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Mucuna pruriens seeds extract boosts growth, immunity, testicular histology, and expression of immune-related genes of mono-sex Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Nutraceuticals have received increased attention in sustainable aquaculture. Consequently, the present study aimed to evaluate the dietary effects of *Mucuna pruriens* (MP) seed extract on growth performance, immune status, hepatic function, biochemical profiles, gonadal histology, and expression of immune-related genes in mono-sex Nile tilapia (*Oreochromis niloticus*). Fish were allocated into four groups and received MP at rates of 0 (control), 2, 4, and 6 g/kg diet, respectively, for 90 days. The results revealed that MP significantly ($P < 0.05$) modulated growth performance (specific growth rate, final length, and length gain rate, body mass gain, and feed conversion ratio), lysozyme activity, and liver enzymes (AST, ALT). However, a non-significant effect on nitric oxide (NO) or immunoglobulin M (IgM) levels was detected, whereas the dietary inclusion of MP had a hypoglycemic effect. In terms of plasma globulin, albumin, globulin/albumin ratio, and cortisol, the MP receiving groups showed insignificant difference ($P < 0.05$) when compared to controls, except for the 2 g MP-supplemented group. The lower inclusion concentration of MP (2 g/kg diet) demonstrated the best result ($P < 0.05$) for gonadosomatic index (GSI) and plasma testosterone level that was consistent with the histological findings reflecting an improvement in the testicular development compared with the control group. Expressions of complement component (C5) and interleukin 1-β (*IL-1β*) genes were significantly up-regulated in MP receiving groups. In conclusion, *M. pruriens* can be used as a safe natural economic feed additive and a low inclusion level of 2 g/kg diet is recommended to improve growth, enhance immunity, maintain liver functioning, improve testicular development, and to modulate immune-related genes in the mono-sex *O. niloticus*.

1. Introduction

Aquaculture is a profitable and rapidly growing industry that contributes to human food security via high-quality meat production and aims to reduce ecological pressure on natural resources [1,2]. Nile tilapia is an ideal fish for aquaculture due to its fast growth, great tolerance to adverse water conditions and diseases, and decent consumer acceptance [3]. A wide range of natural products is currently used

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Table 1
Composition and approximate analysis of basal diet.

Item	g/1000g diet
Fish meal[a]	300
Yellow corn	180
Soybean	360
Wheat bran	50
Vegetable oil	40
Gluten	45
Bi-calcium phosphate	15
NaCl	4
Vitamin and mineral premixes[b]	6
Ingredient	**Approximate analysis (%)**
Crude protein (CP)	38.29
Ether extract (EE)	6.32
Crude fiber (CF)	8.37
Ash	9.02
Digestible energy (Kcal/kg)	3312

[a] Fishmeal (INTRACO, Belgium).
[b] Egavet premix: each 3 kg contain: vitamin A, 11 million IU; vitamin D, 3.5 million IU; vitamin E, 10,000 mg; vitamin K3, 900 mg; vitamin B1, 1100 mg; vitamin B2, 4500 mg; vitamin B6, 2000 mg; niacin, 30,000 mg; biotin, 50 mg; folic acid, 1000 mg; pantothenic acid, 10,000 mg; Mn, 60,000 mg; Zn, 50,000 mg; Fe, 30,000 mg; Cu, 5000 mg; Se, 90 mg; Co, 110 mg; Mn, 250,000 mg.

Table 2
Primers sequences for SYBR green rt-PCR.

Target gene	Primers sequences	Reference
EF-1α	CCTTCAACGCTCAGGTCATC TGTGGGCAGTGTGGCAATC	**Gröner *et al.*, 2015**
IL1B	GCTGGAGAGTGCTGTGGAAGAACATATAG CCTGGAGCATCATGGCGTG	**Castro *et al.*, 2011**
C5	GGACCCGGACCATACAACAG GGGGTTTTGCAGAGATGGGA	**El-abd *et al.*, 2021**

EF-1α: Elongation Factor-1α*; IL1B:* interleukin 1*, beta* (*IL-1β*); and Complement Component (*C5*).

in aquaculture to promote growth and appetite, enhance immunity, increase disease tolerance without any substantial adverse effects [4–10]. Tropical legume *Mucuna pruriens* (MP) is known worldwide as velvet bean and is widespread in Africa and Asia [11]. In addition, it is included in more than 200 drug formulations. The dried beans of MP contain 20–35g crude protein, which is the same as soybean and rice bean, and thus can be used as a cheap alternative source of protein in aquaculture (Sridhar & Bhat, 2009). All parts of the MP own medicinal properties [12]. It has immunostimulatory, aphrodisiac, anthelmintic, and anti-inflammatory properties [13]. *Mucuna pruriens* is economic and available all year round and contains (L-Dopa) 3, 4-dihydroxy-L-phenylalanine, phytochemicals as alkaloids, glycosides, saponins due to its richness in various biological activities [14]. Also, it has been characterized by strong in vitro anti-oxidant activity, and it has been proved that the methanol extracts of MP is a significant source of anti-microbial agents and natural antioxidants [15]. Previous studies addressed the efficacy of mucuna on the growth performance, biochemical profile, and immunity of different fish species including *O. niloticus, Clarias gariepinus* and *Labeo rohita* [13,16,17].

There is a paucity of information available regarding the dietary effect of *M. pruriens* on mono-sex Nile tilapia (*Oreochromis niloticus*) immune, biochemical and related genes expression. Hence, the current study aimed to assess the impacts of *M. pruriens* on growth performance, immune responses, biochemical parameters, testicular function, and gene expression of *O. niloticus*.

