg of testosterone (*Sigma-Aldrich,Co, St Louis, MO, USA*) dissolved in corn oil by subcutaneous injection (26). Group 4: *Spirulina* co-treated group (SP) castrated rats received 5 mg/kg of testosterone dissolved in oil by subcutaneous injection followed by 10 ml/kg orally of *Spirulina* (1000 mg/kg) (*BioAlgues Tunisia*). During the experiment period, all rats were monitored daily for body weight. Behavior tests were performed at the end of the treatments.

Biochemical analyses

Rats were sacrificed by decapitation under slight ether anesthesia. Blood was collected in EDTA tubes and centrifuged at 4°C at 4000 rpm for 15 minutes. Plasma was recuperated and stored at -25°C for further biochemical determinations. Organs, brain, prostate, and seminal vesicles were immediately dissected out, washed in saline solution, weighed, and stored for further oxidative stress measurement. Cholesterol, triglycerides, *alkaline phosphatase* (PAL), *aspartate aminotransferase* (AST) and *alanine aminotransferase* (ALT) were determined using commercial analysis kits (*BioMaghreb, Tunisia*) according to the manufacturer's instructions.

Oxidative stress measurement

Tissue was homogenized in *Tris-buffered saline* (TBS). The homogenate was centrifuged at 4°C at 9000 rpm for 10 minutes and supernatants were collected. Protein level was estimated by *Bradford* method (27). *Superoxide dismutase* (SOD) and *catalase* (CAT) activities were measured in tissue homogenates according to *Misra and Fridovich* (28) and *Aebi* methods (29) respectively. Lipid peroxidation was assessed by *measuring the malondialdehyde* (MDA) level according to the *Draper and Hadley* method (30). *Hydrogen peroxide* (H_2O_2) level was measured according to *Jabri et al.* (31).

Behavioural testing

Animals were habituated to the arena the day following the 20 days of treatment and then submitted to the *object location test* (OLM) (32) and the *novel object recognition test* (NOR) 24 hours later (33). Both behavioural tests were performed between 08:00 am and 03:00 pm (Figure 1).

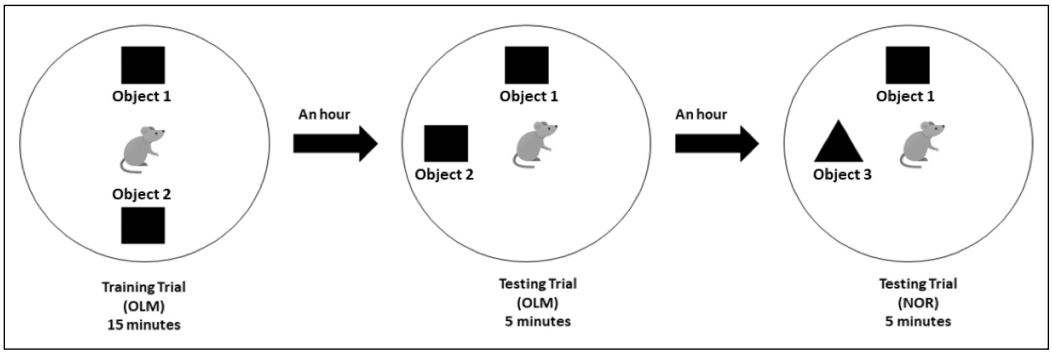

Figure 1. *Object location (OLM) and Novel object recognition (NOR) tests.*

Assessment of spatial memory using object location memory test (OLM)

The arena was a metal circular area with a 50 cm wall and divided into 1 central and 6 peripheral parts of equal surface. Testing consisted of one habituation day and one OLM testing day. On both days, testing was performed between 8:00 am and 04:00 pm and rats were brought to the testing room 15 minutes prior to the start of the testing. During the habituation day, rats were free to explore the vacant arena for 15 minutes. On the OLM testing day, two trials were performed, training and testing trials. During the training trial, two identical objects were placed 5 cm away from the wall such that they are counterbalanced in the arena, each rat was placed then in the centre of the arena and allowed to explore it for 5 minutes. Rats were placed back into their cages following the training trial for a 1h interval between trials. During the testing trial, one of the objects was moved to a quadrant diagonal from the other object, each rat was then replaced in the arena and allowed to explore for 5 minutes. The apparatus was cleaned with 30% alcohol and dried prior to the start of each trial for every rat. Rat movements were tracked and recorded using the Debut video recorder program.

