Pharmacological studies of feed additives (Sanguinarine and Saccharomyces cerevisiae) on growth performance, haematological and intestinal bacterial count with challenge test by *Aeromonas hydrophila* in *Cyprinus carpio*

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ABSTRACT

This study analyzed the effect of dietary supplementation of commercial product Sangrovit® which containing the isoquinoline alkaloid sanguinarine by 500 gm/ton ration, and *Saccharomyces cerevisiae* (yeast probiotic) by 5 g/Kg on common carp (*Cyprinus carpio*) growth performance, hematological and gut microbiota with challenge test by *Aeromonas hydrophila* in *Cyprinus carpio* compared to the control group determined at 15 days intervals during the feeding trial. Each dietary treatment had two replicate aquaria. The results showed that during the period of experiment the using this feed additives showed slight significant increase in growth performance except in sanguinarine plus vitamins which showed high significant ($P<0.05$) increase specially at 45 days meanwhile all groups show non significant changes in Erythrogram and Leucogram except sanguinarine supplemented groups slightly increased. All groups showed significant ($P<0.05$) decrease in gut micobiota (total bacterial and coliform count) and also more resistance to challenge test with *Aeromonas hydrophila*. So these feed additives enhance the fish defense mechanism and elevates the resistance to fish to disease. According to our knowledge this study considers the first record on fish in Egypt.

Key words: Sanguinarine; Saccharomyces cerevisiae; growth performance; gut micobiota and immunity.

INTRODUCTION

The great shortage in the amount of animal protein needed for human consumption require intense research to seal this gap. Fish production can play an important role in providing cheap and safe source of animal protein. Increasing the fish production can be increased either by increasing the size of fish farms which will need more land and water; or improving the resistance of fish to diseases which attack fish industry. Fish play a vital role as a source of animal protein worldwide and increasing role in solving the human nutritional problems worldwide. Fortunately, there are different and extensive water sources that are expected to yield tremendous amounts of fish in Egypt. Also, fish breeding and fish cultures are considered good ways to increase the consumption need from animal protein and major source of income.

Bacterial diseases among cultured fish either primarily or secondarily are considered to be the cause about 80% of fish mortalities **(Austin and Austin 1987).** *Aeromonas hydrophila* is known to be one of the most important bacteria associated with diseases among freshwater fishes. The diseases caused by *Aeromonas hydrophila* ranged from acute rapidly fatal septicemia to latent infections and has been referred as hemorrhagic septicemia or aeromonas septicemia.

In aquaculture, traditional methods for treating infective pathogens include a limited number of government-approved antibiotics and chemotherapeutics. However, the disadvantages such as marginal effectiveness and high cost are obvious

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(Sealey and Gatlin, 2001). These treatments also may cause the accumulation of chemicals in the environment and/or fish, thus posing potential threats to consumers and the environment.

A group of biological and synthetic compounds that enhance the non specific cellular and humoral defense mechanisms in mammals. These substances, such as yeast, vitamin combinations, levamisole, β-glucan, peptidoglycan, chitin and chitosan as well as various products derived from plants and animals are effective in preventing disease **(Villamil *et al*., 2003; Dautremepuits *et al*., 2004).**

Sanguinarine has been incorporated into swine, bovine, and poultry diets to decrease amino acid degradation, increase feed intake, and promote growth. **(Tschirner *et al*., 2003 and Tschirner 2004). Rawling *et al*., (2009)** evaluate the effect of graded dietary supplementation of Sangrovit®, a commercial product containing the isoquinoline alkaloid sanguinarine, on red tilapia (Oreochromis niloticus) growth performance, feed utilisation, hepatic function, haematological parameters and gut microbiota. Compared to the control group, significant elevations in mean daily feed intake (1.19–1.25) were observed in fish fed Sangrovit® diets during the 60-day feeding period. Consequently, the specific growth rate (3.94–4.05% day^{-1}) and weight gain (66.80–71.85 g $fish^{-1}$) were significantly higher in the Sangrovit®-fed groups. With the exception of total leukocyte levels, which were elevated in fish fed Sangrovit® supplemented diets, haematological and immunological parameters remained unaffected. Hepatic alanine aminotransferase activity and hepatosomatic index also remained unaffected in all fish groups. Compared to the control group, the lactic acid bacteria (LAB) populations were lower in fish fed diets containing Sangrovit®. The present study demonstrates that Sangrovit® had a positive effect on tilapia growth performance with no apparent effects on carcass composition, hepatic function and health status.With the increasing concerns about use of antibiotics in aquaculture various pre- and probiotics that had shown potential as alternative treatments **(Nikoskelainen *et al*., 2001).**