2. Materials and methods

2.1. Fish maintenance

Three hundred and sixty mono-sex *O. niloticus* fingerlings (10.32 ± 5.1 g, 6.1 ± 0.5 cm) were obtained from a private fish farm in Kafr Elsheikh Governorate, Egypt. The fish were transferred to the Department of Aquatic Animal Diseases and Management, Benha University, Egypt. The fish were kept in 750 L well-aerated fiberglass tanks for ten days of acclimatization. Water was partially replaced by 25% and the fish was fed a basal diet at the rate of 3% of their body mass. Physiochemical parameters of rearing water including temperature, dissolved oxygen, ammonia, and pH were monitored daily and recorded as 28 ± 2 °C, 6 ± 0.5 mg/L, 0.53 ± 0.07 mg/L, and 7 ± 0.2 respectively.

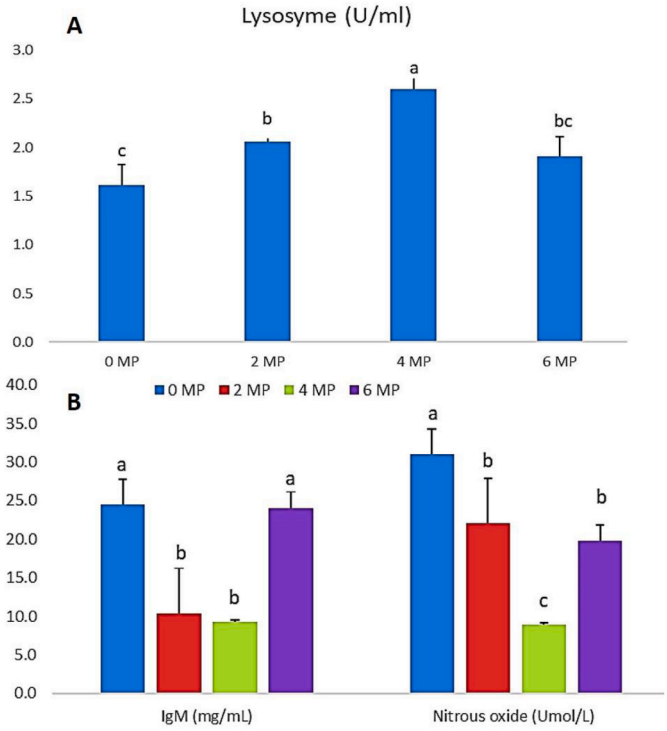

Fig. 1. Lysozyme (A); IgM and nitric oxide (B) activities in *O. niloticus* fed diets incorporated with 0, 2, 4 and 6 g/kg MP for 90 days. Data are presented as mean ± SE (n = 9). Values with different superscript letters are significantly different ($P < 0.05$).

Table 3
Growth parameters of *O. niloticus* fed diets incorporated with 0, 2, 4, and 6 g/kg MP for 90 days.

MP%/kg feed	Initial Wt. (g)	Final Wt. (g)	Initial length (cm)	Final length (cm)	BMG[a]	SGR (%)[a]	FCR[a]	LGR (%)[a]	HSI[a]
0	10.5 ± 0.5	24 ± 1.0^{c}	6.5 ± 0.5	11.5 ± 0.01^{b}	161.5 ± 0.03^{c}	1.5 ± 0.01^{b}	1.0 ± 0.03^{c}	71.1 ± 0.03^{b}	3.5 ± 0.03^{a}
2	10.5 ± 0.5	69.6 ± 0.8^{a}	6.5 ± 0.5	15.3 ± 0.05^{a}	563.0 ± 0.05^{a}	2.0 ± 0.03^{a}	0.3 ± 0.03^{a}	134.8 ± 0.05^{a}	3.3 ± 0.03^{a}
4	10.5 ± 0.5	48.7 ± 0.6^{b}	6.5 ± 0.5	14.5 ± 0.05^{a}	364.0 ± 0.02^{b}	1.9 ± 0.05^{a}	0.5 ± 0.00^{b}	122.6 ± 0.02^{a}	2.0 ± 0.00^{b}
6	10.5 ± 0.5	69.1 ± 0.6^{a}	6.5 ± 0.5	15.3 ± 0.05^{a}	548.0 ± 0.05^{a}	2.0 ± 0.05^{a}	0.3 ± 0.00^{a}	135.8 ± 0.05^{a}	2.2 ± 0.00^{b}

Values are expressed as mean value (n = 30) ± SE. Mean values with different superscript letters are significantly different ($P < 0.05$).
[a] BMG: Body Mass Gain, SGR: Specific growth rate, LGR: Length Gain Rate, FCR: Feed Conversion Ratio, and HIS: Hepato Somatic Index.

