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Original Research Article

Antioxidant, immunostimulant and renal protective activities of tri-herbal combination in African Sharptooth Catfish, *Clarias gariepinus*

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ABSTRACT

Medicinal herbal feed that used as feed additives are widely applied in livestock production and may be applicable to aquaculture production systems. The present study explores the modulatory effects of incorporation of herbal combination of black cumin seeds (*Nigella sativa*), peppermint (*Mentha piperita*) and fennel (*Foeniculum vulgare*), on African Sharptooth Catfish, *Clarias gariepinus*. For this purpose, a total of 72 catfish were randomly distributed into 6 groups of 12 fish each and fed on the experimental diets for 45 successive days. Fish of first group were fed on basic diet only while, those of the second one were fed on basic diet supplemented with 0.5% from each medicinal plant while, other 4 groups were subjected to challenge experiment by injection with *Vibrio vulnificus*. The third group was kept non-injected, fourth group was injected by saline, fifth and sixth groups were injected by *Vibrio vulnificus* after feeding with basic diet and basic diet with herbs, respectively. Results proved that, total antioxidant capacity, serum total proteins, albumins and globulins were observed to be significantly higher in the treated group as compared to the control. Lymphocytes percent increased significantly in herbal fed group comparing to the control group while, neutrophils percent decreased significantly. Although serum urea level was not affected by herbal supplement, serum creatinine level was decreased significantly. Body weight gain of *C. gariepinus* increased significantly after herbal administration. Catfish challenged with a *Vibrio vulnificus* isolate and received the herbal diet showed less mortality than the control group. Fish fed on the herbal diet exhibited normal histological structure of liver, kidney and spleen. In conclusion, based on the current results together with the low cost and the potential antioxidant and immune effects of mixed herbal medicinal plants used in the study, it is recommended to be used in fish feed to diminish the mortalities caused by some aquatic pathogens.

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1. Introduction

Many immunostimulants are being used as feed supplement in fish farms to prevent fish from pathogenic organisms. However, some disadvantages inhibit its use (Sakai, 1999). The extra financial burden on fish raisers of the immunostimulant is important factor (Düğenci et al., 2003). With the outright ban on feed additives, new alternative strategies of ameliorating and conserve the health status of fish and consequently fish production must be explored. The application of naturally occurring immunostimulants such as, herbs, herbal preparations and other botanicals are prioritize over chemical compounds for augmentation of fish health and enhance the activities of non-specific defense mechanisms.

Oxidative stress is a process characterized by alteration of balance between radicals generating and radical-scavengers activities resulting in reduction of antioxidant protection (Bernabucci et al., 2002). Oxidative stress is a primary problem that adversely affects physiological condition and production performance of aquaculture, but synthetic antioxidants have a toxic and carcinogenic effects (Shahidi 2005) so, it is important to sought antioxidant with natural source to ameliorate theses deleterious effects.

The use of plants becomes necessary because of their wide medicinal importance (Zhang, 2002). Among these plants, black cumin seed, *Nigella sativa* which is a spice and used as a preservative in foods. It encompasses many therapeutic properties such as, antibacterial, antifungal, anti-inflammatory (Khan, 1999), immunomodulator, hepatoprotective and renal protective (Ramadan, 2007).

Mint species, *Mentha piperita* (also known as peppermint) belonging to Lamiaceae family (Iscan et al., 2002). Peppermint is an aromatic plant used as a kind of traditional therapy such as gastrointestinal troubles as it can be used as appetizer and exert relaxation on stomach wall (McKay and Blumberg, 2006). Moreover, peppermint has antiseptic properties (Rakover et

al., 2008), antioxidant, antiviral, fungicide and antibacterial activities (McKay and Blumberg, 2006).

Fennel (*Foeniculum vulgare*) is an aromatic herb also used in medicinal plant and as spice in Central Europe and the Mediterranean. Fennel is known as an excellent source of natural antioxidants and participates in daily antioxidant diet (Shahat et al., 2011). From all that, the present study aimed to investigate the influence of mixed tri-herbal medicinal plants on growth rates of African Sharptooth Catfish, *C. gariepinus*, its antioxidant and immunostimulatory activities via changes in various immunological measures. In addition, its impacts on resistance to *V. vulnificus* in challenge infection test. Moreover, the protective effect on reducing the histopathological alteration in structure of kidney, liver and spleen were also considered.

2. Materials and methods

Fish samples

African Catfish (*C. gariepinus*) (n=72) with average weight 50 ± 5 g were collected from private fish farm in El-Dakahlea Governorate, Egypt. The fish were transported to the Aquatic Research Laboratory, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. Acclimatization period was 21 days in porcelain aquaria (260×65×70 Cm) according to the protocol of maintaining bioassay fish as was previously described (Ellsaesser and Clem, 1986). The fish were fed with basal diet without herbal supplement twice daily with a feeding rate of 3% of their body weight (El Kamel and Mosaad, 2012) until the beginning of the experiment. Water change rate was seventy percent daily with dechlorinated stagnant tap water, the fecal matters were removed out daily and dissolved oxygen level maintained by aerators.