Brewer's yeast (*Saccharomyces cerevisiae*) is a natural product from the brewing industry that contains various immunostimulating compounds such as β-glucans, nucleic acids as well as mannan oligosaccharides, and had been used as a diet additive for various animals. It had been observed to be capable of enhancing immune responses **(Ortuño *et al.,* 2002)** as well as growth **(Lara-Flores *et al.,* 2002**) of various fish species and thus may serve as an excellent health promoter for fish culture. **(Nayar *et al*., 1998)** said that Brewer's yeast; S. cerevisiae has been recognized to have potential as a substitute for live food in the production of certain fish or as a potential replacement for fish meal **(Oliva-Teles and Gonçalves, 2001).**

Brewer's yeast consider as source of vitamins and they are one of the most expensive additives to nutritionally complete diets for fish production **(Gaylord *et al*., 1998).**

MATERIAL AND METHODS

(a) Experimental fish

Common carp (*Cyprinus carpio*) fish were purchased from central Laboratory for Aquaculture Research, Suez Canal University. Fish were transported live in air pumped large clear polyethylene bags in dechlorinated, overnight kept tap water. Fish were kept in prepared full glass a aquaria (100 x 40x 50 cm), these aquaria were used for holding the

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experimental fish through the period of study and supplied with chlorine free tap water according to **(Innes, 1966)**, electric air pumping machines and filters were conducted to each glass aquarium. Thermostatic heater (h door, Germany) was used along the course of the study to keep the water temperature 23 ± 1°C. Fish were acclimatized to water environment and kept under observation for 2 weeks before the start of the experiment. Fish were fed a commercial diet; the diet was formulated to contain 30 % crude protein. The diet formulated in small pellets and was provided at 3 % of the body weight as described by **(Eurell *et al*., 1978).**

(b) Tested compound and experimental design:

Fishes were divided into four groups each group contained duplicate sets of 2×25 fish and acclimated for two weeks in the glass aquarium. The first 3 groups received a supplemented (treated) diet for 45 days, while the remaining group served as a control one.

All treatment mixed with the basal diet and egg as a top dressing and a coating agent to prevent detaching of drug. The pellets were prepared weekly, air dried at room temperature and stored in a refrigerator (4^0C) for daily use.

The four dietary groups were:

(1) Group 1 (Control):

Received basal diet free from any additives or treatments.

(2) Group 2 (Sanguinarine):

Received basal diet supplemented with Sanguinarine (Sangrovit® 500 mg/Kg ration) for 45 days **(Rawling *et al*., 2009).**

(3) Group 3 *Saccharomyces cerevisiae* (yeast probiotic) :

Received basal diet supplemented with Saccharomyces cerevisiae by 5 g/Kg ration for 45 days.

(4) Group 4 (Sanguinarine + AD_3E):

Received basal diet supplemented with Sanguinarine plus AD_3E (500 mg/Kg ration plus 2.0 ml/Kg ration), for 90 days.

c) Assessment of growth performance parameters:

Fish were collected from each treatment and control each 2 weeks, and then weighted for determining (Average daily gain (ADG), Body weight gain (WG), feed intake and Feed conversion ratio (FCR) according to (**Ricker 1979).**

**** Weight gain (g) = Wt – Wo**

**** Average daily gain (g) = Wt - Wo / n**

a) Feed intake:

The average feed intake per fish daily and weekly was calculated for each group.

d) Sampling and blood collection:

a- Blood samples:

Blood samples were collected on days 15, 30, 45 from beginning of experiment.