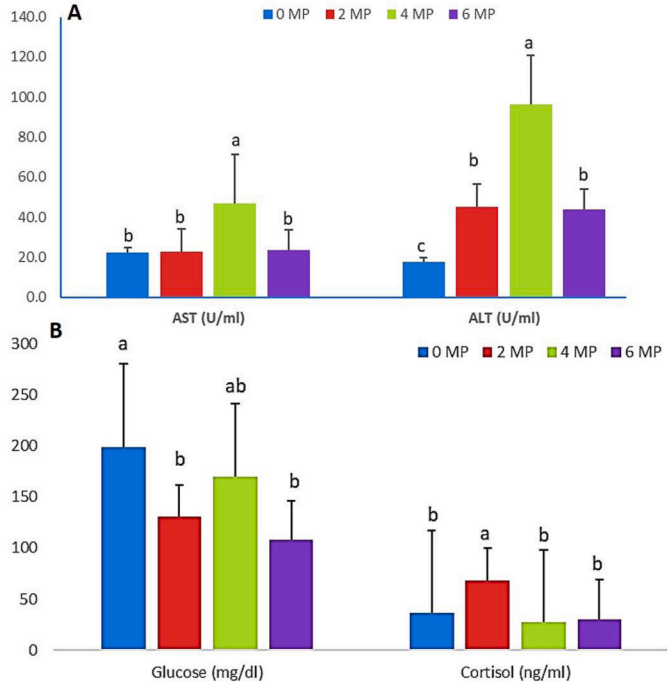

Fig. 2. Effect of 2, 4, and 6 g/kg MP supplemented diets on plasma ALT and AST (A), in addition to glucose and cortisol (B) in *O. niloticus*. Data are presented as mean ± SE (n = 9). The values with different superscript letters are significantly different ($P < 0.05$).

2.2. Diets

The formulation of basal diet and its chemical analysis was carried out following the guidelines of the [18].*Mucuna pruriens* (MP) seed extract in powder form was supplied from Bulk Supplements, USA, and incorporated into the basal diet at required concentrations including 2, 4, and 6 g/kg diet (Table 1). The diet ingredients were evenly mixed, ground to a fine powder, extruded by passing through a 5 mm mesh sieve, and stored at −20 °C until used.

2.3. Experimental design

Fish were divided into four groups in triplicates (30 fish/replicate), and they were fed experimental diets 0 (control), 2, 4, and 6 g MP/kg diet at the rate of 3% of their body weight twice per day for 90 days. Water parameters were maintained at appropriate levels as in the acclimatization period. All procedures were performed according to the approved guidelines for laboratory animal use by the Experimental Animal Use Committee, Benha University, Egypt.

2.4. Growth performance and somatic indices

Growth parameters for fish were assessed for 30 fish per group (10/replicate) including specific growth rate (SGR), feed conversion ratio (FCR), length gain rate (LGR) according to Ref. [19], hepatosomatic Index (GSI), and gonadosomatic index (GSI) using following formulas:

SGR (%g/day) = [(Ln final weight - Ln initial weight) / experimental period (days)] × 100

FCR = Feed intake/Weight gain;

LGR (g) = 100 × [Average terminal body length (cm) − Average initial body length (cm)]/Average initial body length (cm);

HSI (%) = Liver weight/Fish weight × 100

2.5. Blood and tissue sampling

Nine fish/group were sampled and anesthetized with 250 mg/L tricain methanesulfonate (MS222) (Syndel, British Columbia) and blood was drained using a 3 mL heparinized syringe following the procedures reported by Refs. [6,20,21] Plasma was separated by centrifugation at 3600×*g*, 4 °C for 10 min to evaluate aspartate aminotransferase (AST), and alanine transaminase (ALT), glucose, and cortisol concentrations, plasma globulin, albumin, lysozyme activity, IgM and testosterone. After blood collection, liver tissues were sampled in phosphate-buffered saline, pH 7.4 for measuring nitrous oxide activity and in RNAlater (Ambion, USA) for gene expression study. Samples were kept at −80 °C.

2.6. Immune response

Lysozyme detection kits (Sigma-Aldrich, USA) were used for assaying lysozyme activity spectrophotometrically at 450 nm following the manufacturer's (Sigma-Aldrich, USA) protocol according to the following formula: Lysozyme level (U/ml) = ((ΔA450/min assay – ΔA450/min blank) × (df))/(0.03 × 0.001),: df = dilution factor, 0.001 = ΔA450/the unit definition, and 0.03 = enzyme solution volume

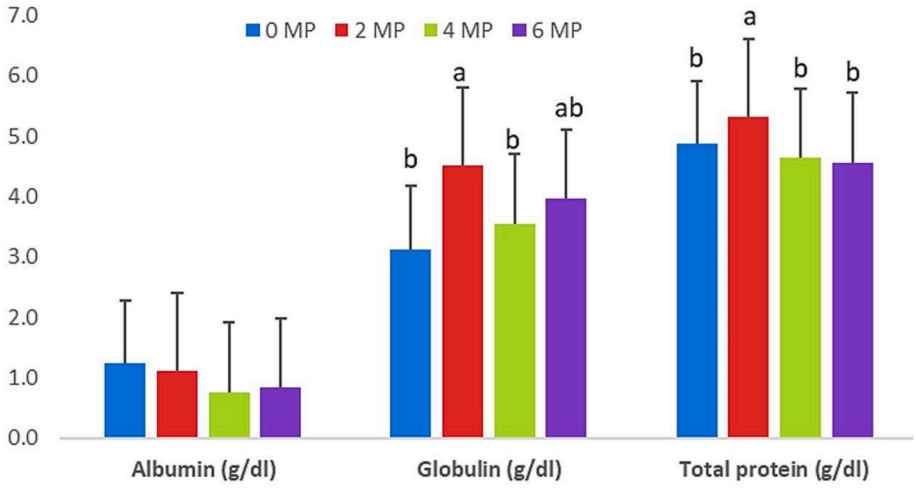

Fig. 3. Albumin, globulin and total protein in *O. niloticus* fed on diets incorporated with 0, 2, 4, and 6 g/kg MP for 90 days. Data are presented as mean ± SE (n = 9). The values with different superscript letters are significantly different ($P < 0.05$).