A rat is considered exploring an object when its nose was within 2 cm from the object. Touching and sniffing activities were counted as exploration, while sitting on the object was not. Rats who don't meet these criteria were excluded from all analyses. To analyse cognitive performance, the following data were measured: the time spent exploring the object moved to a novel place (T1), the object remaining in the familiar place (T2), and the investigation time (%) i.e., which represents the percentage of the time spent in exploring the objects relative to the total time of the trial. Indexes measurements were also considered (34). *Discrimination index* (D1) represented the ability of the rat to distinguish the new object location from the familiar one; this index varies between -1 and +1 with a positive value indicating more preference for the displaced object. D1 is calculated as follows D1 = (T1-T2)/(T1+T2). *Recognition index* (R1) represented the percentage of time spent exploring the displaced object relative to the total exploration time and was calculated as follows: R1=T1/(T1+T2) ×100.

Assessment of spatial memory using novel object recognition memory test (NOR)

An hour after the OLM last testing trial, the familiar object was replaced with a novel object. Rats were placed in the centre of the arena and allowed to explore it for 5 minutes comprising one old object that was used in the last trial of the OLM test and one novel object. The arena was cleaned with 30% alcohol and air-dried prior to the commencement of each trial for every rat. Rat movements were tracked and recorded using the Debut video recorder program. To analyse cognitive performance, the following data were collected: time spent in exploring the novel object(t1), the familiar object(t2), and the investigation time (%), which represents the percentage of the time spent in exploring the objects relative to the total time of the trial. The indexes that were considered were: the *discrimination index* (D1) representing the ability of the rat to distinguish the novel object from the familiar one (this index varies between -1 and +1 with a positive value indicating more preference for the novel object); the *recognition index* (R1) representing the percentage of time spent with the novel object relative to the total exploration time. The indexes were calculated respectively as follows D1 = (t1-t2) /(t1+t2); R1=t1/(t1+t2) ×100 (34).

Statistical analyses

Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison. Data were expressed as mean ± standard error of the mean. A value of $p < 0.05$ was considered statistically significant. Data were analysed using GraphPad Prism 5 software.

RESULTS

Serum biochemical analyses

As shown in Table 1, castrated rats expressed statistically increased plasma levels of ALT, AST, PAL, cholesterol, and triglycerides compared to control group. In contrast, *testosterone replacement* (TT) alone or in combination with and *Spirulina* (SP) restored these parameters to normal levels.

Evaluation of antioxidant enzyme activities

Data showed that castration increased significantly SOD and CAT activities in the prostate, seminal vesicles, and the brain in comparison with the control group (Table 2). Importantly, testosterone replacement significantly ameliorated the abnormal levels of the antioxidant enzymes in the three tissues compared to control levels. However, cotreatment with *Spirulina* did not significantly improve these effects in SP group.

Evaluation of hydrogen peroxide (H_2O_2) and lipid peroxidation levels

Figures 2,3 and 4 showed that MDA levels respectively in prostate, seminal vesicles, and brain, were significantly higher in castrated group in comparison with control group. These increases were associated with a significant increase in H_2O_2 levels as compared with control group. A significant and identical decrease in MDA and H_2O_2 tissue contents was noticed in TT and SP groups as compared to castrated rats. Indeed, there were no remarkable changes in these oxidative stress parameters between control, TT and SP groups.

Table 1.

Biochemical parameters in control, castrated, testosterone and Spirulina treated rats.

Parameters	Control	CT	TT	SP
ALT (U/L)	35.35 ± 3.39	88.90 ± 7.5 *	46.81 ± 6.62 #	41.85 ± 5.28 #
AST (U/L)	17.50 ± 0.95	32.38 ± 3.88 *	23;98 ± 2.66	24.33 ± 3.11
PAL (U/L)	18.70 ± 1.92	56.93 ± 7.39 *	30.02 ± 3.45 #	20.43 ± 1.17 #
Cholesterol (g/l)	1.09 ± 0.12	1.97 ± 0.13 *	1.29 ± 0.07 #	1.06 ± 0.11 #
Triglycerides (g/l)	1.25 ± 0.14	3.10 ± 0.14 *	1.78 ± 0.09 #	1.75 ± 0.15 #

Values are expressed as mean ± SEM.

CT: Castrated group; TT: Testosterone treated group; SP: Testosterone and Spirulina co-treated group.