Preparation of medicinal herbs

Nigella sativa, *Foeniculum vulgare* seeds and *Mentha piperita* leaves were bought from the local market, from Qena, Egypt. The seeds and leaves were washed by water, air dried and

ground before mixing with the basal diet. Basal diet (control) alone or plus tri-herbal supplement were formulated from natural ingredients and herbal mixture as listed in Table 1. The dietary ingredients were thoroughly mixed by a hand palletizer. The pellets allowed to air-dry at room temperature and stored at -5 °C until use. The required amount of the diet was prepared every week.

Fish and experimental design

Seventy-two apparently healthy acclimatized catfish were randomly distributed into six groups each of 12 fish left under laboratory conditions in triplicate manner. This study was carried out into two experiments as described in Table 2. First and second groups (Experiment 1) were used for biochemical analysis while, other groups (Experiment 2) used for challenge experiment.

Table 1: Dietary composition used in the present study

	Basal diet	Herbal diet
<i>Nigella sativa</i>	-	0.5%
<i>Mentha piperita</i>	-	0.5%
<i>Foeniculum vulgare</i>	-	0.5%
yellow corn	34.9%	34.9%
vegetable bean meal	28.6%	28.6%
fish meal	17.0%	17.0%
wheat bran	9.3%	9.3%
mineral mixture	1.7%	1.7%
vitamin mixture	1.0%	1.0%

Table 2: Experimental design used in the present study:

I- Experiment 1 (for biochemical analysis)

Group 1	Fish fed on basal diet (Control) for blood samples.
Group 2	Fish fed on basal diet supplemented with <i>Nigella</i> , peppermint and fennel of 0.5% each of diet weight for blood samples.

II- Experiment 2 (challenge experiment)

Group 3	Fish fed on basal diet (Control) for challenge experiment.
Group 4	Fish fed on basal diet supplemented with <i>Nigella</i> , peppermint and fennel of 0.5% each of diet weight for challenge experiment.
Group 5	Fish fed on basal diet injected with saline (+ve control) for challenge experiment.
Group 6	Fish fed on basal diet (-ve control) for challenge experiment.

Fish were fed twice daily for 45 successive days and body weight was recorded at the beginning and the end of the experiments.

Blood sampling

After 45 days, fish of all groups were quickly anesthetized using tricaine methanesulfonate (MS-222, Argent Chemical Labs, Redmond, Washington, USA). Blood samples were collected from caudal vein of groups 1 and 2 in Eppendorf tubes. The blood was left to clot for 2 hours at 4°C and then centrifuged at 3000 rpm for 15 min, sera were collected and kept at -20°C for the biochemical analysis.

For differential leukocytic count blood smears were made immediately, air dried, fixed with alcohol, stained by Giemsa stain and examined under light microscope. The cells were identified according to the morphological features according to Stoskopf (1993).

Biochemical methods

All biochemical parameters were analyzed spectrophotometrically (Visible Spectrophotometer 723C, Shanghai Phenix Optical Scientific Instrument Co., Ltd, Shanghai, China) according to standard methods using commercial laboratory kits. Total antioxidant capacity (TAC) was carried out according to Koracevic et al. (2001) by using commercial kits (Bio-Diagnostics Ltd., Worcestershire, United Kingdom). Serum total proteins (g/dl) and albumins (g/dl) were estimated according to the methods described by Doumas et al. (1971) and Kaplan and Szabo (1983) respectively. Total proteins and albumins kits (Spectrum Diagnostics, Obour City, Egypt) were used. Serum globulin (g/dl) was calculated according to Busher (1990) by mathematically subtracting albumins value from total proteins value. The methods of young et al. (1975) and Tietz (1986) respectively, were used to determined serum urea and serum creatinine (Egy-chem, Badr city, Egypt).

Challenge experiment

Bacterial strain

Vibrio vulnificus strain was isolated from clinical cases of naturally infected catfish showing signs of septicemia. The strain was identified according to Austin and Austin (2007) and preserved in glycerol at -80°C. To restore its pathogenicity, it was passed three times via intra-peritoneal injection in *C. gariepinus* before using for experimental infection.

Experimental dose

The bacterial count (colony forming units "CFU") was measured by both Spectrophotometric and standard-plate-count method. For spectrophotometry, optical density values were measured to know the counts in bacterial suspensions at wavelength of 600 nm. For standard plate count method, bacterial suspensions were ten-fold serially diluted (Goldman and Green, 2015). The experimental dose was determined depending on preliminary work.

Experimental infection

At the end of each experimental periods (45 days); fish of groups 3 and 4 were ip injected with 0.5 ml of sterile saline containing 1.3×10^5 cfu/ml pathogenic strain of *V. vulnificus*, a +ve control group (n=12) (group 5) were ip injected with sterile saline while, -ve control group (n=12) (group 6) was remained non-injected. The challenge trial lasted for 21 days. Mortality rate was recorded, and dead fish were removed daily and subjected to bacterial re-isolation and identification.

Gross and histopathological study

After body weighing and blood collection, liver, kidney and spleen were inspected for detection of any gross lesions then dissected out and kept in formalin 10% for 24 h. Organs were washed and dehydrated in ascending concentrations of ethanol, cleared in xylene and embedded in paraffin wax. Sections of about 4-5 μm thickness were prepared and then stained

with haematoxylin and eosin for histopathological examination (Bancroft and Gamble, 2008).

Statistical analysis

Results were statistically analyzed using the Graph-Pad Prism (GraphPad Software, San Diego, CA, USA). Values were expressed as mean \pm standard error of mean (SEM) and differences between groups were analyzed by using Student's t-test, results were considered significant at the $P < 0.05$ level.

