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## Safety Evaluation of Prolonged Administration of Stresroak® in Grower Cockerels

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**Abstract:** Stresroak® is a herbal preparation from combination of *Phyllatus emblica*, *Ocimum sanctum*, *Withania*, *somnifera*, *Mangefira indica* and *Shilajit* species. The Ayurvedic drug is used as anti-stress, immunomodulator, adaptogen and performance enhancer in poultry management. with outstanding results. The toxicological effects of prolonged administration of Stresroak in grower Cockerels was evaluated using haematological parameters and serum biochemical assay. Sixty growing Cockerels were used in this study. The birds were randomly but equally divided into 5 groups. Birds in groups A, B, C and D were administered with 109.8mg, 292.8mg, 585.6mg and 951.6mg of the drug dissolved in 2 litres of distilled water, daily for 60 days respectively. While the dose of group A was recommended by the drug manufacturer, the birds in group E were administered with 0.9% Physiological saline. The haematological parameters analyzed were total red blood cell (RBC) count, total white blood cell (WBC) count, haemoglobin concentration (Hb), platelets count and heterophil/lymphocytes ratio. Plasma enzymes and proteins analyzed were total proteins (T.P), albumin (ALB), globulin (GLO), fibrinogen (FIB), total bilirubin (T.Bil), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine Aminotransferase (ALT) and gamma glutamyltransferase (GGT). Stresroak generally improved haematological parameters in chicken administered with the drug when compared with the chicken in the control group. The significant ( $P<0.05$ ) increase in total RBC and WBC counts and MCH both at 30 and 60 days post-administration especially for the therapeutic dose, show that Stresroak® probably enhances erythropoiesis. Lower heterophil/lymphocyte ratio was observed for the groups that received the highest doses of Stresroak® and this was consistent throughout the course of the experiment, which implies that the herbal preparation improved the immunity of the chicken. The plasma levels of total protein, globulin, albumin and fibrinogen increased dose-dependently both at 30 and 60 days post- Stresroak® administration. The plasma levels of ALP and AST were significantly lowered while non-significant changes were observed for plasma levels of ALT and GGT at 30 days post- Stresroak® administration. Conversely, by 60 days post-Stresroak® administration, the plasma levels of ALT and GGT were significantly ( $P<0.05$ ) elevated except in cockerels in group A that received the recommended therapeutic dosage; where the plasma levels was observed to be lower for ALP ( $P<0.05$ ) and AST ( $P>0.05$ ). Histopathological findings did not however reveal any damage to the liver or kidney. It was concluded that Stresroak® exhibits haematinic, hepato-protective and immune stimulation properties and is safest at its recommended therapeutic dose as it was found to have potential tendency to cause hepatic injury when administered for longer period and at higher dosages.

**Key words:** Stresroak, administration, safety, evaluation and cockerels

### Introduction

Stresroak® is a scientifically proven botanical with adaptogenic, immunomodulatory, free radical scavenging and antioxidant rejuvenating actions (Shukla and Srivastava, 1999). Its active ingredients are derived from extract of plants such as *Phyllatus emblica*, *Ocimum sanctum*, *Withania*, *somnifera*, *Mangefira indica* and *Shilajit* extract (Manoharan *et al.*, 2004). It is used as anti-stress, immunomodulator, adaptogen and performance enhancer mostly in poultry management (Rajmane, 1996). Benefits associated with use of include better adaptation of birds to stress thereby minimizing associated losses. It improves specific immune response resulting in optimum antibody production post vaccination. Stresroak® increases non-specific immune response and overall protection against infections and non-infections diseases (Leena *et al.*, 1998).

The immune stimulatory properties of Stresroak® against Infectious Bursa and Newcastle diseases have been described by Mohammed (1996) and Leena *et al.* (1999). Little has been mentioned about the untoward effect of Stresroak® in animals especially since it is usually administered over a prolonged period of time in poultry. This study is aimed at evaluating the effect of prolonged administration of Stresroak® on haematology and plasma biochemical parameters of poultry birds especially cockerels.

