

Comparative Study on the Effect of Fucoïdan and Levamisole on Some Selective Biochemical and Hematological Parameters in Heat Stress Rabbits

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Abstract: This study was planned to investigate the effect of fucoïdan and levamisole on some hematological and biochemical parameters in growing rabbits, in both normal and heat stress conditions. One month aged rabbits were orally treated with fucoïdan at a dose of 100 and 200 mg/kg body weight daily for 4 weeks and levamisole with 8 mg/kg as a single dose every 2 weeks. This study was conducted at winter and summer. Fucoïdan and levamisole treated groups returned the increased RBCs count and stress leukogram picture to normal at the end of the study. Also, biochemical profile in fucoïdan treated groups, showed significant decrease in ALT, AST, urea and creatinine with increased level of total protein and globulin when compared with heat stress group. We concluded that fucoïdan express good immunomodulating, hepatoprotective and renoprotective effect against stress induced by high temperature.

Keywords: Fucoïdan , Biochemical & Hematological Parameters, heat stress , Rabbits.

1. Introduction

Fucoïdan refers to a type of polysaccharide containing substantial percentages of L-fucose and sulfate ester groups mainly derived from brown seaweed and some marine invertebrates. Now it is named as “fucoïdan” according to IUPAC rules, but some also called it fucan, fucosan or sulfated fucan [1]. Chemical compositions of most fucoïdians are complex, mainly being composed of fucose and sulfate. They also contain other monosaccharides (mannose, galactose, glucose, xylose, etc.) and uronic acids, even acetyl groups and protein. Furthermore, the structures of fucoïdians from different brown algae vary from species to species[1, 2].

For the past decade fucoïdians isolated from different species have been extensively studied due to their varied biological activities, including anticoagulant and antithrombotic, antiviral, antitumor and immunomodulatory, anti-inflammatory, antioxidant, gastric protective effects and therapeutic potential in surgery [1,2]. Levamisole is a synthetic imidazothiazole derivative which is a highly acceptable antinematodal drug because of its broad range of activity in a large number of hosts . The drug appears to restore depressed immune function rather than to stimulate response to above normal levels. Levamisole stimulate formation of antibodies to various antigens, by stimulating T-cell activation and proliferation, potentiate monocyte and macrophage functions including phagocytosis and chemotaxis and increase neutrophil adherence. [3]. The drug is well absorbed and widely distributed and can be detected in all tissues and fluids, with the highest levels in liver and kidneys [3]. Our work was planned to study the effect of fucoïdan and levamisole on some selective hematological and biochemical parameters in growing rabbits, under heat stress conditions.

2. MATERIALS AND METHODS

2.1. Experimental Rabbits:

Eighty (80) unsexed one month old apparently healthy rabbits were obtained from a local commercial farm in Mansoura governorate (Egypt). The rabbits were housed in batteries and fed commercial diet and water which were supplied all the day along four weeks, the duration of experiment.

2.2. Treatment Drugs

- 1- Fucoïdan extracted from brown algae *Laminaria* species, (Leili Natural Products Co., Ltd, China).
- 2- Levamisole , (ADWIA Co., Egypt)

2.3. Experimental Design

Two experiments were conducted to investigate the effect of fucoïdan and levamisole on rabbits. The first one was during winter, optimum temperature for raising rabbits. While the second was in summer where heat stress is prevalent.

2.4. First Experiment

The experiment was applied in one month old rabbits, after treatment with fucoïdan and levamisole in winter (January) and extended for four weeks, where the temperature was (16 ± 2 C). Forty (40) rabbits were assigned randomly and divided into four groups, where a group was kept as negative control, non treated (Control) , and another three groups treated orally with fucoïdan (F 100) , (F 200) and levamisole (Leva.) in a dose (100, 200 and 8) mg/kg b.w respectively .