3. Results

During the 45 days of feeding trials, there was no mortality or abnormal fish behavior in all groups.

Results of the current study are presented in Table 3. It appears from Table 3 and Fig. 1A

that serum level of TAC significantly increased ($P < 0.05$) in catfish fed on tri-herbal mixture compared with those fed on basic diet. total proteins, globulins and lymphocytes percent showed significant increase after feeding on herbal mixture (Figs. 1B and C respectively). Besides, significant increase ($P < 0.05$) of albumins after herbal combination was also detected (Fig 1B). Although globulins were significantly increased in herbal treated fish, there is no change in A/G ration between treated and control groups (Table 3). Serum creatinine level decreased significantly ($P < 0.001$) in herbal treated catfish compared with those fed on basic diet while, serum urea level almost showed no change between treated and control groups (Fig. 2).

Table 3: Biochemical analysis in herbal and basic diet fed fish:

	Control	Herbs treated
Total antioxidant capacity (mmol/L)	0.32 \pm 0.01	0.41 \pm 0.03*
Globulins (g/dl)	6.05 \pm 0.16	6.53 \pm 0.16*
A/G ratio (%)	0.34 \pm 0.01	0.34 \pm 0.02
Total proteins (g/d)	8.11 \pm 0.14	8.86 \pm 0.20**
Albumins (g/dl)	2.06 \pm 0.04	2.33 \pm 0.07**
Lymphocytes (%)	70.08 \pm 1.76	87.42 \pm 0.38***
Neutrophils (%)	26.67 \pm 1.73	9.50 \pm 1.15***
Monocytes (%)	2.42 \pm 0.70	1.92 \pm 0.38
Esinophils (%)	0.25 \pm 0.13	0.33 \pm 0.14
Basophils (%)	0.58 \pm 0.19	0.75 \pm 0.22
Creatinine (mg/dl)	0.45 \pm 0.02	0.34 \pm 0.01***
Urea (mg/dl)	8.23 \pm 0.29	8.35 \pm 0.25
Body weight gain (g)	41.12 \pm 1.42	54.96 \pm 6.23*
Mortality rate (%)	41.7 \pm 1.25	75.00 \pm 1.46*

(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Fig. 3A showed significant increase ($P<0.05$) in body weight gain after administration of tri-herbal mixture compared with control. The percent of survival and mortality of *C. gariepinus* infected with pathogenic strain of *V. vulnificus* showed that, mortality rate significantly lower ($P<0.05$) in herbal fish (group 4) than the basic diet fed fish (group 3) (Fig. 3B) while, there is no mortality rates in +ve and -ve groups. Post mortem findings of dead fish showed signs of septicemia, however the clinical signs reported were less severe in the herbal treated group. Macroscopically, the scarified catfish of the control group demonstrated normal brown discoloration of the liver suffered from some paleness in some areas. Kidney was apparently

healthy. Spleen appeared slightly congested and moderately enlarged. Herbal-treated fish showed liver, kidney and spleen with normal appearance either sizes or shapes.

Microscopically, the liver of the control catfish exhibited mild degree of vacuolar and hydropic degeneration of the hepatocytes with moderate congestion in the blood vessels mainly central vein (Figs. 4 a & b). Treated liver showed normal arranged hepatocytes within normal shapes and sizes; as well as focal aggregation of the melanomacrophages was detected adjacent to blood vessels (Fig. 4 c). In addition, the hepatic blood vessels appeared apparently intact; therein central vein seemed within normal shapes and sizes (Fig. 4 d).

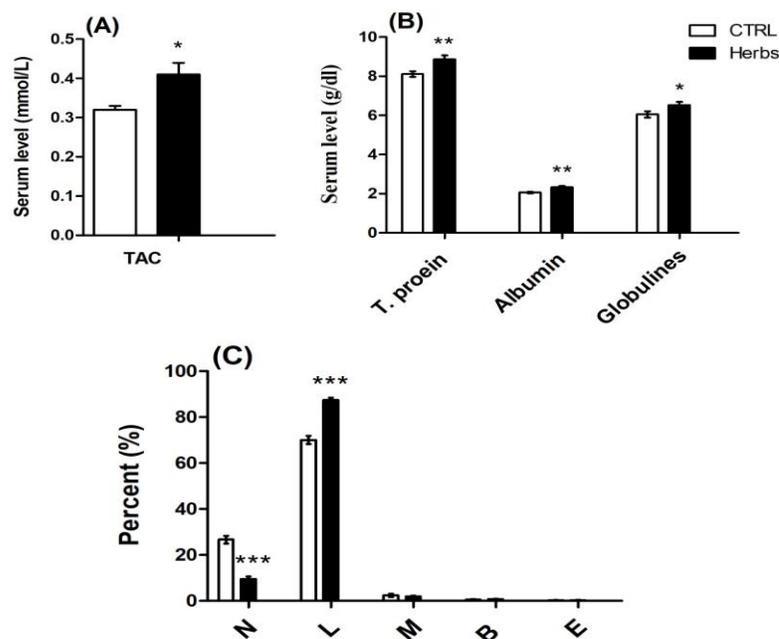


Fig. 1: Serum level of total antioxidant capacity (A), total proteins, albumins and globulins (B) as well as differential leukocytic count (C) in response to administration of *Nigella Sativa*, *Mentha Piperita* and *Foeniculum Vulgare* for 45 successive days (n=12, * $P<0.05$, ** $P<0.01$).