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PAL: Alkalin phosphatase.

** $p < 0.05$ as compared to control group. # $p < 0.05$ as compared to CT group.*

Table 2.
Effect of testosterone replacement in combination or not with Spirulina on antioxidant enzymes activities in prostate, seminal vesicles, and brain tissues in castrated rats.

	Prostate		Seminal vesicles		Brain	
	SOD (U/mg proteins)	CAT (umol/min/mg protein)	SOD (U/mg proteins)	CAT (umol/min/mg proteins)	SOD (U/mg proteins)	CAT (umol/min/mg proteins)
C	6.73 ± 1.32	74.02 ± 8.65	13.28 ± 1.59	93.65 ± 10.56	12.67 ± 1.82	73.90 ± 4.1
CT	13.4 ± 1.45 *	165 ± 15.13 *	40.72 ± 4.26 *	199.4 ± 23.83 *	54.57 ± 5.67 *	140.8 ± 10.89 *
TT	7.45 ± 1.58	130.8 ± 3.35 *	30.49 ± 1.65 *	137.7 ± 18.88	22.28 ± 0.99 #	97.63 ± 3.23 #
SP	11.68 ± 1.83	109.2 ± 6.18 #	23.92 ± 1.83 #	124 ± 19.05 #	22.48 ± 1.41 #	96.82 ± 4.31 #

Values are expressed as mean ± SEM. C: Control; CT: Castrated group; TT: Testosterone treated group; SP: Testosterone and Spirulina co-treated group. SOD: Superoxide dismutase; CAT: Catalase.
** p < 0.05 as compared to control group. # p < 0.05 as compared to CT group.*

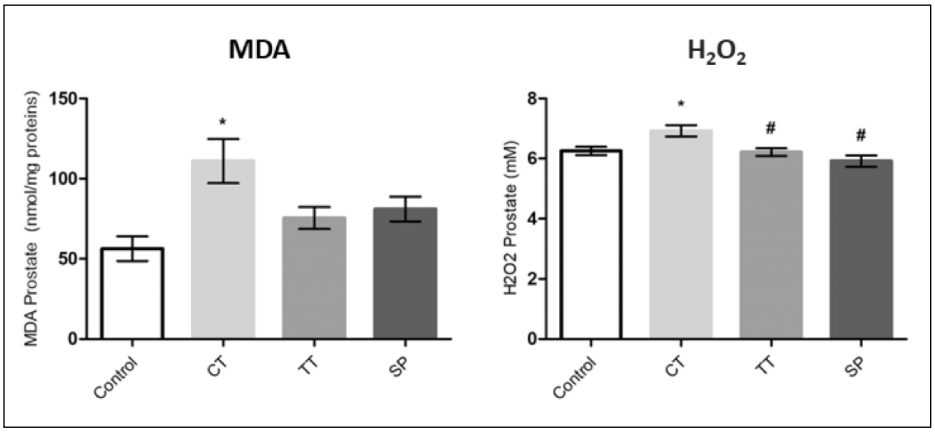

Figure 2.
Effect of testosterone replacement in combination or not with Spirulina on prostate MDA and H_2O_2 levels of castrated rats.

Values are expressed as mean ± SEM.
CT: Castrated group;
TT: Testosterone treated group;
SP: Testosterone and Spirulina co-treated group.
MDA: Malondialdehyde;
H_2O_2: Hydrogen peroxide;
** p < 0.05 compared to control group.*
 # p < 0.05 as compared to CT group.

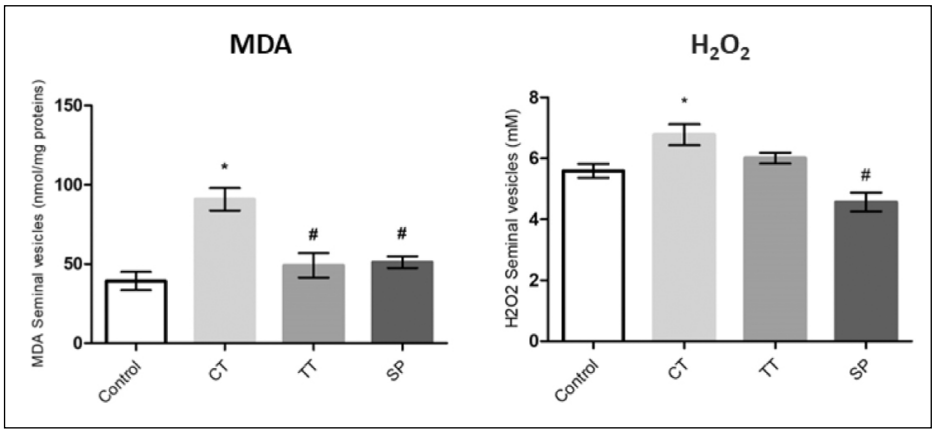

Figure 3.
Effect of testosterone replacement in combination or not with Spirulina on seminal vesicles MDA and H_2O_2 levels in castrated rats.