2.5. Second Experiment

The experiment was performed in one month old rabbits exposed to heat stress after treatment with fucoïdan and levamisole in summer (July) and extended for four weeks, where the temperature was (38 ± 2 C). Forty (40) rabbits were assigned randomly and divided into four groups, where a group was kept as positive control, non treated heat stressed (H

stress), and another three groups treated orally with fucoidan (HS+F100), (HS+F200) and levamisole (HS+Leva.) in a dose (100, 200 and 8) mg/kg b.w respectively.

In both experiment light was supplied continuously and rabbits were fed commercial diet, and all rabbits have free access to feed and water, with regard that neither the diet nor the water contains any drug.

2.6. Blood Sampling

At the end of the 2nd, 4th two separate blood samples were collected. One sample was taken in epindorf tubes at which mixed with EDTA (0.5mg/ml blood) as anticoagulant for hematological examination RBCs, Hb, PCV, MCV, MCH, MCHC, TLC and differential leucocytic count. The second sample was taken in test tubes without anticoagulant for clear serum separation which is carefully collected and stored in epindorf tubes at - 20 °C until estimation of serum chemistry including ALT, AST, , total protein, albumin, globulin, urea and creatinine which estimated spectrophotometer by commercial kits (Spine React Co. Spanish) according to enclosed pamphlet.

2.7. Statistical Analysis

Hematological values and serum biochemical parameters were analyzed by one way analysis of variance followed by Dunn's multi range test (ANOVA) using soft ware program SPSS version 20 for window .

3 RESULTS AND DISCUSSION

Optimal temperature for raising in rabbits is 15-18°C. Rabbits exposed to the ambient temperature of 25°C for 12h daily had lower weight gains than rabbits kept at 15°C. Environmental temperatures above 28°C cause heat-induced physiological stress [4].

The erythrogram data in the present work (table-1) for the rabbits treated with fucoidan (F100 and F200) showed insignificant change between all experiment groups. In the same aspect, erythrogram date revealed insignificant change for rabbits treated with levamisole in the 2nd week. However, in the 4th week RBCs count was increased significantly for rabbits treated with levamisole our results partially agreed with [4] who observed an increase in RBC on day 7 of levamisole administration with an increasing trend in RBCs value throughout the experimental period.

In this work erythrogram results revealed insignificant change between heat stress group and control group in the 2nd week while RBCs count in the 4th week was increased in heat stress group, this may be attributed to role of glucocorticoids and ACTH in stimulation of erythropoiesis in animals, where cortisol has a stimulatory effect on erythropoietin. Glucocorticoid may stimulate the proliferation of hemopoietic precursors, change the hemopoietic microenvironment [5].

TABLE 1: Erythrogram picture (mean ± S.E.) in rabbits treated with fucoidan and levamisole in optimum and heat stress conditions.

