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Effect of some medicinal plants on fertility in cocks

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List of Abbreviations

Abbreviations	Meaning
3-AT	3-Amino-1,2,4-Triazole
AIDS	Acquaired immunodeficiency syndrom
ALA	α-linolenic acid
ALT	Alanine transaminase
ALP	Alkaline phosphatase
AST	Aspartate transaninase
B.Wt	Body weight
CAT	Catalase
CYP17 A1	Cytochrome P450 17A1
DHA	Docosahexaenoic acid
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DM	Dry matter
DNA	Deoxyribonucleic acid
cDNA	Complementary DNA is a DNA copy of a messenger RNA
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FCR	Feed Conversion Rate
EPA	Eicosapentaenoic acid
FSH	Follicular Stimulating Hormone
g/dl	Gram per decilitre
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GGT	Gamma Glutamyltransferase
GIT	Gastrointestinal tract.
GnRH	Gonadotropin-releasing hormone
GP	Ginger powder
GPx & GSH-Px	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione

H&E	Hematoxylin and Eosin
Hb	Hemoglobin
I/V	Intra-venous
L	Liter
LH	Lutinizing hormone
LHR	Lutinizing hormone receptors
LIN	Linseed
LO	Linseed oil
MAGI	Male accessory gland infection
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MDA	Malondialdhyde
MCV	Mean Corpuscular Volume
mg/dl	Milligram per dieciliter
mg/kg	Milligram per kilogram
mmol/l	millimoles per litre
ng/ml	Nano gram per milliliter
nmol/l	Nanomole per liter
PCR	Polymerase chain reaction
PCV	packed cell volume
PUFA	Polyunsaturated fatty acids
RBCs	Red blood corpuscles
RNA	Ribonucleic acid
ROS	reactive oxygen species
r.p.m	Round per minute
SO	sunflower oil
SOD	superoxide dismutase
TAC	Total antioxidant capacity
U/L	Unite per liter
WBCs	White blood corpuscles

Summary

This study was performed to evaluate the potential ameliorative effect of linseed oil and zingiber oil on male fertility problems induced by fluconazole in cocks and their effects on some performance parameters, some antioxidant markers, testosterone hormone level, semen analysis and expression of fertility related genes in adult cocks. Moreover histopathological examination of testes and epididymis was done. Furthermore, their effect on some hematological and biochemical parameters were investigated.

This study was conducted on 42 clinically healthy mature cocks (1500-1700 gm) were divided into 6 groups each group contains 7 cocks as the following:

1st group: Cocks were served as non-treated group (Control negative).

2nd group: Cocks were given the therapeutic dose of fluconazole (5 mg/kg b.wt /day) orally in drinking water for a week (Control positive).

3rd group: Cocks were administered the therapeutic dose of fluconazole (5 mg/kg b.wt /day) orally in drinking water for a week, then followed by linseed oil (60 ml/kg DM) for two weeks.

4th group: Cocks were administered the therapeutic dose of fluconazole (5 mg/kg b.wt /day) orally in drinking water for a week, then followed by zingber oil (20 ml/kg DM) for two weeks.

5th group: Cocks received linseed oil (60 ml/kg DM) for two weeks, then followed by the therapeutic dose of fluconazole (5 mg/kg b.wt /day) orally in drinking water for a week.

6th group: Cocks received zingber oil (20 ml/kg DM) for two weeks, then followed by the therapeutic dose of fluconazole (5 mg/kg b.wt /day) orally in drinking water for a week.

Blood samples were collected from 5 cocks of each group from wing vein on 1day, 1st week and 2nd weeks post drug administration. The last blood samples were collected on 3rd week post drug administration by slaughtering with sharp knife. Samples were collected in three test tubes, the 1st and 2nd tubes were mixed with salt of ethylene diamine tetra-acetic acid (EDTA). 1st one was centrifuged at 3000 /15 r.p.m to get a clear plasma sample for some anti-oxidant markers estimation, while the 2nd sample subjected to hematological test. Finally, the 3rd blood sample were collected in the blank tube, after that were put in the centrifuge at 3000 /15 rpm to get a clear serum samples. The obtained sera were kept in deep freezer at – 20º C until assayed for serum total testosterone and other biochemical parameters.

In the 3rd week post dosing, testis of each cock were collected immediately after slaughtering, cleaned and minced in ice-cold phosphate-buffered saline (PBS, 1:2 w/v, pH 7.4; 37 °C), and squeezing it gently to obtain the fresh undiluted semen in a clean Petri dish and incubated at 37 °C for half an hour for liquefaction to proceed the following examinations. Sperm quality was determined by three parameters: sperm motility, morphology, and viability.

In the 3rd week post drug administration, 5 cocks (from each group) were slaughtered to obtain testes and epididymis were fixed in 20% formalin for histopathological examination. Another part of the testis was kept at -80 °C for conducting quantitative real time polymerase chain reaction (real-time PCR) for gene expression investigation.

After collection of all data, it has been analyzed statistically by computerized SPSS program (Version 19) using one way ANOVA.

From the obtained results, fluconazole induced non- significant changes on the performance parameters (testicular weight, somato-gonadal index and B.Wt/comb and wattles index) of treated cocks, while oil supplemented groups before drug administration (G5 & G6) showed a significant increase in weight of testes and weight of comb and wattles when compared with the control group.

The effect of the tested preparations on some heamatological parameters of the treated cocks. The results showed that there was no significant variation in all hematological parameters in fluconazole and other oils supplemented groups either after or before fluconazole administration.

The obtained data reflected a significant increase in ALT, AST and urea levels in fluconazole treated cocks, while creatinine was not significantly altered. Moreover, Fluconazole significantly increased MDA while decreased CAT and GSH, decreased serum testosterone level, decreased mass motility% and live/dead ratio, while significantly increased abnormal sperm % which accompanied by some histopathological alteration in testes and epididymis. Furthermore, the expression of CYP17A1 and LHR genes in testicular tissue showed 2 -3 fold significant decrease, while aromatase gene showed 3 fold significant elevation when compared with the control negative group.

In the presented study, linseed and zingiber oil supplemented groups revealed a significant decrease in ALT, AST and urea levels while creatinine was not significantly changed except in 1st week post fluconazole dosing. Furthermore, oils supplemented groups showed a

significant decrease in MDA but significantly increased CAT and GSH, increased serum testosterone H level and mass motility% of examined semen samples. Also there was a significant decrease in abnormal sperm % and no significant alterations in live/dead ratio in oils supplemented groups when compared with fluconazole treated group G2. The histopathological examination of testes and epididymis of oil supplemented groups revealed a considerable improvement in comparison with fluconazole treated group.

On level of gene expression of fertility related genes, only zingiber oil supplemented group after fluconazole medication, showed 2 fold significant elevation in expressed amount of LHR gene while other oil supplemented groups showed no significant alteration in comparison with fluconazole treated group. All oil given groups showed 1-2 fold significant decrease in aromatase gene expression while significantly increased the expressed CYP17A1 amount by less than l fold when compared with FCZ treated group fluconazole treated group.