five fish randomly collected from each treatment groups and control group, blood samples from each group were taken by disposables syringes from caudal vessels according to (**Rowley, 1990)** divided into 2 parts., The 1st small part of blood sample was heparinized for the determination of some haematological parameters, 2nd part is used for serum collection.

a- Total erythrocytic and leucocytic counts:-

The total red and white blood cells were counted, by New bauer Double haemocytometer using Natt-Herrick's solution and WBCs diluting fluid counted the total red and white cells per µl of blood according to (**Miller and Seward, 1971).**

b- Estimation of blood haemoglobin

c- Packed cell volume (PCV %)

e- Differential leucocytic

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count (DLC):-
The percentage and absolute values for each type of cells were calculated according to (**Schalm, 1986; Jain, 1986).**

e) Bacterial count:
Fish samples were collected at days 15, 30, 45 for bacteriological examination under complete aseptic conditions. One gram of intestine was grind with 9 ml peptone
then ten fold serial dilutions carried out. Each dilution was inoculated in three Petri dishes; from which two received plate count agar for total bacterial count and the other received MacConky agar for coliform count. **(Shalaby, et al., 2006).**
Counting of colonies for the plates showing 30 -300 colonies per plates.

f) Determination of biochemical parameters in serum:

1- Determination of liver transmitters:
1-1 Determination of serum aspartate aminotransferase (sAST) and serum alanine amino transferase (sALT) activities according to (**Bergermeyer et al., 1986).**

2-Determination of serum total proteins
2-1 Determination of serum total proteins, according to (**Henry 1964)**
2-2 Determination of serum albumin according to **(Doumas., 1971)**
2-3 Determination of serum globulin.

3- Kidney function test
3-1 Determination of serum urea according to **(Numann *et al.*, 1977)**
3-2 Determination of serum creatinine according to **(Faulkner and King 1976)**

g) Challenge experiment:
Acclimated (15 days) experimental fish were divided into 4 groups. Ten (l0) fish from each treated group were injected I/P with 0.2ml hrs broth culture of *Aeromonas hydrophila* strain containing 3 x 10^7 CFU/ml^{-1} on days 45 (**Gopalakannan and Arul, 2006).**The bacterial count in each concentration was determined by pour plate method. **(Selvaraj *et al.*, 2009**). 20 fish from control group were collected, 10 fish were injected I/P **(Schaperclaus *et al.*, 1992)** with A.hydrophila strain containing 3x 10^7CFU/ml^{-1}, the other 10 were injected I/P with 0.2 ml phosphate buffered saline (PBS).
Clinical signs and Postmortem findings were also monitored and recorded.

h) Data analysis:
Statistics were calculated with SPSS for windows version 16.0; the means values obtained in the different groups were compared by One Way ANOVA.

RESULTS and DISCUSSION:

I) **Assessment of growth performance parameters:**
The results showed that during the period of experiment the using this feed additives showed mild significant increase in growth performance (Average daily gain-Weight gain-Feed intake- Feed conversion ratio) except in sanguinarin plus vitamins which showed more significant ($P<0.05$) increase specially at 45 days (table 1,2).

II) Effects of Sanguinarine, *Saccharomyces cerevisiae* and combination Sanguinarine plus Vitamins AD_3Eon Leucogram
Total leucocytic count of common carp fed on diet containing Sanguinarine, *Saccharomyces cerevisiae* and combination Sanguinarine plus Vitamins $AD_3E$was illustrated in table that (4,5). It has been shown from this table that:
All treated groups showed non significant changes in total leucocytic count except Sanguinarin plus vitamins meanwhile found mild changes in all group in lymphocytic and monocytic count; finally non significant changes in all groups in eosinophiles and basophiles count.

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All treated groups showed non significant changes in total leucocytic count at first 15 days but significant increase observed at 30 days Sanguinarine plus vitamins AD_3E (9.33×103/ µL) when compared to the control group 5.67 ×103/ µL and at 45 days (8.67 ×103/ µL) when compared to the control group 4.33 ×103/ µL.

All groups expose increase in erythrocytic count, hemoglobin content and PCV% in our opinion the increase in haemogram in fish might be due to the nutritive value of these substances.