### Materials and Methods

**Experimental animals:** Sixty day-old chicks (cockerels) were purchased from FOL-HOPE Farms Limited, Alakia, Ibadan, Nigeria. They were brooded for four weeks during which Newcastle and Gumboro vaccines (National Veterinary Research Institute, Vom, Nigeria)

Table 1: Mean haematological parameters of cockerels at 30 days post- Stresroak<sup>®</sup> administration

	PCV (%)	BC (10 <sup>9</sup> /mm <sup>3</sup> )	Hb (g/dl)	WBC (10 <sup>9</sup> /mm <sup>3</sup> )	Platelet (10 <sup>9</sup> /mm <sup>3</sup> )	MCV (fl)	MCHC (%)	MCH
A	26.80±1.47	2.42±0.53	8.32±0.50 <sup>a</sup>	12.40±2.53 <sup>a</sup>	286.00±29.93	115.40±20.90	30.97±1.54 <sup>a</sup>	36.04±7.66
B	26.00±2.10	2.20±0.36	8.60±0.75	15.68±4.15	274.80±30.68	118.83±16.26	33.03±1.01	33.74±6.42
C	29.80±2.56	2.80±0.73	9.86±0.45	15.30±3.16	105.20±8.09 <sup>a</sup>	112.37±23.69	37.09±0.81 <sup>b</sup>	33.46±2.60
D	26.20±1.94	2.65±0.16	8.76±0.84	14.56±1.42	253.20±28.02	100.23±13.91	37.16±8.80 <sup>c</sup>	33.33±4.61
E	27.80±1.17	2.38±0.34	9.24±0.67 <sup>a</sup>	16.56±2.43 <sup>a</sup>	274.40±24.63 <sup>a</sup>	118.84±16.46	33.00±1.04 <sup>a,b,c</sup>	39.45±5.71

Same superscripts within each column are statistically significant at P<0.05.

were administered. The cockerels had free access to water and were fed *ad lib* with chick's mash which was later replaced with growers mash for the later part of the experiment. After brooding for four weeks, the birds were randomly but equally divided into five groups A, B, C, D and E. Each group contains 12 birds. The birds were not given access to any multivitamin or anti-stress formulation.

**Drug administration:** Stresroak<sup>®</sup> is presented in concentration of 122mg/ml. The chicks in group A received the recommended therapeutic dose of 109.8mg, while chicks in groups B, C and D were administered with 292.8mg, 585.6mg and 951.6mg of Stresroak<sup>®</sup> in two liters of distilled water respectively, daily for the period of sixty days. Chicks in group E received normal saline for the same period of time.

**Blood sample collection and analyzes:** Blood samples were collected on 30th and 60th day post-administration of Stresroak<sup>®</sup>. Blood was collected from right jugular vein into Lithium heparinized tubes for haematological and biochemical analysis. They were later decanted into clean sample bottles and centrifuged at the rate of 1,000 revolutions per minutes (RPM) for 10 minutes. The blood collected was analyzed for haematological parameters. The Red Blood Cell (RBC) and the White Blood Cell (WBC) counts were determined by haemocytometer and packed cell volume (PCV) estimated using the micro-haematocrit methods described by Coles (1974). Haemoglobin concentration was determined by the cyanomethaemoglobin method as described by Jain (1986). Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) were calculated from original data obtained (Jain, 1986). Blood smears were stained with Giemsa stain for differential WBC count (Gueye *et al.*, 1988).

Plasma obtained from the blood samples were analyzed for alkaline phosphatase (Tietz and Shuey, 1986), aspartate aminotransferase (Bergmeyer *et al.*, 1985), alanine aminotransferase (Klauke *et al.*, 1988), gamma glutamyltransferase (Abicht, 2001), total protein (Keller, 1984), globulin, albumin (Tietz, 1995) and total bilirubin (Schlebusch *et al.*, 1995).

**Histopathology:** Some chicken were randomly selected from each group in the study. The birds were

anaesthetized with ether and thereafter killed by cervical dislocation. Samples of liver, kidney and spleen were collected and fixed in 4% Formaldehyde. The tissues were processed by usual method for paraffin embedding and stained with haematoxylin and eosin (H and E). The slides prepared from these organs were observed under light microscope for pathological changes.

**Statistical analysis:** The data were expressed as Mean ± standard error of means (SEM). The test of significance between groups was determined by the student t-test at P < 0.05 (Bailey, 1992).