Values are expressed as mean ± SEM.
CT: Castrated group;
TT: Testosterone treated group;
SP: Testosterone and Spirulina co-treated group.
MDA: Malondialdehyde;
H_2O_2: Hydrogen peroxide;
** p < 0.05 compared to control group.*
 # p < 0.05 as compared to CT group.

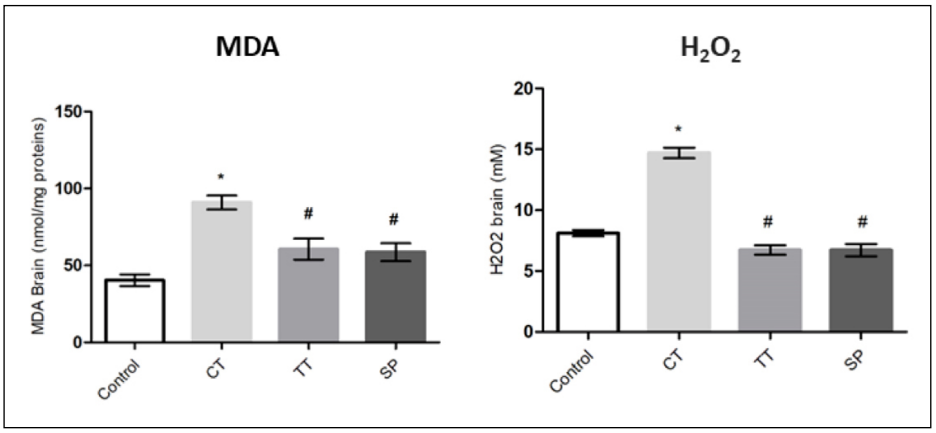

Figure 4.
Effect of testosterone replacement in combination or not with Spirulina on brain MDA and H_2O_2 levels in castrated rats

Values are expressed as mean ± SEM.
CT: Castrated group;
TT: Testosterone treated group;
SP: Testosterone and Spirulina co-treated group.
MDA: Malondialdehyde;
H_2O_2: Hydrogen peroxide;
** p < 0.05 compared to control group.*
 # p < 0.05 as compared to CT group.

Table 3.
Effect of testosterone replacement in combination or not with Spirulina on castrated rats' behaviour during OLM and NOR tests.

	Object location memory test				Novel object recognition test			
	Control	CT	TT	SP	Control	CT	TT	SP
Investigation time (%)	11 ± 0.83	5.61 ± 0.37 *	8.22 ± 0.97	8.67 ± 0.83	14.47 ± 1.19	3.94 ± 0.57 *	11.80 ± 0.44 #	11.94 ± 0.43 #
Recognition index	78.31 ± 0.43	38.50 ± 3.15 *	77.51 ± 0.46 #	77.85 ± 0.97 #	81.27 ± 1.65	34.35 ± 2.43 *	75.66 ± 1.37 #	77.85 ± 1.95 #
Discrimination index	0.56 ± 0.008	-0.23 ± 0.07 *	0.55 ± 0.009 #	0.56 ± 0.01 #	0.63 ± 0.03	-0.31 ± 0.04 *	0.51 ± 0.02 #	0.55 ± 0.03 #

Values are expressed as mean ± SEM. CT: Castrated group; TT: Testosterone treated group; SP: Testosterone and Spirulina co-treated group.
** $p < 0.05$ as compared to control group. # $p < 0.05$ as compared to CT group.*

Assessment of memory performances

In the OLM trial, castrated rats exhibited statistically decreased investigation time as compared to control group, while no significant difference in the investigation time was noticed between the control, TT and SP groups (Table 3). Compared with the other groups, castrated rats displayed less preference for the displaced object in the novel place since they spent equal time exploring the object remained in the familiar location and the displaced one. Moreover, the comparison of the discrimination index between control and castrated group revealed a significant lower index in CT group as compared to control, TT and SP treated groups.