These result also agree with (**Rawling *et al*., 2009)** who reported that total leukocyte levels were elevated in fish fed Sangrovit® supplemented diets, but on the other hand these results were not in accordance with him where he reported that haematological and immunological parameters remained unaffected

III) Effects of Sanguinarine, *Saccharomyces cerevisiae* and their combinations on Total bacterial count:

Group treated with Sanguinarine plus vitamins AD_3E showed highly significant decrease in total bacterial count 43.44×10^6 CFU followed by Sanguinarine, and finally *Saccharomyces cerevisiae* 53.10, 63.49, 66.11, 79.44×10^6 CFU respectively when compared to the control group 83.33 $\times10^6$ CFU (Table 6).Group treated with Sanguinarine showed highly significant decrease in total coliform count 7.43×10^5 CFU followed by Sanguinarine plus vitamins AD_3E, finally *Saccharomyces cerevisiae* 7.83, 9.49, 9.73, 11.53×10^5 CFU respectively when compared to the control group 16.44×10^5 CFU.

The obtained results are compatible with those reported by (**Dzink and Socransky, 1985)** who stated that Quaternary benzo[c] phenanthridine alkaloids have antimicrobial activity at minimum inhibitory concentrations for several bacteria. Many of these bacteria belong to the genus commonly found in the gastrointestinal tract.

Furthermore, these results were supported by **(Newton *et al;* 2002)** who found that natural compounds extracted from plants, such as the quaternary benzo[*c*]phenanthridine alkaloids (**QBA**) sanguinarine and chelerythrine, are known to have antimicrobial effect.

IV) Effects of Sanguinarine, *Saccharomyces cerevisiae* and combination Sanguinarine plus Vitamins AD_3E on biochemical parameter:

All treated groups showed mild significant changes in biochemical parameters when compared to the control group (Table 7, 8).

Comparing to the total serum globulin of the control group it was cleared that there was significant increasing in the total serum globulin of the all treated group but it was highly significant in that group treated with Sanguinarine plus vitamins AD3E.

The results are compatible with those reported by with (**Rawling *et al.,* 2009)** who reported that hepatic alanine aminotransferase activity remained unaffected in fish fed Sangrovit® diets.

Table 1: Effects of Sanguinarine (500 g/ton ration), *Saccharomyces cerevisiae* (5g/Kg ration) and combination Sanguinarine plus Vitamins AD_3E on Average daily gain (ADG), weight gain (WG) (Means±S.E.) N=5.

Time / Groups	At 15 days		At 30 days		At 45 days	
	ADG	WG	ADG	WG	ADG	WG
Control	$0.49^{a}\pm0.006$	$7.32^{a}\pm0.09$	$0.42^{c}\pm0.006$	$6.36^{c}\pm0.09$	$0.52^{c}\pm0.014$	$7.82^{c}\pm0.22$
Sanguinarine	$0.50^{a}\pm0.043$	$7.57^{a}\pm0.65$	$0.48^{bc}\pm0.014$	$7.24^{bc}\pm0.22$	$0.71^{ab}\pm0.010$	$10.67^{ab}\pm0.16$
Saccharomyces cerevisiae	$0.57^{a}\pm0.03$	$8.85^{a}\pm0.50$	$0.59^{ab}\pm0.05$	$8.90^{ab}\pm0.86$	$0.76^{ab}\pm0.08$	$11.36^{ab}\pm1.28$
Sanguinarine + AD_3E	$0.53^{a}\pm0.060$	$7.96^{a}\pm0.72$	$0.68^{a}\pm0.066$	$10.23^{a}\pm0.54$	$0.88^{a}\pm0.086$	$13.19^{a}\pm0.34$

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Means within the same row having different letters (a,b,.) are significantly different at P≤ .05

Table 2 Effects of Sanguinarine (500 g/ton ration), *Saccharomyces cerevisiae* (5g/Kg ration) and combination Sanguinarine plus Vitamins AD$_3$E on feed intake and Feed conversion ratio (FCR) (Means±S.E.) N=5.