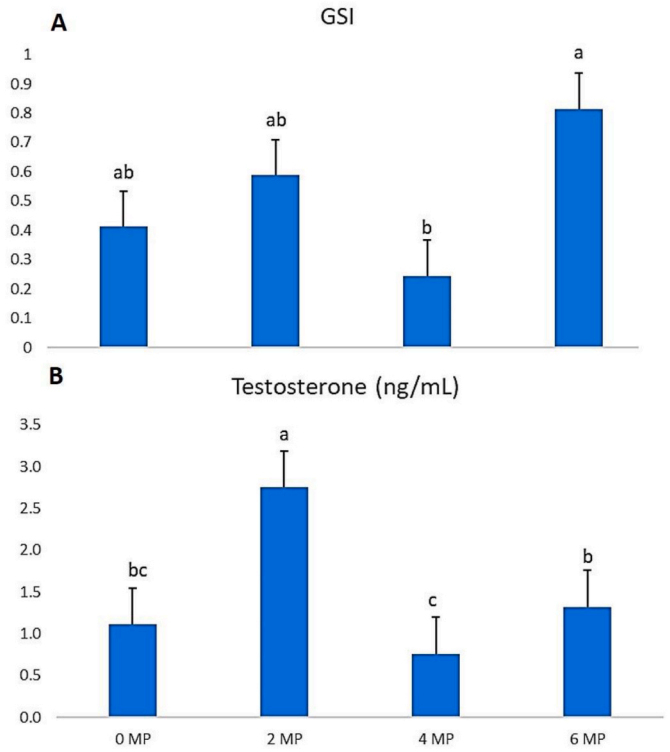

Fig. 4. GSI (A) and plasma testosterone level (B) in *O. niloticus* fed with 2, 4, and 6 g/kg MP incorporated diets. Data are presented as mean ± SE (n = 9). The values marked with superscript letters are markedly different ($P < 0.05$).

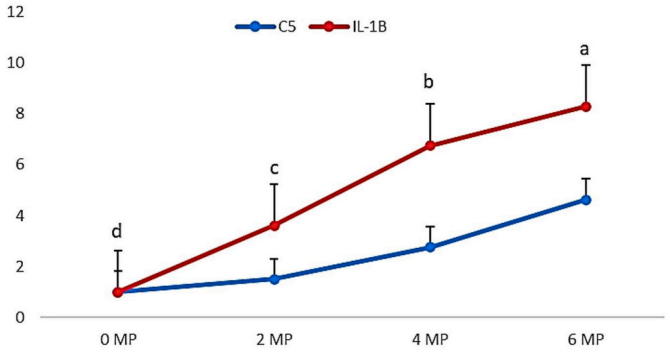

Fig. 5. Photomicrograph of testis at 30 days showing: 5A is the control group which observed with higher connective tissue (Ct) between seminiferous lobules (L), appearance of spermatogenic cyst as spermatid (St). 5B is 2 g/kg MP fed group showing testicular lobulation (L), spermatid cyst (St), primary spermatocyte (thin arrow) and secondary spermatocyte (thick arrow). 5C is 4 g/kg MP fed group demonstrating spermatozoa (Sz) in the lumen of testicular lobules. 5D is 6 g/kg MP fed fish showing spermatozoa (Sz) and degeneration and sloughing of some spermatogenic cells in some cysts (arrows). Scale bar 50 μm.

(milliliters); and the same procedures illustrated by Refs. [6,21,22]. Nitrous oxide was also assayed spectrophotometrically at 570 nm by mixing 85 μl Griess reagent with 85 μl sample for detecting nitrite oxidation correlated with NO amount (Sigma-Aldrich, USA), using the formula: NO (Umol/L) = A sample/A standard × 50 following [21,23]. A commercial immunoglobulin M (IgM) ELISA kit (CUSABIO, China) was used for assaying IgM (mg per dl) at 450 nm according to the company protocol.

2.7. Biochemical parameters

Glucose, cortisol concentrations; AST, and ALT activities were measured coloremetrically at 546 nm using commercial kits (BioMed Diagnostic commercial kits, Egypt) protocols. Plasma globulin and albumin were determined using a readymade chemicals kit (Spinreact, Spain) at 620 nm following the company's instructions by a chemistry analyzer (RA-50, Bayer).

2.8. Testicular indexes

Gonadosomatic index was measured according to Ref. [24] using the following formula:

$$\text{GSI (\%)} = \text{Weight of gonad / Weight of fish} \times 100$$

Testosterone was measured using RIDASCREEN® ELISA kits at 450 nm according to the manufacturer's instructions.

2.9. Testicular histological examination

To investigate testicular histology study, testis samples from nine fish/group were collected and fixed in Bouin's solution (Merck, USA) for 24 h, then dehydrated in an ascending alcohol series, cleared and paraffin wax embedded. 5 μm sections were stained with Hematoxylin and Eosin (H and E) and histologically examined using a digital imaging system (Leica, Germany) following [25].

2.10. Expression of complement component and interleukin 1-beta genes

Total RNA was isolated from liver samples (nine fish/group; 3 fish/replicate) using the Trizol method (1 ml Trizol/50 mg sample) following company's protocol (Invitrogen, USA). RNA quantity was assessed using a Nano-Drop spectrophotometer and a 260/280 nm absorption ratio of 1.80: 2.00 confirmed purity. Reverse transcription was performed using a high-capacity cDNA reverse transcription kit (Invitrogen, USA) following the instructions and procedures in our previous work [26]. The rt-PCR reaction was performed in Stratagene MX3005P, primers (Metabion, Germany) are listed in Table 2 and RT-PCR run included 1 cycle (94°C-15 min), and 40 cycles (94°C-5 min, 62 °C −30s and 72°C-30s). The relative expression of the *C5* and *IL-1β* genes was calculated using the $2^{-\Delta\Delta Ct}$ method [27].