Time /Week	Group	RBC ×10 ⁶ /μL	Hb g/dl	PCV %	MCV Fl	MCH Pg	MCHC %
2 nd week	Control	4.79±0.31 ^a	10.88±0.52 ^a	31.8±1.20 ^a	66.87±2.01 ^a	22.84±0.64 ^a	34.19±0.58 ^a
	F 100	5.13±0.18 ^a	10.75±0.18 ^a	31.4±0.51 ^a	61.42±1.71 ^a	21.06±0.86 ^a	34.25±0.54 ^a
	F 200	4.92±0.15 ^a	10.95±0.11 ^a	31.5±0.45 ^a	64.13±1.65 ^a	22.31±0.68 ^a	34.77±0.25 ^a
	Leva.	4.81±0.19 ^a	10.71±0.26 ^a	30.9±0.95 ^a	64.73±1.44 ^a	21.97±0.52 ^a	33.97±0.89 ^a
	H stress	4.98±0.11 ^a	11.34±0.09 ^a	33.0±0.35 ^a	66.37±1.35 ^a	22.81±0.61 ^a	34.36±0.27 ^a
	HS+F100	5.05±0.23 ^a	11.12±0.25 ^a	31.8±0.58 ^a	63.71±3.88 ^a	22.29±1.41 ^a	34.96±0.17 ^a
	HS+F200	5.31±0.30 ^a	11.32±0.33 ^a	33.0±1.10 ^a	62.50±1.84 ^a	21.46±0.73 ^a	34.32±0.20 ^a
	HS+Leva.	5.12±0.15 ^a	11.09±0.29 ^a	33.0±0.88 ^a	64.76±0.88 ^a	21.60±0.41 ^a	33.58±0.01 ^a
4 th week	Control	5.06±0.14 ^a	10.79±0.14 ^a	31.90±0.40 ^a	63.26±1.57 ^a	21.39±0.46 ^a	33.84±0.11 ^a
	F 100	5.27±0.15 ^{ab}	10.89±0.33 ^a	31.95±0.61 ^a	60.73±0.74 ^{ab}	20.67±0.20 ^{ab}	34.06±0.56 ^a
	F 200	5.29±0.13 ^{ab}	10.75±0.20 ^a	32.20±0.37 ^a	61.01±1.75 ^{ab}	20.33±0.41 ^{ab}	33.38±0.68 ^a
	Leva.	5.20±0.19 ^b	10.93±0.17 ^a	32.20±0.86 ^a	62.13±1.98 ^{ab}	21.13±0.85 ^{ab}	34.01±0.78 ^a
	H stress	5.56±0.11 ^b	11.10±0.20	32.60±0.51 ^a	58.67±0.38 ^b	19.98±0.08 ^b	34.05±0.12 ^a
	HS+F100	5.34±0.19 ^{ab}	10.67±0.37 ^a	31.20±1.07 ^a	58.50±1.46 ^{ab}	19.99±0.41 ^{ab}	34.21±0.43 ^a
	HS+F200	5.49±0.14 ^{ab}	10.91±0.21 ^a	32.40±0.81 ^a	59.12±1.89 ^{ab}	19.92±0.64 ^b	33.70±0.51 ^a
	HS+Leva.	5.40±0.18 ^{ab}	10.86±0.18 ^a	32.00±0.84 ^a	59.31±1.06 ^{ab}	20.16±0.46 ^{ab}	33.98±0.48 ^a

F (Fucoidan), Leva (Levamisole), H stress (Heat stress). Means in the same column not followed by the same letter differ significantly ($P < 0.05$).

Cortisone stimulates erythropoiesis possibly by increasing the oxygen consumption of tissues and thereby promoting tissue hypoxia which in turn stimulate erythropoietin [6]. From erythrogram results in our work, it was obvious that both (HS+F100) and (HS+F200) groups were insignificantly changed from (H stress) group or control group along the whole experiment and the same did (HS+Leva.) group.

Our leukogram data (table-2) for the rabbits treated with fucoidan (F100 and F200) showed insignificant change from the control along the whole experiment except for the lymphocytosis in (F200) which may be due to the immunostimulant effect of fucoidan. Fucoidan of *L. japonica* can restore the immune functions of immunosuppressed mice, and it was an immunomodulator acting directly on macrophage and T lymphocyte.[1].

From leukogram results in this work, leukocytosis and heterophilia were prevailed in heat stress group along the whole experiment in addition to lymphopenia in the 4th week. This is attributed to the effect of stress induced by exposure of animals to heat (stress leukogram) in addition to the role of increased corticosteroides associated with heat stress. Our results partly agreed with Gregory [7], who reported that the control sheep had significantly higher cortisol concentrations than the sheep fed, an extract from brown seaweeds *Ascophyllum nodosum*, during the hottest part. Our results agreed with Tara *et al.*, [8], who reported that summer stress significantly raised the heterophil: lymphocyte (H:L) ratio in rabbits in the period from March to July. In the same line Özge *et al.*, [9], who reported an increase in heterophil and basophil ratios and H/L ratio and decreased monocyte and lymphocyte proportions after the exposure of broilers to acute heat stress. A corrective effect for the stress leukogram was obvious along the whole experiment in fucoidan treated groups which may be due to the antioxidant and immunomodulating effect of fucoidan on decreasing cortisol level of transport. In this work leukogram showed leukocytosis in the 2nd week and lymphocytosis along the whole experiment in levamisole (Leva.) treated groups. Also a corrective effect of levamisole was observed in the 2nd week in (HS+Leva.) group, while in the 4th week there were insignificant change in TLC but with lymphocytosis and decrease in heterophils which may be due to the immunomodulatory activity of levamisole. Our results are in accordance with Zia *et al.*, [4], who recorded that lymphocyte percentage and neutrophils decreased monocyte count increased on days 7 and 14 after levamisole (single dose) administration while. Our results revealed that ALT and AST serum levels were significantly elevated in the Heat stress (H stress) groups along the whole experiment indicating heart and or liver damage this is may be due to the damaging effect of oxidative stress caused by heat stress.