Time / Groups	At 15 days		At 30 days		At 45 days	
	Feed intake	FCR	Feed intake	FCR	Feed intake	FCR
Control	1.052^{ab}±0.01	2.16^{a}±0.03	1.272^{a}±0.01	3.00^{a}±0.05	1.463^{b}±0.01	2.81^{a}±0.10
Sanguinarine	1.044^{b}±0.01	2.10^{a}±0.17	1.271^{a}±0.02	2.63^{ab}±0.03	1.488^{ab}±0.03	2.09^{b}±0.07
Saccharomyces cerevisiae	1.072^{a}±0.01	1.83^{a}±0.10	1.338^{a}±0.02	2.29^{bc}±0.20	1.604^{a}±0.04	2.16^{b}±0.20
Sanguinarine + AD$_3$E	1.060^{ab}±0.01	2.06^{a}±0.27	1.299^{a}±0.02	1.93^{c}±0.15	1.606^{a}±0.05	1.85^{b}±0.13

Table (3): Effects of Sanguinarine (500 g/ton ration), *Saccharomyces cerevisiae* (5g/Kg ration) and combination Sanguinarine plus Vitamins AD$_3$E on erythrogram (Means±S.E.) N=5.

Time / Group	At 15 days			At 30 days			At 45 days		
	RBCs ×10^{6}µL	HB (g/dL)	PCV%	RBCs ×10^{6}µL	HB (g/dL)	PCV %	RBCs ×10^{6}µL	HB (g/dL)	PCV%
Control	1.54^{bc} ±0.01	8.59^{bc} ±0.3	24.67^{b} ±1.2	1.83^{a} ±0.08	9.67^{a} ±0.2	32.33^{a} ^{b}±0.3	1.80^{a} ±0.27	8.50^{bc} ±0.5	27.33^{b} ±0.7
Sanguinarine	1.66^{ab} ±0.07	9.41^{a} ±0.3	29.67^{a} ±1.5	2.01^{a} ±0.21	10.00^{a} ±0.9	33.00^{a} ^{b}±1.2	2.07^{a} ±0.08	9.77^{ab} ±0.7	31.67^{ab} ±1.7
Saccharomyces cerevisiae	1.47^{c} ±0.00	7.85^{c} ±0.3	27.00^{ab} ±0.6	2.13^{a} ±0.20	8.95^{ab} ±1.4	29.33^{b} ±1.8	1.88^{a} ±0.34	8.82^{b} ^{c}±1.1	27.67^{b}± 2.7
Sanguinarine + AD$_3$E	1.54^{bc} ±0.03	8.52^{bc} ±0.0	26.33^{b}± 0.3	2.40^{a} ±0.48	10.63^{a} ±1.6	34.33^{a} ±0.9	2.24^{a} ±0.38	10.50^{a} ±0.9	33.67^{a}± 2.3

Table (4): Effects of Sanguinarin(500 g/ton ration*Saccharomyces cerevisiae* (5g/Kg **ration) and combination Sanguinarine plus Vitamins AD$_3$E on Total leucogram count ×10^3 / µL (Means±S.E.) N=5.**

Time / Group	At 15 days (Relative %)			At 30 days (Relative %)			AT 45 days (Relative %)		
	T.L.C ×10^3/ µL	Lymphocyte count×10^3/ µL	Neutrophiles count 10^3/ µL	T.L.C ×10^3/ µL	Lymphocyte count×10^3/ µL	Neutrophiles count 10^3/ µL	T.L.C ×10^3/ µL	Lymphocyte count×10^3/ µL	Neutrophiles count 10^3/ µL
Control	3.67^{a} ±0.88	2.59^{a} ±0.66 (69.8)	0.58^{a} ±0.13 (16.9)	5.67 ±0.88	2.84^{b} ±0.40 (50.7)	2.04^{bc} ±0.48 (35.0)	4.33^{b} ±0.33	2.20^{a} ±0.28 (50.3)	1.38^{a} ±0.08 (32.3)
Sanguinarine	4.33^{a} ±0.33	2.89^{a} ±0.21 (66.9)	0.69^{a} ±0.16 (15.8)	7.00^{bc} ±0.58	3.53^{ab} ±0.34 (50.3)	2.06^{bc} ±0.25 (29.3)	7.00^{a} ±0.58	3.76^{a} ±0.36 (54.1)	2.07^{a} ±0.36 (29.2)
Saccharomyces cerevisiae	3.67^{a} ±0.33	2.69^{a} ±0.21 (73.5)	0.34^{a} ±0.07 (9.2)	5.67^{c} ±0.33	2.87^{b} ±0.23 (50.7)	2.04^{bc}± 0.18 (36.0)	6.00^{a} ±1.29	3.21^{a} ±0.96 (54.7)	1.77^{a} ±0.57 (29.7)
Sanguinarine + AD$_3$E	4.00^{a} ±0.58	2.76^{a} ±0.48 (69.2)	0.65^{a} ±0.26 (16.2)	9.33^{a} ±0.33	4.44^{a} ±0.30 (47.5)	3.23^{a} ±0.21 (34.9)	8.67^{a} ±1.40	4.16^{a} ±1.05 (49.4)	3.13^{a} ±0.05 (34.3)