2.11. Statistical analysis

IBM SPSS Statistics, Version 22 (IBM Corp, Armonk, NY, USA) was used for data analysis. Statistical assumptions including normality of data distribution and homogeneity of variances were ascertained by Shapiro-Wilk's and Levene's tests, respectively, before conducting one-way ANOVA. Duncan's test was used for multiple comparisons. Statistical significance level was set at $P < 0.05$ and the results are represented as mean ± standard (SD).

3. Results

3.1. Growth performance

Growth parameters are depicted in Table 3. Dietary supplementation with all MP concentrations (2, 4 and 6 g/kg diet) showed a marked improvement in growth performance compared to the control group where MP significantly increased the specific growth rate, final length, and length gain rate ($P < 0.05$). In addition, dietary inclusion of 2 and 6 g MP gave the most a substantial improvement in the final weight, body mass gain, and feed conversion ratio. While the value of HSI showed a significant difference only at the concentration of 2 g MP/kg diet compared to controls ($P < 0.05$).

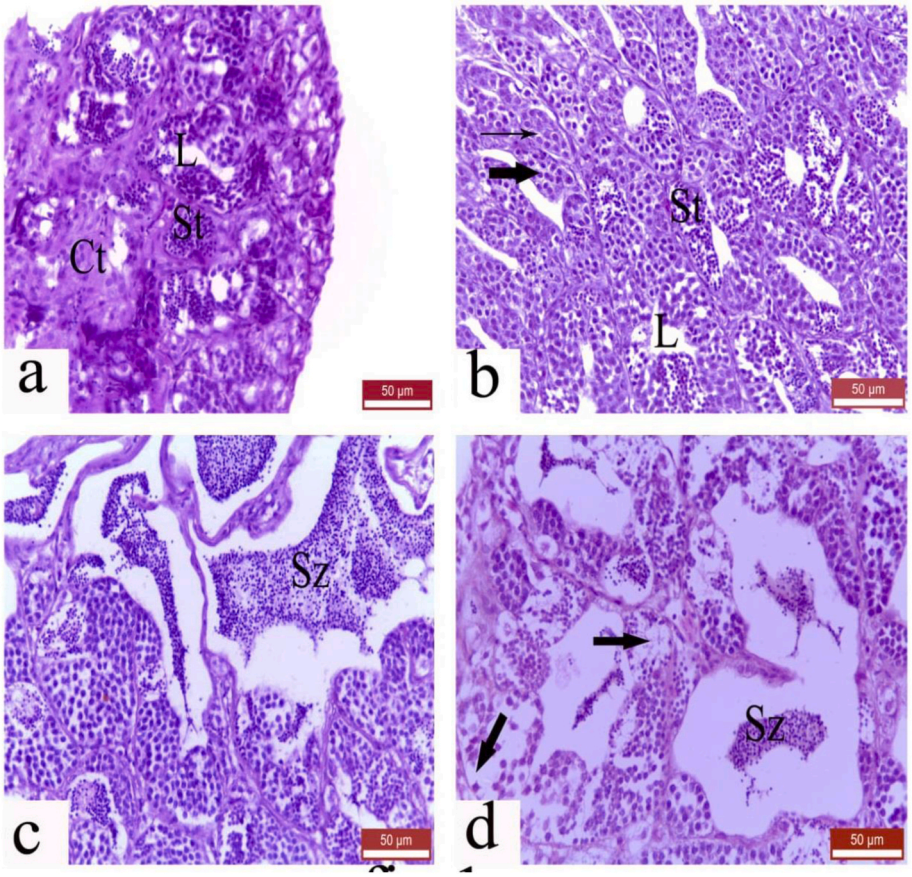

Fig. 6. Photomicrograph of testis at 60 days showing: 6A is control group which is observed by appearance of most of spermatogenic cysts, primary spermatocyte cyst (arrow), secondary spermatocyte (St) spermatid (St) and spermatozoa in lumen of lobules (Sz). 6B is 2 g/kg MP group demonstrating a high amount of spermatozoa (Sz) in lumen. 6C is 4 g/kg MP fed fish showing low spermatozoa (Sz) in the lumen of testicular lobules and some cysts were degenerated (arrows). 6D is 6 g/kg MP fed group showing that most of spermatogenic cysts are degenerated (Dc) and congestion in interlobular connective tissue (arrow). Scale bar 50 μm.

3.2. Immune response

The dietary incorporation of MP revealed significant improvement ($P < 0.05$) in lysozyme activity with the highest value recorded in the group receiving the 4 g MP/kg diet. However, there was a marked decrease in NO or IgM readings, except for the 6 g MP/kg group that maintained the same NO level as in the control group without any reduction (Fig. 1 A and B).

3.3. Biochemical assays

The AST activity showed a significant difference ($P < 0.05$) in the groups fed with MP-incorporated diets (2 and 6g/kg), except for the group supplemented with a 4 g MP/kg diet, which demonstrated a significant increase ($P < 0.05$) in the AST level than the control group. While the ALT levels were higher in MP groups with the highest value for the 4 g MP/kg group compared to the control group (Fig. 2 A).

Plasma glucose levels were significantly ($P < 0.05$) decreased in MP groups with the most substantial decrease in the 4g MP/kg groups; however, cortisol level did not demonstrate any change in the MP-treated groups (Fig. 2 B).