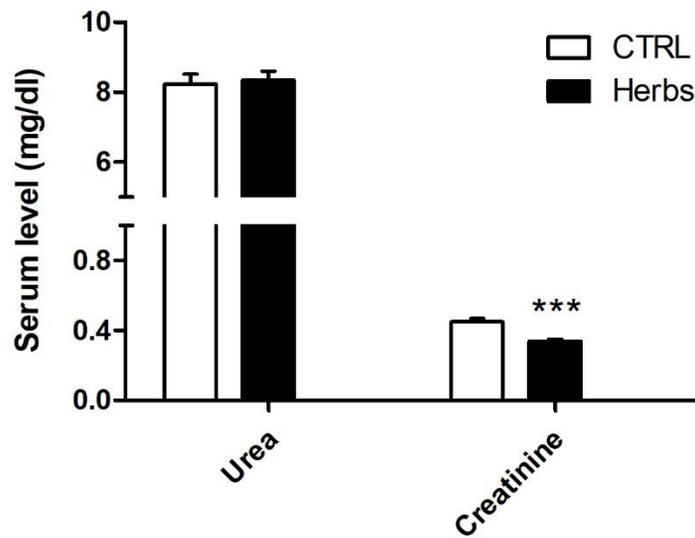


Fig. 2: Serum level of urea and creatinine represents kidney function after 45 days administration of tri-herbal combination (n=12, *** P<0.001).

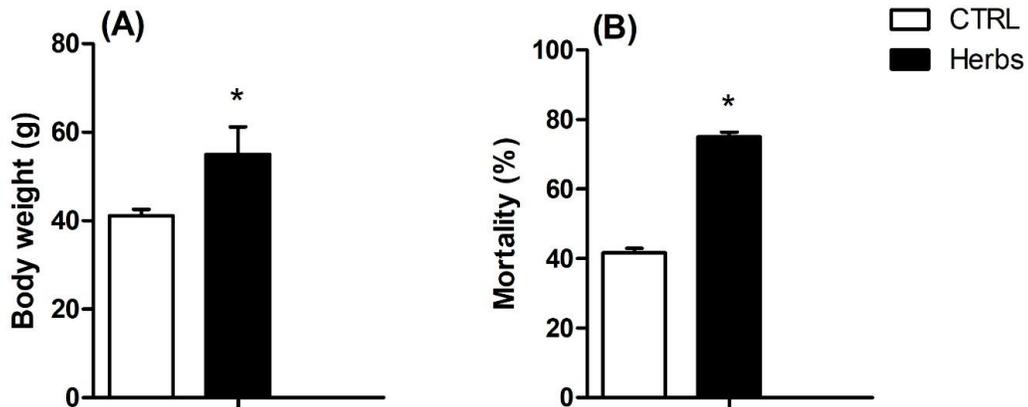


Fig. 3: Body weight gain in control and treated fish after administration of tri-herbal combination for 45 days (A). Mortality rate in control and treated fish after infection with *V. vulnificus* (n=12, * P<0.05).