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Table (5): Effects of Sanguinarin(500 g/ton ration), *Saccharomyces cerevisiae* (5g/Kg ration) and their combination on Total leucogram count ×10³ / µL (Means±S.E.) N=5.

Time / Group	At 15 days (Relative %)			At 30 days (Relative %)			AT 45 days (Relative %)		
	Monocyte count ×10³/ µL	eosinophiles count ×10³/ µL	Basophiles count ×10³/ µL	Monocyte count ×10³/ µL	eosinophiles count ×10³/ µL	Basophiles count ×10³/ µL	Monocyte count ×10³/ µL	eosinophiles count ×10³/ µL	Basophiles count ×10³/ µL
Control	0.35^{a} ±0.08 (9.7)	0.073^{ab} ±0.02 (2.0)	0.07^{a} ±0.014 (1.7)	0.64 ^{b} ±0.13 (11.7)	0.073^{a} ±0.02 (1.3)	0.08^{a} ±0.013 (1.3)	0.66 ^{a} ±0.14 (15.0)	0.043^{a} ±0.00 (1.0)	0.06^{a} ±0.012 (1.3)
Sanguinarine	0.61 ^{a} ±0.01 (14.3)	0.103^{ab} ±0.02 (2.3)	0.03^{a} ±0.015 (0.7)	1.26^{a} ±0.09 (18.0)	0.090^{a} ±0.02 (1.3)	0.07^{a} ±0.021 (1.0)	0.99 ^{a} ±0.13 (14.0)	0.107^{a} ±0.05 (1.7)	0.08^{a} ±0.026 (1.0)
Saccharomyces cerevisiae	0.55^{a} ±0.10 (14.7)	0.070^{ab} ±0.04 (2.0)	0.02^{a} ±0.012 (0.7)	0.60 ^{b}±0.03 (10.7)	0.093^{a} ±0.02 (1.7)	0.06^{a} ±0.020 (1.0)	0.84^{a} ±0.38 (13.0)	0.093^{a} ±0.05 (1.3)	0.09^{a} ±0.023 (1.3)
Sanguinarine + AD$_3$E	0.48 ^{a} ±0.09 (12.0)	0.063^{b} ±0.01 (1.7)	0.04^{a} ±0.006 (1.0)	1.44^{a} ±0.13 (15.3)	0.160^{a} ±0.04 (1.7)	0.06^{a} ±0.023 (0.7)	1.23 ^{a} ±0.36 (14.7)	0.133^{a} ±0.011 (1.3)	0.01^{a} ±0.013 (0.3)

Table (6): Effects of Sanguinarin (500 g/ton ration) *Saccharomyces* cerevisiae by 5 g/Kg ration) and combination Sanguinarine plus Vitamins AD$_3$E on total bacterial count and coliform count (CFU) (Means±S.E.) N=5.