Plasma albumin demonstrated insignificant differences in the MP groups compared to the control. Nonetheless, globulin level was significantly ($P < 0.05$) higher in groups supplied with 2 and 6 g MP/kg diets compared to the control group. Regarding the value of total protein, it was significantly elevated ($P < 0.05$) in the supplemented group with a 2 g MP/kg diet in comparison to controls. However, the groups receiving 4 and 6g MP/kg diet did not reveal a significant ($P < 0.05$) difference than the control group (Fig. 3).

3.4. Testicular indexes

The highest ($P < 0.05$) male GSI value was recorded in the group receiving 6 g MP/kg diet, followed by 2 g MP/kg supplemented group, and finally the control group. The lowest value ($P < 0.05$) was recorded for the group supplied with a 4 g MP/kg diet (Fig. 4 A). Groups incorporated with 2 and 6 g MP/kg diets also showed a substantial ($P < 0.05$) increase in testosterone levels compared to the control group (Fig. 4 B).

3.5. Testicular histology

After 30 days, the control group demonstrated a significant amount of connective tissue and lobulation, most of the spermatogenic cells were present at the periphery of the testicular lobules and some spermatogenic cysts appeared (Fig. 5 A). In the 2 g MP/kg supplemented group, the amount of connective tissue decreased, testicular lobulation was more obvious, and different developmental stages of spermatogenic cells were arranged in form of cysts, each cyst contained one stage which could be a primary or secondary spermatocyte and spermatid (Fig. 5 B). In the case of the group provided with a 4 g MP/kg diet, more development of testicular tissue was detected. In addition, spermatozoa appeared in the lumen of lobules (Fig. 5 C). While the supplemented group with a 6 g MP/kg diet, it exhibited the appearance of spermatozoa in the lumen of lobules; and some primary, secondary spermatocyte, and degeneration in the spermatid cysts (Fig. 5 D).

At the end of 60 days, the control group showed less amount of interlobular connective tissue, testicular lobulation was clear, most spermatogenic cysts were present, and some lobules had spermatozoa in their lumen (Fig. 6 A). A combined diet with 2 g MP showed greater testicular development and the number of spermatozoa in the lumen increased (Fig. 6 B). On the contrary, in the supplemented group with 4 g MP, the number of spermatozoa in the testicular lumen was decreased

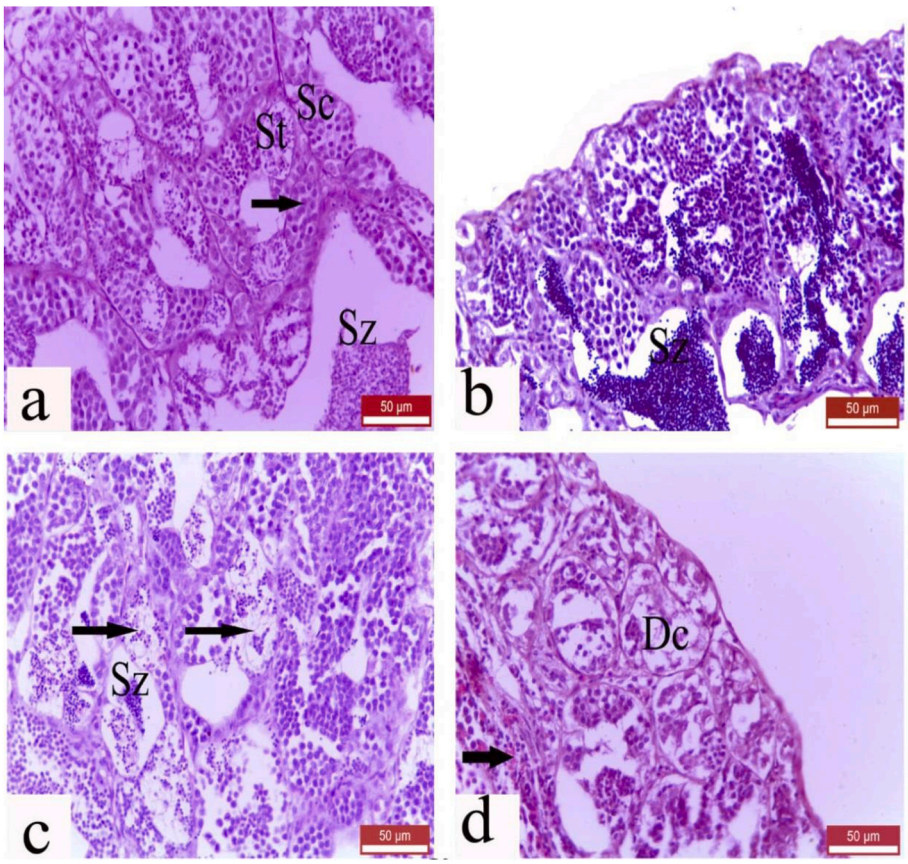

Fig. 7. Photomicrograph of testis at 90 days showing: 7A is he control group where there were scattered spermatogonia (arrow), primary spermatocyte cyst (Ps), secondary spermatocyte cyst (Sc) and spermatid (St). 7B is 2 g/kg MP fed group showing spermatozoa in lumen of seminiferous lobules (Sz). 7C is 4 g/kg MP fed group showing vacuolation of spermatogenic cells (arrow), 7D is 6 g/kg MP fed fish showing increased amount of degeneration (Dc) and more vacuolated cells were present (arrow). Scale bar 50 μm.

and some spermatogenic cysts were degenerated (Fig. 6 C). The cleared degenerative changes were more pronounced in the 6 g MP/kg group showing exfoliation of spermatogenic cells from the cyst, atrophy of some spermatogenic cysts rather than congestion of interlobular connective tissue (Fig. 6 D).