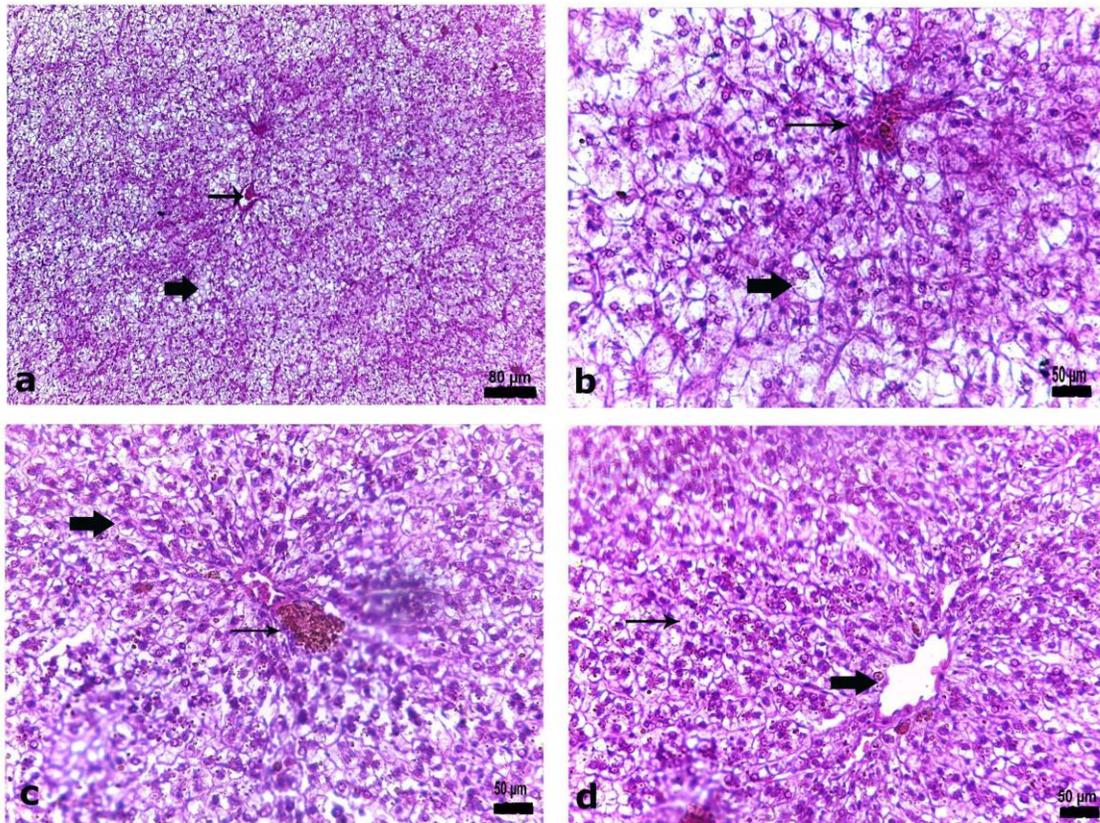


Fig. 4 (a-b): Photomicrograph of the liver of the control catfish showing cytoplasmic vacuolation of the hepatocytes (thick arrow) with mild congestion in the central vein (thin arrow) (a). High power of plate a to show cytoplasmic vacuolation of the hepatocytes (thick arrow) with mild congestion in the central vein (thin arrow) (b). Fig. (c-d): Photomicrograph of the liver of the treated catfish showing normal arranged hepatocytes (thick arrow) and focal infiltration of the melanomacrophage (thin arrow) (c), normal architecture of the liver with normal hepatocytes (thin arrow) and normal blood vessels (thick arrow) (d). (H&E., bar = 50 & 80 μm).

The posterior renal tissues of the control group displayed necrosis with sloughing of the epithelial lining renal tubules toward the lumen (Fig. 5 a). While, the glomeruli appeared apparently healthy in addition, there was focal aggregation the melanomacrophages and hemosiderin (Fig. 5 b). On the contrary, the treated group showed normal nephrons with intact renal corpuscles, besides normal haemopoietic tissues (Fig. 5 c). Moreover, mild distribution the melanomacrophages and hemosiderin was detected (Fig. 5 d).

Spleen of the control catfish revealed mild degree of lymphoid depletion of the white pulp with focal infiltration the melanomacrophage (Figs. 6 a & b). Mild congestion in the blood vessels was observed in the control spleen (Fig. 6 c). On the contrast, the red pulp displayed normal histological architecture (Fig. 6 d). The treated catfish exhibited normal splenic architecture of normal white and red pulps (Fig. 6 e); besides mild aggregation the melanomacrophages was noticed (Fig. 6 f).

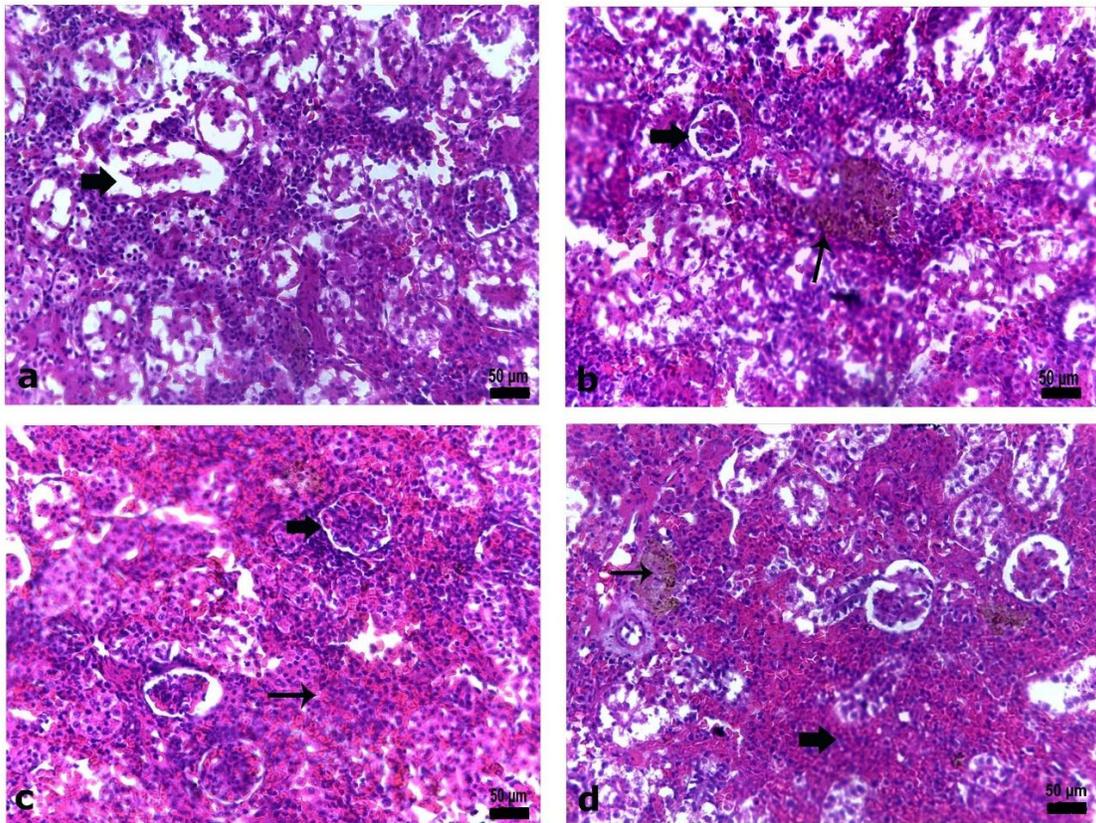


Fig. 5 (a-b): Photomicrograph of the posterior kidney of the control catfish showing renal tubular necrosis with sloughing of the epithelial lining (a), normal glomeruli (thick arrow) with focal aggregation the melanomacrophages and hemosiderin (thin arrow) (b). **Fig. 5 (c-d):** Photomicrograph of the posterior kidney of the treated catfish showing normal renal corpuscles (thick arrow) and normal haemopoietic tissues (thin arrow). (c), mild aggregation the melanomacrophages and hemosiderin (thin arrow) and normal haemopoietic tissues (thick arrow) (d). (H&E., bar = 50 & 80 µm).