Crude commercial fucoidan was more active than the purified fucoidan at inhibiting the proliferation of vascular

smooth muscle cells, and then they speculated that a specific structure in the crude fucoidan may mediate its biological activities. Indeed, the content of the sulfated groups in fucoidan determines its anti-proliferative and anti-coagulant activities in fibroblasts. Identification of the structures of fucoidan that protect hepatocytes from hepatotoxins and that inhibit hepatic stellate cell growth is needed for the development of fucoidan as an anti-fibrotic agent [10].

In this work, fucoidan treatment significantly reduced ALT and AST serum levels in heat stressed rabbits. This is may be due to the hepatoprotective effect of fucoidan and its antioxidative effect. This results are approved histopathologically by apparently normal hepatic architecture except few degenerative. Our result agreed with Kum *et al.*, [11] reported that fucoidan extracted from *Undaria pinnatifida* sporophylls and *Laminaria japonica* and each were injected intraperitoneally at dose of (100 mg/kg) caused significant decrease in serum ALT and AST levels in rats exposed to CCl₄-induced oxidative stress due to role of fucoidan as a potential scavenger of free radicals generated by lipid peroxidation of the liver cells of CCl₄-treated rats. In the same aspect, Shinji *et al.*, [10] noticed significant decrease in levels of ALT and AST in fucoidan (crude) treated mice against CCl₄-induced acute (IV injection of fucoidan 25 and 50 mg/kg body weight) and chronic (IV injection of fucoidan 50 mg/kg body weight twice a week for 8 weeks). The anti-fibrogenic activity of fucoidan is due, at least in part, to attenuation of hepatic stellate cell activation by inhibition of transforming growth factor- β and/or by scavenging of reactive oxygen species, which can suppress the cascade of events that leads to hepatic stellate cell activation. Levamisole treatment significantly reduced ALT and AST serum levels in heat stress group, (table-3) this is may be due to the hepatoprotective effect of levamisole and its antioxidant effect of levamisole where Guifeng *et al.*, [12] recorded significant increase in SOD and lysozyme activities but decrease MAD activity.

Our results showed significant decrease in serum total protein level in heat stress group along the whole experiment which associated with decrease in both albumin and globulin in the 4th week only. This may be associated with decreased feed intake associated with high environmental temperature and also, may be due to liver damage. Our results showed significant increase in globulin (HS+F100) (HS+F200) in the 2nd week and in (F100), (F200), (HS+F100) and (HS+F200) in the 4th. Fucoidan has both humoral and cell-mediated immune responses under in vitro and in vivo conditions. A higher population of large B cells in spleen could be observed after treatment with the fucoidan, in mice infected with herpes simplex virus type 1. These results imply that the fucoidan enhance cellular immune response[13].

TABLE 2: Leukogram picture (mean \pm S.E.) in rabbits treated with fucoidan and levamisole in optimum and heat stress conditions.