At 90 days, the control fish showed more development of testicular tissue, cysts of primary, secondary spermatocytes, and spermatids were more predominant, spermatogonia were scattered through the lobules and spermatozoa were present in the lumen of testicular lobules (Fig. 7 A). The group supplemented with 2 g MP/kg diet showed an elevated amount of spermatid cysts, and an increased number of spermatozoa in the lumen (Fig. 7 B). In the case of the group given a 4 g MP/kg diet, it revealed a degree of degeneration, and some cells were vacuolated (Fig. 7 C). Furthermore, 6 g MP/kg group showed increased degeneration and more vacuolated cells were present (Fig. 7 D).

3.6. Expression of C5 and IL-1β genes

The expression pattern of both *C5* and *IL-1β* genes revealed significant up-regulation ($P < 0.05$) for the MP incorporated groups in a pattern of 2 < 4<6 g MP/kg diet (Fig. 8).

4. Discussion

In aquaculture, it is more economic and beneficial to use traditional medicine in order to overcome the disadvantages of chemical therapies [28–34]. The present investigation is the first to assess the impact of a nutraceutical, *M. pruriens*-enriched diet, on the growth, immune, liver function, biochemical responses, testicular development, and associated-gene expression of mono-sex Nile tilapia (*O. niloticus*). Growth efficacy on a nutraceutical supplement is assessed by considering the growth performance parameters which indicate fish health and the physiological status [35]. In the current investigation, the groups fed various concentrations of *M. pruriens*-enriched diets showed significantly enhanced growth performance. Among the enriched diets, dietary intervention with 2 g MP demonstrated the best growth performance, followed by 6 g MP. Likewise [13], reported that the *M. pruriens* meal substantially enhanced growth performance, and metabolic activity, in *Labeo rohita* at low concentration. Also [17], reported that processed mucuna seed meal improved growth efficacy when replaced the fishmeal by approximately 10% of *Clarias gariepinus* diets. These effects can be attributed to the enhancement of palatability and digestibility of nutrients like proteins and carbohydrates found in MP which in turn enhance growth rate as recorded by Ref. [36].

Nitric oxide is a key signaling molecule responsible for a wide range of cellular immunity [37], lysozymes and IgM are the primary defense molecules that mediate the immune response in freshwater fish [38,39]. Herein, the dietary incorporation with MP demonstrated a significant improvement ($P < 0.05$) in lysozyme activity. This could be attributed to the anti-lipid peroxidation effect, which contributes to the removal of hydroxyl and superoxide radicals as reported by Ref. [40] or due to the presence of anti-nutrients as protease inhibitors [36]. Also, the enhanced immune response could be returned to the richness of MP in alkaloids like prurienine, prurienidine, and prurieninine as reported by Ref. [41]. On the same manner [13,42], detected the ability of *M. pruriens* to enhance cellular immunity through elevating lysozyme activity in *Labeo rohita* and common carp. The liver is the fundamental fish organ responsible for binding, storage, and detoxification processes in freshwater fish [4,26]. In the current context, there was a non-significant difference in the AST and ALT activities in groups fed MP-rich diets. Modulation in hepatic enzymes was more pronounced in the diet supplemented with 2 g MP which could be attributed to the ability of MP-incorporated diets to protect liver tissues from oxidative damage and keep AST and ALT levels [14,43]. Likewise [13], reported