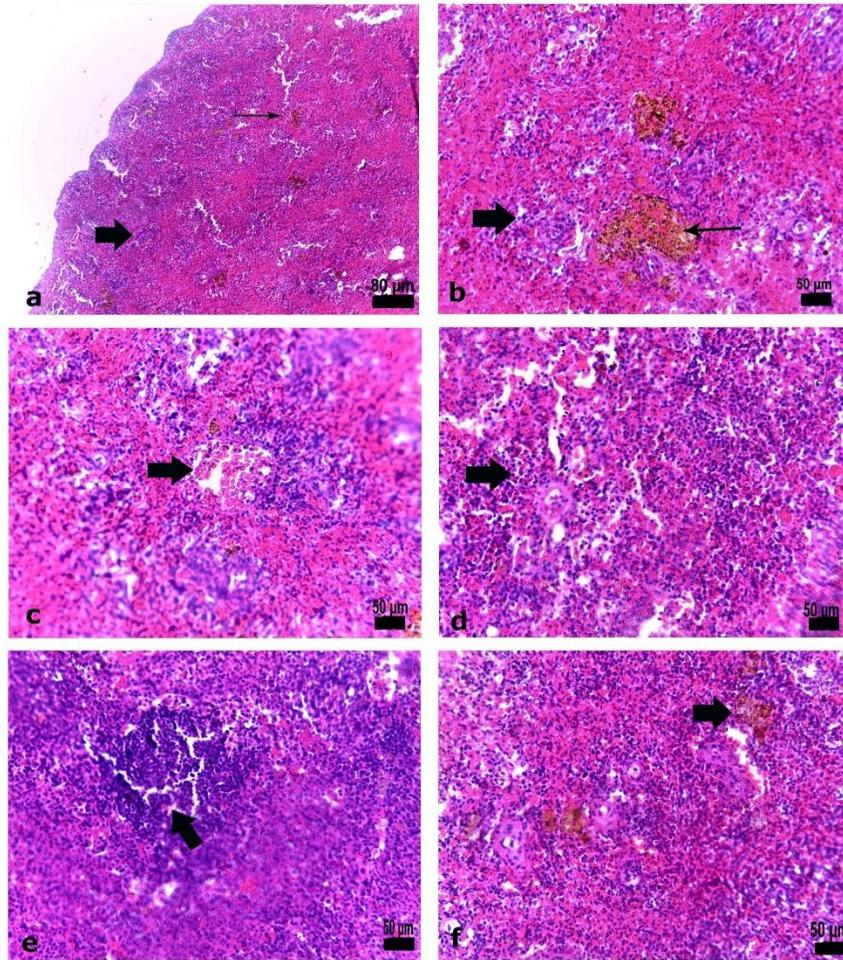


Fig. 6 (a-d): Photomicrograph of the spleen of the control catfish showing mild lymphoid depletion of the white pulp (thick arrow) with focal aggregation the melanomacrophages (thin arrow) (a). High power of **plate a** to show mild lymphoid depletion of the white pulp (thick arrow) with focal aggregation the melanomacrophages (thin arrow) (b), mild congestion in the blood vessels (c), normal histological architecture of the red pulp (d). **Fig. 6 (e-f):** Photomicrograph of the spleen of the treated catfish showing normal splenic architecture (e), mild infiltration of the melanomacrophages (f). (H&E., bar = 50 & 80 μm).

4. Discussion

Antimicrobials and vaccination for disease control strategy have suppressed the fish growth with subsequent generation of bacterial resistance and accumulation of antimicrobial agents in fish products. Therefore, their use in aquatic life become criticized (Gulec et al., 2013). For these important considerations, one of the most auspicious methods of curing or preventing diseases in fish farms is by modulating the defense mechanism of fish

through prophylactic application of products originated from natural plants (Agarwal and Singh, 1999 and Devasagayam and Sainis, 2002) The present study aimed to investigate the potential effects of black cumin seed, peppermint and fennel combinations on the antioxidant capacity, immune response and renal protective activities in *C. gariepinus*, with consequent study of the disease resistance ability. Moreover, histopathological

investigation of liver, kidney and spleen were also considered.

Plants contain antioxidant activity that gives protection against risky oxidative deterioration (Charles, 2013). Black cumin seed, peppermint and fennel are considered excellent source of natural antioxidant, and this could be proved in the current study. Increased level of antioxidants in laboratory rats with acute toxic hepatitis was recorded by Katikova et al. (2001) after feeding on the extract of peppermint leaf. Level of serum glutathione peroxidase and catalase activities as enzymatic antioxidant showed significant increase in *C. gariepinus* fed on diets contained either fenugreek or thyme compared to the control group (Emeish and Saad El- Deen, 2016).