In NOR tests, the investigation time was also significantly decreased in castrated group as compared to control group. This effect was totally reversed in testosterone and testosterone plus *Spirulina* treated groups. Furthermore, control, TT, and SP treated groups showed a clear tendency to explore the novel object rather than the familiar object. In fact, these rats showed a significantly higher recognition index in comparison to castrated group and spent more than 75% of their investigation time with the novel object. Castrated rats showed no preference for the novel object as they displayed a decreased recognition index as compared to control group.

The comparison of the discrimination index between castrated and control group showed that castrated rats had a significantly decreased index as compared to control group which showed a non-distinguish of the novel object. However, treated rats with testosterone alone or in combination with *Spirulina* displayed a comparable discrimination index to the control group and a significant increase in the discrimination index value as compared to the castrated group.

Discussion

Testosterone is a key hormone that has been known for its major role in carbohydrates, lipids, and proteins metabolism (35, 36). Testosterone deficiency has been linked to an increase in body fat mass, impairment in glucose and lipid tolerance, as well as oxidative stress imbalance; all these factors can contribute to metabolic disorders (37). In this regard, we assessed castration effect on adult male rats and evaluated whether testosterone could mitigate physiological perturbations induced by testosterone deficiency. In addition, we investigated the possible potential of the filamentous cyanobacterium, *Spirulina platensis*, to enhance the androgen effect in castrated rats. Our results revealed that plasma levels of cholesterol and triglycerides were remarkably increased after castration in CT rat group in comparison with the control group. Our findings are in harmony with previous studies of testosterone deficiency effect on lipid profiles in aging male rats (38) and orchiectomized rats (39). Testosterone level appears to have complicated relationship with cholesterol metabolism regulation and its associated anomalies, in a matter of facts low testosterone levels is associated with pro atherogenic lipid profiles in men (2), particularly lower levels of the *high-density lipoprotein cholesterol* (HDL-C). As steroid hormones can bind and interact with specific DNA domains it has been suggested that testosterone is involved in the molecular metabolism of cholesterol within the liver through the upregulation of several genes namely the *hepatic lipase* (HL), the scavenger B1 receptor (SR-B1) (40), and the nuclear liver X receptor (LXR) (41). Both HL and SR-B1 mediate and facilitate the uptake of HDL into hepatocytes, thereby stimulate the cholesterol uptake and efflux. Studies demonstrated that increased activity of SR-B1 and HL were linked to cholesterol level lowering effect of testosterone administration (40). It was also suggested that testosterone is involved in the liver uptake of *low-density lipoprotein cholesterol* (LDL-C) through the modulation of the PCSK9-LDLR pathway, thus the clearance of LDL-C from circulation (42). Importantly, our study showed that plasma lipid profile perturbations were reversed by testosterone replacement either alone or in association with *Spirulina* in castrated rats. Our results are in line with previous studies which have demonstrated that testosterone replacement therapy (43) and *Spirulina platensis* supplementation (44) ameliorated serum cholesterol and triglycerides levels respectively in castrated or high fat diet-fed rats.

Liver enzymes such as transaminases (ALT, AST) and ALP are sensitive biomarkers widely used to assess liver injury (45). Thus, these intracellular proteins are released into the blood upon hepatocyte damage. Our results showed that in castrated rats, these enzymes greatly increased in plasma above normal value. However, supplementation with testosterone or testosterone plus *Spirulina* were effective in improving these liver damage biomarkers.

Oxidative stress is a major mechanism of tissue injury, it is induced by the imbalance between the production of the *oxygen reactive species* (ROS) and the antioxidant system (46). It is generally caused by lipid accumulation, and DNA damages and leads to the loss of organ functions. The occurrence of oxidative stress is linked to aging, aging-related diseases and diverse clinical conditions including diabetes and heart diseases (47, 48). Previously, it has been shown that low testosterone level

is correlated to an imbalance of the oxidative stress status. In the study of *Mancini et al.* (49), sixteen patients with hypogonadism were compared to ten healthy patients to investigate the role of testosterone in the oxidative stress mechanism showing that a lipid antioxidant enzyme *Coenzyme Q10* (CoQ10) was reduced in the hypogonadism condition and that the testosterone replacement therapy resulted in an increase in CoQ10 serum level (49). Furthermore, in vitro assays showed that low testosterone treatment could decrease lipid peroxidation and the ROS production in TM3 Leydig cells (50).