Time /Week	Group	TLC 10 ³ /μL×	Lymphocytes ×10 ³ /μL	Heterophils ×10 ³ /μL	Eosinophils ×10 ³ /μL	Basophils ×10 ³ /μL	Monocytes ×10 ³ /μL
2 nd week	Control	8.43±0.85 ^a	4.86±0.57 ^a	3.17±0.24 ^a	0.03±0.02 ^a	0.06±0.04 ^a	0.31±0.03 ^{ac}
	F 100	8.73±0.55 ^a	5.46±0.33 ^{ab}	2.81±0.16 ^a	0.06±0.04 ^a	0.07±0.03 ^a	0.33±0.03 ^{ac}
	F 200	9.40±0.09 ^{ad}	6.09±0.12 ^{bc}	2.84±0.09 ^a	0.04±0.02 ^a	0.06±0.02 ^a	0.38±0.004 ^a
	Leva.	10.62±0.67 ^{bd}	7.24±0.63 ^c	2.87±0.56 ^a	0.04±0.02 ^a	0.06±0.03 ^a	0.42±0.03 ^a
	H stress	19.65±0.53 ^c	5.86±0.26 ^{abd}	13.71±0.38 ^b	0.12±0.05 ^a	0.08±0.05 ^a	0.12±0.08 ^b
	HS+F100	11.50±0.77 ^b	5.82±0.42 ^{ab}	5.24±0.45 ^c	0.06±0.03 ^a	0.11±0.03 ^a	0.18±0.05 ^b
	HS+F200	11.83±0.68 ^b	5.89±0.31 ^{ab}	5.64±0.37 ^c	0.09±0.04 ^a	0.05±0.03 ^a	0.22±0.05 ^{bc}
	HS+Leva.	12.08±0.71 ^b	6.89±0.31 ^{cd}	4.84±0.33 ^c	0.05±0.03 ^a	0.13±0.09 ^a	0.36±0.02 ^a
4 th week	Control	10.19±0.29 ^a	6.05±0.19 ^a	3.83±0.12 ^{ac}	0.04±0.02 ^a	0.04±0.03 ^a	0.23±0.06 ^{ad}
	F 100	10.54±0.44 ^a	7.08±0.86 ^{ab}	3.07±0.78 ^{ac}	0.05±0.03 ^a	0.05±0.03 ^a	0.30±0.05 ^{ab}
	F 200	11.53±0.65 ^{ab}	8.48±0.71 ^{bd}	2.59±0.43 ^a	0.05±0.03 ^a	0.07±0.03 ^a	0.35±0.02 ^{bc}
	Leva.	11.64±0.78 ^{ab}	7.79±0.43 ^b	3.21±0.4a ^c	0.06±0.04 ^a	0.04±0.03 ^a	0.45±0.04 ^c
	H stress	13.42±0.91 ^b	4.25±0.36 ^c	8.89±0.86 ^b	0.08±0.04 ^a	0.12±0.06 ^a	0.12±0.05 ^a
	HS+F100	10.721±1.3 ^a	6.32±0.71 ^{ad}	4.17±0.79 ^{ac}	0.04±0.03 ^a	0.04±0.02 ^a	0.16±0.02 ^{aa}
	HS+F200	12.46±0.70 ^{ab}	6.67±0.53 ^{ad}	3.86±0.52 ^{ac}	0.06±0.02 ^a	0.04±0.02 ^a	0.19±0.02 ^{ad}
	HS+Leva.	12.18±0.52 ^a	4.56±0.29 ^c	7.53±0.60 ^{ab}	0.04±0.03 ^a	0.05±0.03 ^a	0.27±0.04 ^{aa}

Means in the same column not followed by the same letter differ significantly (P<0.05).

TABLE 3: Some selective biochemical parameters mean (±S.E.) in rabbits treated with fucoidan and levamisole in optimum and heat stress conditions.