Different types of white blood cells (WBCs) contribute in the cellular immunity, including lymphocytes and other granulocytes (Nakanishi, 1999) and play important role in the cellular immune response and resistance to infectious pathogens (Whyte, 2007). Their functions can be boosted by natural immunostimulants as was previously reported in gold fish that were supplemented with mixtures of chosen herbs (Harikrishnan et al., 2010). Although, percent of lymphocytes significantly increased in the present study, the percent of other WBCs types (neutrophils, monocytes, basophils and eosinophils) decreased in herbal treated group compared with control one owing to the high percent of lymphocytes. Monocytes and eosinophils showed no significant differences in fry Caspian white fish (*Rutilus frisii kutum*) fed on peppermint extracts (Adel et al., 2015), while the percent of neutrophils and lymphocytes increased and decreased, respectively.

Serum proteins and immunoglobulin levels are a major indicator of humoral part of innate immune system of fish (Secombes and Fletcher, 1992; Magnadóttir, 2006) which is the primitive line of defense against infectious pathogens. Serum total proteins and total globulins levels in herbal group showed significantly higher levels than the control one, indicating the immune

stimulating effects of tri-herbs used. Similar results were reported that total proteins in serum was elevated in fish after administration of 1% ginger (Dugenci et al., 2003) and in rainbow trout fed on *Nigella sativa* (Dorucu et al., 2009). IgM level was increased in fry Caspian white fish (*Rutilus frisii kutum*) fed on peppermint extracts (Adel et al., 2015).

Urea level which is an indicator of kidney function is not affected by mixed herbal immunostimulant used. On the other hand, creatinine level was significantly decreased in herbal diet group. Feeding rats on *Nigella sativa* seeds leads to significant decrease in serum urea and creatinine level during exposure to lead acetate toxicity (Farrag et al., 2007) and anticancer drugs (Ali and Blunden, 2003). *Nigella sativa* increases serum total proteins, decrease level of amino acids circulating in the blood subsequently, declines deamination lead to less formation of non-proteins nitrogenous compound including creatinine (Al-Logmani and Zari, 2011). Moreover, peppermint also, has the nephroprotective effect due to its phytochemicals as menthol (35.9%) and menthone (25.6%) (Chaves et al., 2011). Ullah et al. (2014) and Khalil et al. (2015) agree with the present results as serum creatinine level decreased after administration of peppermint alone or mixed with other medicinal plants in experimental animals. Likewise, *Nigella Sativa*, Fennel has the ability to elevate serum level of total proteins and albumins subsequently, attenuates level of non-protein nitrogenous compounds including urea and creatinine (Al-Masri and Ali, 2013). From the previous findings it is clear that *Nigella Sativa*, peppermint and fennel had individual nephroprotective effect so, in the current study the tri-herbal combination augments and improves the renal function.

The body weight gain among treatment group fed herbal supplemented diet implies that they had effect on growth. Weight gain was increased in fry Caspian white fish (*Rutilus frisii kutum*) fed on peppermint extracts (Adel et al.,

2015). Also, *Nigella Sativa* increased serum level of total proteins and albumins as it is expected its anabolic role in tissue growth and development and reflecting as increase body weight gain. In addition, the present results match with the previous findings which proved increase body weight gain after administration of fennel (Al-Masri and Ali, 2013) and peppermint (Khalil et al., 2015).

From the result of challenge experiment, it was revealed antibacterial and immune modulatory activities of herbal combination used. They have positively impacted the resistance of fish to *V. vulnificus* infection as was indicated by the decreased mortality and severity of clinical signs in fish fed on the herbal combination. Fennel extracts exerted the protection activity against acute and subacute inflammatory disorders and showed a central tranquilizer effect (Choi and Hwang, 2004). Peppermint has antiseptic, antimicrobial and anti-inflammatory characteristics. The major component of peppermint essential oil was menthol which is characterized by antimicrobial effect (Iskan et al., 2002). It possesses excellent antimicrobial activities against *E. coli*, *Staphylococcus aureus*, and *Candida albicans* (Yadegarinia et al. 2006). Also, alcoholic extract of the *Nigella* seed was shown to have antibacterial activity against *E. coli* and *Micrococcus pyogenes* var. *aureus* (Pruthi, 2001). Elkamel and Mossad (2012) stated that, the mortality rate of Nile tilapia fed with black cumin ration and challenged with *A. hydrophila* was significantly lower than those that received the basic diet. Also, the mortality rate of *C. gariepinus* fed with either fenugreek or thyme rations and challenged with *A. hydrophila* was significantly lower than those that received the basic diet (Emeish and Saad El-Deen, 2016).

The treated catfish with herbal plants restored normal histological appearance and architecture either grossly or microscopically as observed in liver, kidney and spleen. It is attributed to wide potential specific protective effect of the herbal plants on tissues. *Nigella sativa* grant protection

against hepato-nephrotoxicity induced either by disease or chemicals; as well as it could provide cardioprotective, neuroprotective, lung protective and gastroprotective activities (Darakhshan et al. 2015). *Nigella sativa* is high in biological active compounds; in addition to it offered an antioxidant activity in different organs lead to neutralize the oxygen free radicals generated (Burits and Bucar, 2000). Moreover, it has variety of therapeutic effects as anti-inflammatory, anti-cancer, antihistaminic and antibacterial. Also, it has been mentioned that *Nigella sativa* possess immunomodulatory and anti-neoplastic effects in many previous experimental studies (Morsi, 2000).