## Results

### Haematological parameters

**30 Days Post- administration of Stresroak<sup>®</sup>:** At the 30th day post administration of Stresroak, the mean value of PCV (27.80±1.17%) in the control group was higher than those of groups A (26.80±1.47%), B (26.00±2.10%) and D (26.20±1.94%). The difference of means is not significant (P>0.05) between any two groups. The value of RBC was lower in the control group (2.38±0.34) than in groups A (2.42±0.53), C (2.80±0.73) and D (2.65±0.16) but higher than that of group B (2.20±0.36). The mean value of Hb for the control group (9.24±0.67gdl<sup>-1</sup>) was higher than those of groups A (8.32±0.50gdl<sup>-1</sup>), B (8.60±0.75gdl<sup>-1</sup>) and D (8.76±0.84gdl<sup>-1</sup>) but lower than that of group C (9.86±0.45gdl<sup>-1</sup>). The difference of the means was significant (P<0.05) between the values of groups A and the control. The mean values of total WBC count, MCV and MCH of the control group were higher than the values for each of the test groups but the difference of means is only significant for WBC values between groups A and control. There was significant reduction (P<0.05) in the platelet counts of group C (215.40±24.63) compared to that of control group E (274.40±24.63). Likewise, there was significant (P<0.05) increase in the mean value of MCHC of group A (30.97±1.54) compared to that of the control group (33.00±1.04) (Table 1). The heterophil/lymphocyte ratio decreased dose dependently with the control having the highest ratio as follows; groups A (0.41), B (0.40), C (0.38), D (0.32) and E (0.42) respectively (Table 3).

**60 days post- administration of stresroak<sup>®</sup>:** There was significant increase (P < 0.05) in the total WBC counts

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Table 2: Mean haematological parameters of cockerels at 60 days post- Stresroak® administration

	PCV(%)	RBC( $10^6/mm^3$ )	Hb(g/dl)	WBC( $10^3/mm^3$ )	Platelet	MCV(fl)	MCHC(%)	MCH
A	31.00±3.85	2.65±0.47	10.20±2.56	19.56±0.74	184.00±26.68 <sup>a</sup>	118.62±5.91	32.94±0.77	39.15±4.92
B	29.60±1.20	2.64±0.45 <sup>a</sup>	9.84±0.71	22.84±2.54 <sup>a</sup>	228.40±21.79	181.62±9.57 <sup>a</sup>	32.08±1.23	57.59±12.33 <sup>a</sup>
C	29.80±1.20	2.33±0.24	9.72±0.54	19.44±2.42	237.20±35.68	128.71±8.35	32.62±0.45	41.12±1.91
D	29.80±1.17	2.76±0.2	9.36±0.42	19.10±1.57	206.20±30.44	106.48±7.31	32.06±0.81	34.12±1.10
E	29.60±1.62	2.50±0.44 <sup>a</sup>	9.48±2.61	19.34±1.11 <sup>a</sup>	238.00±39.76 <sup>a</sup>	121.57±4.85 <sup>a</sup>	32.00±0.85	38.94±5.18 <sup>a</sup>

Same superscripts within each column are statistically significant at P<0.05.

Table 3: Mean values of heterophyte/lymphocyte ratio of cockerels at 30 and 60 days post- Stresroak® administration

Parameters	30 days post-administration					60 days post-administration				
	A	B	C	D	E	A	B	C	D	E
Heterophils (%)	27.80	27.60	26.60	23.80	29.00	31.8	27.80	26.50	24.80	36.20
Lymphocytes (%)	68.00	69.80	69.20	74.80	69.00	55.4	62.20	63.80	67.60	52.00
Heterophil/Lymphocyte (H/L) Ratio	0.41	0.40	0.38	0.32	0.42	0.57	0.45	0.41	0.37	0.70

Table 4: Mean values of plasma total bilirubin and proteins of cockerels at 30 and 60 days post- Stresroak® administration.

Groups	30 days post-administration					60 days post-administration				
	T.BIL (μmol/l)	T.P (g/dl)	ALB (g/dl)	GLO (g/dl)	FIB (g/dl)	T.BIL (μmol/l)	T.P (g/dl)	ALB (g/dl)	GLOB (g/dl)	FIB (g/dl)
A	0.44±0.12	3.70±.38	1.26±0.08	2.07±0.23	0.37±0.08	0.26±0.08	3.22±0.34	1.16±0.19	1.74±0.44	0.32±0.11
B	0.45±0.19	3.61±.42	1.28±1.17	2.20±0.45	0.13±0.06 <sup>a</sup>	0.38±0.07	3.06±0.17	1.20±0.09	1.59±0.67	0.27±0.05
C	0.45±0.29	3.60±.42	1.28±0.47	2.21±0.43	0.11±0.07 <sup>a</sup>	0.40±0.11	3.18±0.32	1.20±0.11	1.66±0.34	0.32±0.09
D	0.44±0.21	3.88±.25	1.34±0.25	2.15±0.22	0.39±0.10	0.42±0.11	3.24±0.14	1.20±0.12	1.75±0.23	0.29±0.08
E	0.38±0.08	3.56±.34	1.17±0.17	2.01±0.31	0.38±0.11 <sup>a,b</sup>	0.38±0.13	3.26±0.29	1.20±0.11	1.73±0.12	0.33±0.13