Time / Groups	At 15 days		At 30 days		At 45 days	
	Total bacterial count (CFU) ×10⁶	Total coliform count (CFU)×10⁵	Total bacterial count (CFU) ×10⁶	Total coliform count (CFU)×10⁵	Total bacterial count (CFU) ×10⁶	Total coliform count (CFU)×10⁵
Control	40.10^{a} ±0.06	7.00^{a} ±0.06	92.57^{b} ±0.07	15.50^{ad} ±0.26	117.33^{a} ±1.45	26.83^{a} ±0.44
Sanguinarine	29.10^{e} ±0.06	0.85^{e} ±0.03	62.10^{e} ±0.06	4.60^{f} ±0.06	68.10^{d} ±0.06	16.83^{a} ±0.12
Saccharomyces cerevisiae	37.10^{b} ±0.06	4.83^{b} ±0.33	100.23^{a}± 0.12	8.93^{b} ±0.03	101.00^{b}±0 .58	20.83^{a} ±0.44
Sanguinarine + AD$_3$E	25.10^{f} ±0.06	2.27^{d} ±0.12	53.10^{f} ±0.06	6.70^{e} ±0.06	52.13^{e} ±0.09	14.53^{a} ±0.09

Table (7): Effects of Sanguinarin(500 g/ton ration), *Saccharomyces cerevisiae* and combination Sanguinarine plus Vitamins AD$_3$E on on biochemical parameters. Means±S.E.) N=5.

Time / Group	At 15 days			At 30 days			At 45 days		
	ALT (U/ml)	AST (U/ml)	Total Protein	ALT (U/ml)	AST (U/ml)	Total Protein	ALT (U/ml)	AST (U/ml)	Total Protein
Control	34.43^{a} ±0.44	59.12^{b} ±0.32	4.690b^{c} ±0.06	32.67^{a} ±1.84	61.95^{a} ±0.69	4.425^{abc} ±0.28	34.14^{a} ±0.76	59.35^{b} ±1.73	3.974^{b} ±0.08
Sanguinarine	31.02^{a} ±0.70	56.00^{d} ±0.13	4.427^{d} ±0.04	33.29^{a} ±1.06	62.00^{a} ±1.15	3.796^{c} ±0.11	32.47^{a} ±0.74	58.50^{bc} ±0.26	4.910^{a} ±0.42
Saccharomyces cerevisiae	31.50^{a} ±1.05	56.55^{cd} ±0.53	3.933^{e}± 0.09	29.80^{a} ±0.65	57.93^{b} ±0.53	4.850^{a}± 0.49	30.74^{a} ±1.37	57.10^{bc} ±0.38	4.023^{b}± 0.12
Sanguinarine + AD$_3$E	31.16^{a} ±1.48	57.18^{c} ±0.41	4.446^{cd} ±0.14	32.34^{a} ±0.50	60.63^{ab} ±0.81	4.678^{ab} ±0.15	31.56^{a} ±1.31	55.99^{c} ±0.87	5.286^{a} ±0.25

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Table (8): Effects of Sanguinarin(500 g/ton ration), *Saccharomyces cerevisiae* and combination Sanguinarine plus Vitamins AD$_3$E on biochemical parameters. Means±S.E.) N=5.