The restoration of the normal tissues structure induced by Peppermint suggested its protective activity which may be attributed to the free radical-scavenging properties of flavonoids (Kalpana and Alkal, 2012). Mint has antioxidant property prevent oxidative stress in tissues (Ortmann et al., 2004) since; it has scavenging compounds like monoterpene ketones, menthone, and isomenthone. Peppermint enhances regeneration and increase rate of restoration of liver cells through increase number of hepatic nucleus and intracellular RNA concentration (Vokovic-Gacic and Simic, 1993). It intended to fasten recovery of the dead and damaged tissues through rapid removal of the cellular debris (Samarth and Kumar, 2003). So, it offered protection against oxygen derived-free radicals and cellular damage (Baliga et al., 2003). Also, it considered as an effective antiallergic, antispasmodic plant (Samarth et al., 2006) and anticarcinogenic mechanism (Jain et al., 2011).

Fennel is medicinal herbal plant can give a protective power against many chemically induced damages in liver and kidney cells because it contains adequate amount of many antioxidants (Hanafi et al., 2004). The phytochemical composition of fennel revealed presence of many active components such as thymol, carvacrol, terpinenes, P-thymene and thymol methyl ether, phenolic glycosides,

flavonoids, phytosterols-triterp and saponins which have antioxidant and free radical scavenging activity. It led to reduce the amount of reactive oxygen species generated by fatty acid peroxidation; it also minimized the DNA damage produced (Naglaa et al., 2010).

Conclusions

It could be concluded from the current study that combination of black cumin seeds (*Nigella sativa*), peppermint (*Mentha piperita*) and fennel (*Foeniculum vulgare*) in the fish diet can influence the total antioxidant capacity, immune response and renal activity which subsequently stimulated the resistance of fish to *V. vulnificus* infection. Moreover, addition of these herbal mixture as herbal feed additives could reestablish the normal histological structure of tissues.

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الملخص العربي

نشاط مزيج ثلاثي الأعشاب الطبية كمضاد للأكسدة ومحفز للمناعة والحماية الكلوية في القرموط

الأفريقي

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اكتسبت المكملات الغذائية العشبية الطبية قبولاً واسعاً في الإنتاج الحيواني وقد تكون قابلة للتطبيق على أنظمة إنتاج تربية الأحياء المائية. تستكشف الدراسة الحالية التأثيرات البيولوجية لدمج المزيج العشبي من بذور الكمون الأسود (حبة البركة) ، والنعناع والشمر ، على القرموط الأفريقي . لهذا الغرض ، تمت هذه الدراسة علي ٧٢ قرموط أفريقي بمتوسط وزن 50 ± 5 جرام تم تقسيمها إلى ٦ مجموعات وتغذى على الوجبات الغذائية التجريبية لمدة ٤٥ يوماً متتالية. المجموعة الأولى ضابطة تم تغذيتها علي العليقة العادية بدون أي إضافات أما المجموعة الثانية تم تغذيتها علي العليقة العادية مضافا إليها ٠.٥% من حبة البركة وأوراق النعناع والشمر وبعد انتهاء التجربة تم سحب عينات الدم للتحليل البيوكيميائي والمناعي وأخذ الكبد والكلية والطحال لدراسة التغيرات النسيجية. المجموعات الأخرى تم استخدامها في حقن بكتريا فيبريو فولنيكاس حيث انهتم حقن البكتريا مع المجموعة التي تتغذي علي العليقة العادية والمجموعة التي تتغذي علي العليقة العادية مضافا إليها مخلوط الأعشاب الثلاثة ومجموعة أخرى تم حقنها بمحلول ملح والأخيرة بدون حقن. أثبتت النتائج أن إجمالي قدرة مضادات الأكسدة ، ومستوي البروتين في الدم ، والألبومين والجلوبيولينات لوحظت أنها أعلى بشكل ملحوظ في مجموعة الأسماك التي تتغذي علي مزيج الأعشاب مقارنة بالمجموعة الضابطة. زادت الخلايا الليمفاوية بنسبة كبيرة في مجموعة التغذية العشبية مقارنة بالمجموعة الضابطة بينما انخفضت نسبة الخلايا المتعادلة بشكل ملحوظ. على الرغم من أن مستوى اليوريا في الدم لم يتأثر بالمكملات العشبية ، فقد انخفض مستوى الكرياتينين في الدم بشكل ملحوظ كما أن معدل وزن الجسم من القرموط الأفريقي زادت بشكل كبير بعد تناول الأعشاب. أظهرت المجموعى التي تم حقنها بالبكتريا وتتغذي بمزيج الأعشاب أقل وفيات (٤١.٧%) من المجموعة الضابطة. الأسماك التي تلقت النظام الغذائي العشبي أظهرت بنية نسيجية طبيعية للكبد والكلية والطحال. استنادا إلى النتائج الحالية جنباً إلى جنب مع انخفاض التكلفة ومضادات الأكسدة المحتملة والآثار المناعية من النباتات الطبية العشبية المختلطة المستخدمة في الدراسة ، فمن المستحسن أن تستخدم في تغذية الأسماك للحد من الخسائر التي تسببها بعض مسببات الأمراض المائية.