In the present study we revealed that castrated rats showed a remarkable increase in SOD and CAT activities in the prostate, seminal vesicles, and the brain in comparison with the control group. Whereas testosterone replacement result in a significant increase in the level of these antioxidant enzymes. However, cotreatment with *Spirulina* did not significantly improve these effects towards TT group. Testosterone deficiency also significantly increased both MDA and H_2O_2 levels in prostate, seminal vesicles, and brain, in comparison with control group. Interestingly, administration of testosterone either alone or in combination with *Spirulina* restored these changes induced by castration. Our results are consistent with previous studies that have showed that oral administration of *Spirulina* prevented repro-toxicity by mitigating lipid peroxidation in testis of furan-intoxicated rats (23) and counterbalancing the perturbation of antioxidant enzymes in cadmium-intoxicated mice (51). However, the fact that *Spirulina* cotreatment did not enhanced the positive effects of testosterone in castrated rats suggested that androgen actions might involve other mechanisms unrelated to oxidative stress.

The loss of bioavailable testosterone in male is associated with a dysfunction in androgen responsive tissues including the brain. A vast majority of human and animal studies demonstrated that testosterone deficiency causes a decline in cognitive performance (52) and affective behaviour in males (53). Some data reported conflicting results and supported a non-consistent effect of androgens (54). Studies on molecular mechanism of androgen action on the brain indicated that testosterone have a direct impact on glial cells thereby can modulate the myelinisation mechanism, synapse, and dendritic branching number as well as neuron growth (55). It has also been shown that even though gonadotropic neurons do not express androgen receptors, testosterone can modulate these neurons through a neuropeptide called kisspeptin which is not only expressed in the *hypothalamic-pituitary-gonadal axis* (HPG) but also in the limbic regions of the brain implicated in the emotional and cognitive behaviour (*Mills et al.*, 2018. However, the exact causality of the relationship between testosterone levels and brain functions is still not firmly established.

In our study we performed OLM and NOR cognitive tests to assess the spatial memory performance in rats. Our data demonstrated that castration caused less interest in exploring objects in both the OLM and NOR trials. Likewise, the discrimination index of CT group displayed a negative value which indicate the incapacity of castrated rats to distinguish the novel object and the novel location of the object. These findings are along with previous results of *Pintana et al.* (56), that showed a cognitive decline in rats with testosterone deprivation. Whereas rats who received testosterone replacement of 0.20 mg/kg spent significantly more time exploring objects than castrated group and showed a significantly greater preference for the displaced and the novel object in comparison to castrated rats. Coherently, testosterone replacement displayed a positive discrimination index and a higher recognition index in comparison to CT group. Our findings contradicted the results of *Borbélyovà et al.* (57) who reported no effect of low testosterone concentration neither acute testosterone treatment on the exploratory behaviour and memory performance assessed by the Open Field test in aged and castrated male rats. This difference in results can be explained by several limitations namely the difference in the treatment period and the behavioural tests used.

Castrated rats who received *Spirulina* co-treatment also exhibited significantly more time exploring the objects in the arena, they displayed a higher discrimination and recognition index as compared to castrated group. In agreement with our results, previous data showed that *Spirulina* could improve memory deficit induced by scopolamine in rats through modulation of oxidative stress imbalance (58). Further, *Wang et al.* (59), reported that *Spirulina* could impart appreciable relief in L-methionine induced cognitive deficit in rats by counterbalancing the acetylcholinesterase activity and brain oxidative enzymes activity.

Conclusions

This study demonstrated that testosterone deficiency led to an increase in plasma cholesterol and triglycerides as well as AST, ALT, and PAL levels associated with unbalanced oxidative status and cognitive impairment. Testosterone replacement could counteract these castration-induced changes. However, there were no notable effects when testosterone was combined with *Spirulina* in castrated rats. Further investigations are needed to understand the underlying mechanisms of testosterone deficiency-induced alterations.

Acknowledgements

Authors express their sincere thanks to *Professor Youssef Krichen, CEO of Bio Algae Tunisia company* for the free supply of phycocyanin and *Dr Sihem Ben Hassine* for her technical assistance.

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Conflict of interest: The authors declare no potential conflict of interest.