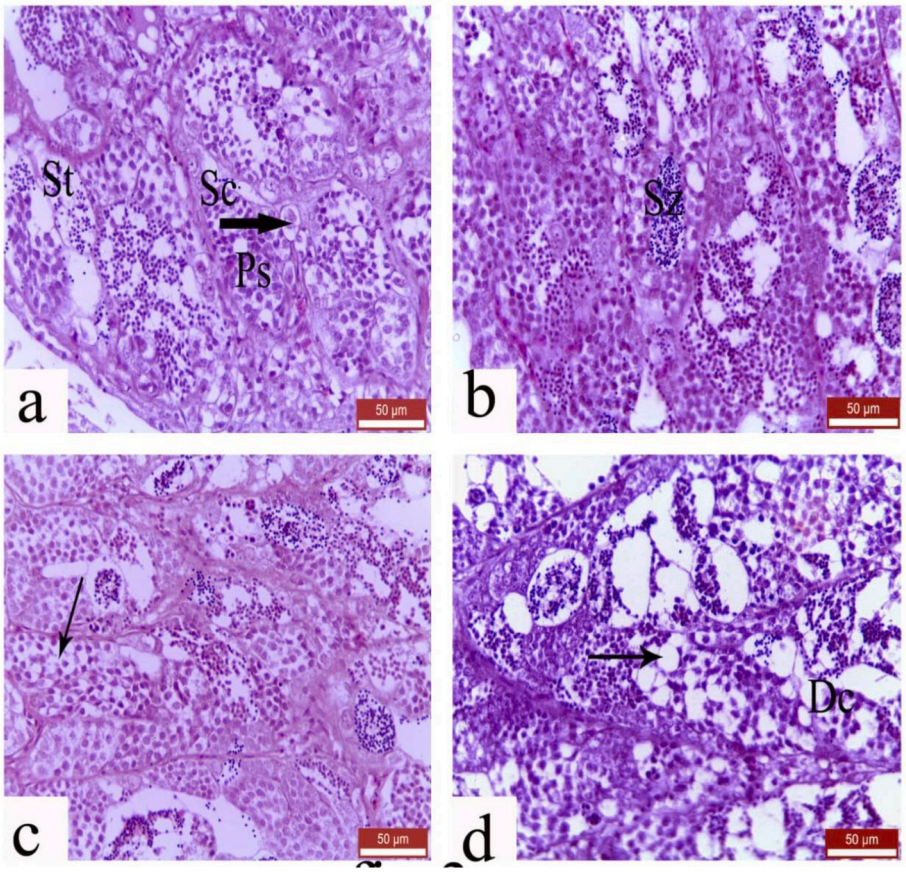

Fig. 8. Expression levels of *C5* and *IL-1β* genes in *O. niloticus* fed with 2, 4, and 6 g/kg MP. Data are presented as mean ± SE (n = 9). The values with different superscript letters are substantially different ($P < 0.05$).

varied levels of AST and ALT activities of *Labeo rohita* fed on MP-enriched diets. Plasma glucose levels were significantly ($P < 0.05$) decreased in the MP groups, which is compatible with [13,14,44] who reported the potential of *M. pruriens* to decrease glucose levels indicating the synergistic effect *M. pruriens* seed extract in decreasing blood sugar levels [45] through releasing glucagon and glucose convert to pyruvate by glycolysis [14]. Our findings were supported by Ref. [46] who reported that the seeds of MP lessened plasma glucose level at a dose of 500 mg/kg. Regarding plasma proteins assessment, MP dietary inclusion did not reveal significant differences in plasma proteins, and cortisol except for a concentration of 2 g MP which could indicate the protective properties of a low concentration of MP that decreases and diminishes with the high concentrations. It is opined that the modulation in the cortisol level due to the potent antioxidant activity of MP. [47], confirmed this finding and reported that MP had a potential antioxidant activity through the *in vivo* model of lipid peroxidation that induced by stress. Likewise [13], reported modulated serum glucose level in *L. rohita* fed an MP supplement.

Results of testicular indexes like GSI and testosterone levels reveal the best significant increase for the dietary incorporation with 2 g MP due to action of MP on the hypothalamus-pituitary-gonadal axis, L-Dopa content, and the anabolic effect to increase testosterone secretion as recently recorded by Refs. [14,48]. The results pattern showed a diminished activity at higher dosages.

Testicular histology reading is correlated with previous testicular indexes, revealing the best reading for the group enriched with 2 g MP that induced a well-pronounced development of the testicular histological structure that continued to progress over time throughout the entire experimental period compared to the control group. With increasing concentration and time, degenerative changes started to appear that may be related to some inhibitory substances that were concentrated with higher doses that would be accompanied by reverse effects of L-Dopa at higher concentrations. Similarly [49], reported a negative impact of melastoma leaf extract in the Nile tilapia fish due to the bioactive compound cytosterol. The improved histological structure of dietary supplementation with 2 g MP might be because of the ability of MP incorporation to enhance the development of spermatocytes to sperm. And improve overall fertility by acting on the hypothalamus–pituitary-gonadal axis, indicating dose-dependent histological alterations. It is suggested that the potent antioxidant capacity of MP maintains the testicular architecture. Our results were supported by Ref. [50] who revealed that the MP extract contains huge amounts of phenolic constituents that are characterized by free radical scavenging and high anti-oxidant activities. In the same context [51], reported that dietary incorporation with *Aspilia mossambicensis* and *Azadirachta indica* leaf powders altered the gonadal histological characteristics of *O. niloticus* by alkaloids and flavonoid contents.

Assessment of immune-associated genes is vital for indicating and confirming the immunological status in freshwater fish [5,21,26,52]. Expression of the immune-related genes *C5* and *IL-1β* was correlated with the lysozyme activity results, where the MP diets enhancing gene expression in a pattern of $2 < 4 < 6$ g MP/kg diet. This could be attributed to the ability of *M. pruriens* to enhance cellular immunity as previously reported in earlier studies [13,14,53], and correspondingly, up-regulate the expression of immune-related genes.

Conclusively, MP incorporation at a level of 2 g/kg diet revealed overall improved growth, immune, testicular histology, and related-gene expression in mono-sex *O. niloticus*. Consequently, MP can be economically used for enhancing fish performance and increasing its production capacity for sustainable aquaculture.

5. Conclusion

The present findings highlight the importance of *M. pruriens* as a growth promoter, immunostimulant, and reproductive enhancer indicating the best reproductive performance in the meantime. Hence, considering the experiment expenses and the ease of handling, we propose *O. niloticus* as an animal model for the future primary discovery of herbal extracts and other bioactive compounds for sustaining aquaculture. Further studies are needed to determine the significance of MP on other fish species and to test other provocative impacts.

Data availability statement

All data are available in the manuscript.

Research ethics

Current study was performed in agreement with the approved guidelines for the Use of Laboratory Animals by the Experimental Animal Use Committee, Benha University, Qalubia, Egypt. The experiment was performed according to the guidelines for use and care of laboratory animals approved by the National Institutes of Health (NIH Publications No. 8023, revised 1978).

CRediT authorship contribution statement

Hiam Elabd: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources, Writing – original draft, Writing – review & editing. **Caterina Faggio:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Heba H. Mahboub:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources, Writing – original draft, Writing – review & editing. **Mahmoud Abdelghaffar Emam:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Samar Kamel:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Reda El Kammar:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Noha S. Abdelnaeim:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Adel Shaheen:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Nikola Tresnakova:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources. **Aya Matter:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Resources, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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