T.BIL = Total bilirubin., T.P = Total protein., ALB = Albumin., GLO = Globulin., FIB = Fibrinogen, Same superscripts within each column are statistically significant at P<0.05.

Table 5: Mean values of plasma enzymes of cockerels at 30 and 60 days post- Stresroak® administration

Groups	30 days post administration				60 days post administration			
	ALP (U/L)	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	GGT (U/L)
A	1316.80±52.64 <sup>a</sup>	266.20±35.26	3.00±0.89	30.00±3.58	758.80±55.51 <sup>a</sup>	239.40±2350	4.00±1.41	28.40±2.24
B	1478.60±55.94 <sup>b</sup>	216.40±45.95 <sup>a</sup>	5.80±1.83	31.60±3.77	868.00±56.90	227.60±10.40 <sup>a</sup>	4.00±1.10	27.00±5.25
C	1461.20±78.45 <sup>b</sup>	225.40±41.57 <sup>a</sup>	5.00±2.90	41.60±9.42	930.00±55.42	244.00±33.54	6.40±1.02 <sup>a</sup>	33.40±0.29 <sup>a</sup>
D	1626.80±56.18	234.60±15.79 <sup>a</sup>	4.40±1.62	36.60±4.85	1139.00±45.34 <sup>b</sup>	258.20±13.61	5.60±2.25	31.20±3.48 <sup>b</sup>
E	1638.40±65.26 <sup>b,c</sup>	282.60±20.19 <sup>b,c</sup>	3.40±1.50	34.80±7.73	866.80±42.58 <sup>b</sup>	248.40±13.71 <sup>a</sup>	3.40±0.85 <sup>a</sup>	25.40±3.26 <sup>b</sup>

Same superscripts within each column are statistically significant at P<0.05.

(22.84±2.54), MCV (181.62±9.57) and MCH (57.59±12.33) of groups C compared to the control group E. The increase recorded in the mean value of PCV, RBC, Hb and MCH of the various test groups compared to that of the control group at day 60 p.a of Stresroak were not significant (P>0.05). There was also significant reduction (P<0.05) in platelet counts of group A (184.00±26.68) compared to that of the control group E (238±39.76) (Table 2). The heterophil/lymphocyte ratio reduced dose dependently while the control group E recorded the highest Heterophil./Lymphocyte ratio (Table 3).

### Biochemical parameters

**30 days post- administration of stresroak®:** There was no significant (P>0.05) difference in the plasma total bilirubin, total protein and albumin of various test groups compared to that of the control group (Table 4). There was a steady increase in the plasma levels of globulin (P>0.05) and fibrinogen (P<0.05) in the test groups when compared with that of control group (Table 4).

There was significant (P<0.05) reduction in the mean values of ALP of groups A (1316.80±52.64), B (1478.60±55.94) and C (1461.20±78.45) and non-significant increase in group D (1646.80±56.18) compared to that of the control group E (1638.40±65.26) respectively. The mean values plasma level of AST in each of the test groups was lower than that of the control group; the difference of means is significant (P<0.05) for groups B, C and D. There was no significant (P>0.05) difference in the mean values of ALT and GGT of various test groups compared to that of the control group (Table 5).

**60 days post- administration of stresroak®:** There was no significant difference in the plasma total bilirubin, total protein, globulin, fibrinogen and albumin of various test groups compared to that of the control group (Table 4). There was increase in the values of ALP in groups B (868.00±56.90), C (930.00±55.42), D (1139.00±45.34) compared to that of the control group E (866.80±42.58) while group A (758.80±55.51) significantly (P<0.05)

decreased. The mean values AST of group B (227.60±10.40) was significantly ( $P<0.05$ ) lower compared to that of the control group E (248.40±13.71). The mean value of ALT increased non-significantly ( $P>0.05$ ) for all the test groups when compared with the control group. The mean values of GGT increased non-significantly for groups A (28.40±2.24) and B (27.00±5.25) but significantly ( $P<0.05$ ) for groups C (33.40±0.29) and D (31.20±3.48) when compared with the value obtained for the control group (25.40±3.26) respectively (Table 5).