Time / Week	Group	ALT U/L	AST U/L	T. Protein g/dl	Albumin g/dl	Urea mg/dl	Creatinine mg/dl
2 nd week	Control	26.0±0.71 ^a	20.2±0.7 ^{ac}	6.9±0.19 ^{ab}	3.48±0.07 ^a	23.6±1.3 ^a	0.63±0.02 ^a
	F 100	26.2±1.43 ^a	19.6±0.9 ^{ac}	6.8±0.04 ^{ab}	3.49±0.03 ^a	24.8±1.4 ^a	0.66±0.01 ^a
	F 200	24.8±1.35 ^a	18.8±1.5 ^a	6.7±1.2 ^{ab}	3.46±0.05 ^a	23.2±1.1 ^a	0.61±0.02 ^a
	Leva.	26.4±2.06 ^a	19.4±1.2 ^{ac}	7.2±0.17 ^a	3.49±0.11 ^a	22.0±0.71 ^a	0.66±0.02 ^a
	H stress	30.2±0.78 ^b	25.4±0.93 ^b	6.5±0.17 ^b	3.43±0.14 ^a	36.4±1.01 ^b	0.86±0.03 ^b
	HS+F100	20.2±0.80 ^c	22.0±0.76 ^c	7.1±0.19 ^a	3.37±0.05 ^a	30.6±0.92 ^c	0.76±0.03 ^c
	HS+F200	18.4±0.68 ^c	15.5±0.85 ^d	6.9±0.83 ^{ab}	3.26±0.13 ^a	28.2±1.01 ^c	0.76±0.01 ^c
	HS+Leva.	20.0±1.0 ^c	21.0±0.9 ^{ac}	6.8±0.78 ^{ab}	3.36±0.02 ^a	28.5±1.10 ^c	0.79±0.02 ^c
4 th week	Control	24.6±1.21 ^a	23.6±1.1 ^{ad}	7.09±0.12 ^a	3.7±0.10 ^{ab}	36.2±1.15 ^a	0.75±0.01 ^a
	F 100	25.2±1.53 ^a	20.8±1.2 ^{ac}	7.79±0.08 ^b	3.99±0.09 ^a	34.2±0.5 ^{ad}	0.73±0.01 ^a
	F 200	26.0±0.71 ^a	24.4±2.5 ^{ad}	7.4±0.06 ^{bd}	3.81±0.04 ^a	31.1±0.8 ^b	0.71±0.02 ^a
	Leva.	24.1±0.95 ^a	26.2±1.4 ^{bd}	6.95±0.01 ^a	3.5±0.06 ^{bc}	33.4±1.2 ^{bd}	0.74±0.04 ^a
	H stress	39.4±1.10 ^c	28.8±0.86 ^b	6.45±0.26 ^c	3.37±0.18 ^c	39.9±0.71 ^c	0.92±0.02 ^b
	HS+F100	23.4±0.93 ^a	25.6±1.3 ^{bd}	6.9±0.09 ^a	3.44±0.06 ^c	32.2±1.1 ^{bd}	0.80±0.08 ^c
	HS+F200	18.0±0.89 ^b	22.2±1.6 ^{ac}	7.1±0.15 ^{ad}	3.38±0.07 ^c	25.8±0.8 ^e	0.75±0.18 ^a
	HS+Leva.	25.6±0.51 ^a	18.6±0.51 ^c	7.08±0.12 ^a	3.45±0.03 ^c	31.0±1.0 ^{bd}	0.80±0.06 ^c

Means in the same column not followed by the same letter differ significantly (P<0.05) .

The present results showed significant increase in serum urea and creatinine indicating renal damage in heat stress groups. Fucoidan treatment groups showed significant decrease in serum urea and creatinine level (F200) in the 4th week, (HS+F100) and (HS+F200) along the whole experiment this may be attributed to the renoprotective effect of fucoidan. Our results agreed with *Zhang et al.*, [14], who reported that fucoidan showed renoprotective effects. Also, we agreed with *Zhang et al.*, [15] who revealed that the elevated urinary protein excretion and plasma creatinine due to the induction of nephritis were significantly reduced by fucoidan at doses of 100 and 200 mg/kg.

4. CONCLUSION

In conclusion the fucoidan express good immunomodulating, hepatoprotective and renoprotective effect in growing rabbits against stress occur due to high temperature.

Conflict of Interests

The authors declare that they have no conflict of interests.

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