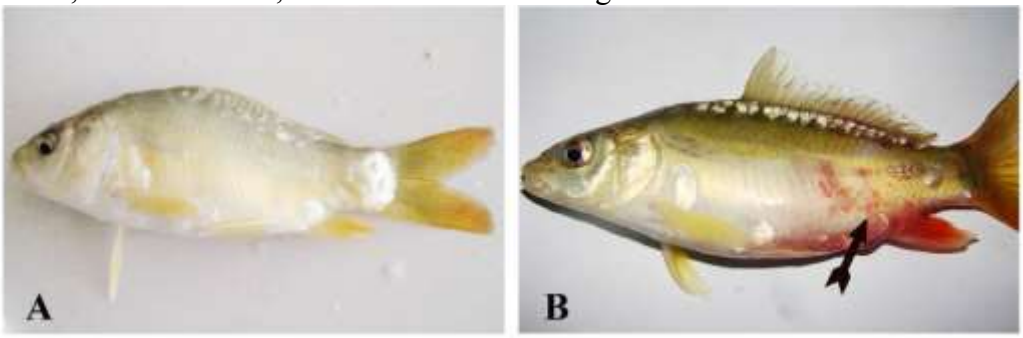
time / Group	At 15 days			At 30 days			At 45 days		
	Albumin g/dL	**Urea mg/ dl.**	**Creatinine mg/ dl.**	**Albumin g/dL**	**Urea mg/ dl.**	**Creatinine mg/ dl.**	**Albumin g/dL**	**Urea mg/ dl.**	**Creatinine mg/ dl.**
Control	**1.824^{a} ±0.042**	**5.08^{ab} ±0.15**	**0.703^{a} ±0.034**	**1.533^{c} ±0.048**	**5.30^{ab} ±0.13**	**0.720^{a} ±0.012**	**1.434^{c} ±0.009**	**4.96^{a} ±0.17**	**0.760^{a} ±0.021**
Sanguinarine	**1.361^{d} ±0.036**	**4.74^{b} ±0.04**	**0.713^{a} ±0.018**	**1.427^{d} ±0.019**	**5.52^{ab} ±0.39**	**0.703^{a} ±0.003**	**1.495^{c} ±0.020**	**5.03^{a} ±0.24**	**0.683^{b} ±0.00**
Saccharomyces cerevisiae	**1.697^{ab} ±0.006**	**4.99^{ab} ±0.12**	**0.717^{a} ±0.015**	**1.916^{a} ±0.031**	**5.69^{ab} ±0.35**	**0.667^{a} ±0.027**	**2.074^{a} ±0.054**	**5.18^{a} ±0.30**	**0.687^{b} ±0.009**
Sanguinarine + AD$_3$E	**1.282^{cd} ±0.158**	**4.84^{ab} ±0.25**	**0.713^{a} ±0.018**	**1.555^{c} ±0.014**	**6.09^{a} ±0.01**	**0.687^{a} ±0.026**	**1.517^{c} ±0.007**	**5.51^{a} ±0.30**	**0.670^{b} ±0.015**

V) Clinical signs and postmortem lesions due to challenge test:-

The clinical signs appear in ***Cyprinus carpio*** in response to I/P injection of Aeromonas ***hydrophila*** were similar but varied in the severity of developed lesions darkening of the body color (Fig.1), with large red irregular hemorrhages on the body surface. Also appearance of congestion and hemorrhage of all fins. Abdominal distension due to accumulation of ascetic fluid in abdominal cavity with protruded anal opening (Fig.2).The epidermis was eroded leaving skeletal muscle exposed (loosed leaving ulcers) (Fig .3), finally loss of all reflexes just prior to death.

Internally, congestion of all internal organs with yellowish serous fluid in the abdominal cavity (Fig.4), was found enlarged liver with hemorrhagic patches, inflamed foci, necrotic areas and distended gallbladder with bile (Fig.5). The intestine may be inflamed and hyperaemic containing yellowish mucus and was voided of food (Fig.6). Kidney appeared swollen and congested. Also, these results confirmed by (**West *et al.,* 1991)** who found Vitamin A has a number of significant effects on innate and specific immune responses.**(Rebeca *et al.,* 2009)** suggested that dietary vitamin D$_3$ administration has an effect on the innate immune parameters of gilthead seabream. The immunostimulant effect was greater on the cellular innate immune parameters.

The obtained results are compatible with those reported by **(Montero et al., 1999)** who said Vitamin E provides additional health protection through its immunostimulant property, and it seemed to have more protective role against stress.



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Conclusion:

In response to our consumer demands the government regulation now a day the intensive fish production industry must adapt to producing fish without antibiotic residue, so that this study revealed the importance of feed additives in fish culture to face different stress and act as alternative to some antimicrobial to manage Fish health.

Plants have always been a traditional source of medicine and have the potential to provide new drug. Plants that contain pharmacological active components may require greater attention.

The present study recommended using sanguinarine plus vitamins as feed additives to achieve an excellent growth performance, biochemical parameters and may be use as supportive therapy in fish farms.

Finally, we can conclude that we must increase researches about nontraditional therapy for prevention and controlling of diseases to avoid side effect of using antibiotic including drug residue.

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