**Histopathology:** Histological sections of liver and the kidney samples from the different test groups and control did not reveal any pathological changes under the light microscope.

### Discussion

Administration of varying doses of Stresroak<sup>®</sup> (122mg/ml) at 109.8mg, 292.8mg, 585.6mg and 951.6mg increased PCV, total RBC count and haemoglobin concentration in dose-dependent manner both at 30 and 60 days post-administration. The increase in PCV, total RBC count and haemoglobin concentration obtained especially for the therapeutic dose, though not statistically significant; show that Stresroak<sup>®</sup> probably enhances erythropoiesis and it can therefore be used as haematinic for domestic animals. Ziauddin *et al.* (1996) also reported significant increase in haemoglobin concentration, red blood cells post-Stresroak<sup>®</sup> administration in mice. In this study, lower heterophil/lymphocyte ratio was observed for the groups that received the highest doses of Stresroak<sup>®</sup> and this was consistent throughout the course of the experiment. Lower heterophil/lymphocyte ratio is a strong pointer to increased potentiation of the immune system (Chattopadhyay *et al.*, 1993; Rajmane, 1996). It was clearly established in this study that this effect of Stresroak<sup>®</sup> was dose-dependent within the range of concentration of Stresroak<sup>®</sup> used. Immuno-stimulation is one of the major reasons for which Stresroak<sup>®</sup> remains a valuable tool in the hands of its users (Pradhan *et al.*, 1995; Leena *et al.*, 1998; Shukla and Srivastava, 1999; Deka *et al.*, 2004; Manoharan *et al.*, 2004). The herbo-mineral constituents of Stresroak<sup>®</sup> include *Shilajit* species, *Phyllatus emblica*, *Ocimum sanctum*, *Mangifera indica* and *Withania somnifera*. Each of these plants has been reported singly potent enough to boost immunity in poultry (Semblulingam *et al.*, 1998; Makare *et al.*, 2001; Prakash *et al.*, 2002; Rajak *et al.*, 2004). In which case, the combination of the five active ingredients in the formulation of Stresroak<sup>®</sup> is expected to be of far greater potency and efficacy owing to additive and synergistic effects. The increase in total WBC count in test groups in this study is similar to the

findings of Agarwal *et al.* (1999) in cyclophosphamide-induced myelosuppression in rat administered with extracts of *Withania somnifera* which is one of the active ingredients of Stresroak<sup>®</sup>.

In this study, administration of Stresroak<sup>®</sup> resulted in increase in the total protein, globulin and albumin and albumin in animals administered with Stresroak<sup>®</sup>. This increase in levels of plasma proteins and its component have been ascribed to its hepato-protective effect owing to the anti-stress, adaptogenic, free radical scavenging and antioxidant rejuvenating actions of Stresroak<sup>®</sup> (Rajak *et al.*, 2004). The plasma levels of ALP and AST were significantly lowered while non-significant changes were observed for plasma levels of ALT and GGT as at 30 days post-Stresroak<sup>®</sup> administration. Conversely, by 60 days post-Stresroak<sup>®</sup> administration, the plasma enzymes were found to have increased in cockerels in the test groups almost dose-dependently, except in cockerels in group A that received the recommended therapeutic dosage; where the plasma levels was observed to be consistently lower for ALP ( $P<0.05$ ) and AST ( $P>0.05$ ). These findings show that as much as the administration of Stresroak<sup>®</sup> can be of immense benefit in poultry management, this study is pointing to the fact that the drug might also be toxic to the liver when administered beyond four weeks or at higher doses than the therapeutic dose. It should however be noted that this hepatotoxic disposition of higher doses of Stresroak<sup>®</sup> appears to be mild, in that elevation of the plasma enzymes in chicken administered with Stresroak<sup>®</sup> was not significant and histopathological findings did not reveal any pathological changes in the liver.

It is therefore concluded that administration of Stresroak<sup>®</sup> has haematinic, hepato-protective and immune stimulation properties and is safest at its recommended therapeutic dose as it was found to have potential tendency for hepatic injury when administered for longer period and at higher dosages.

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