Assessing the effect of Thyme and Rosemary as antiaflatoxicosis on fertility in male rats

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Abstract: Thyme and rosemary play a role in hepatoprotectivity and act as antiaflatoxicosis. This study has attempted to investigate effect of subacute dose of aflatoxin AFB1 on fertility in albino rats and possibly to predict any benefits or harms of rosemary and thyme leaves powder. Aflatoxin were detected and extracted from *Aspergillus flavus* isolated from poultry rations and processed animal feeds. One hundred samples of poultry rations and processed animal feeds (50 of each) were collected from markets at Cairo governorate for investigation of fungal contamination and detection of aflatoxin. The maximum levels of toxin were obtained from *A. flavus* isolated from processed animal feeds followed by those isolated from poultry rations. Forty eight apparently health male albino rats weighed (150-170 g) were divided randomly into 6 equal groups. Animals of the first group were given healthy commercial pelleted basal diet and kept as a negative control. The animals of groups 2, 4 and 6 were injected intraperitoneal with AFB1, 1.5 mg/kg body weight. Then on the second day the diet of rats were supplemented with 5% commercial thyme leaves powder for groups 3 & 4 and 2.5 % commercial rosemary powder for groups 5&6. The second group was left without any treatment and kept as a positive control. The period of feeding was continued for 28 days. Aflatoxin injected rats showed unexpectedly increase in weight gain comparing to all tested groups, a significant decrease in somatic index of testis, no significant effect on seminal vesicles, prostate gland weights, sperm count and motility between intoxicated rats and controls. Though a significant increase in testosterone and estradiol levels in intoxicated rats compared to the control group, no significant difference in testosterone estradiol ratio T/E_2 was observed. A significant decrease in total antioxidant capacity (TAC) and a significant increase in lipid peroxides (LPO) were observed. Thyme leaves powder fed to healthy or aflatoxin injected rats resulted in significant decrease in weight gain and no significant in testis weight**.** Thyme leaves improved sperm count and motility, no significant difference in testosterone and progesterone levels. Thyme supplemented to the diet of intoxicated rats did not exhibit any improvement in TAC&LPO. Rosemary leaves powder increased the weight gain significantly in comparable to control group, but reverse effect on weight gain occurred in intoxicated rats. No significant effect on testis and prostate gland weights, sperm count and motility either in healthy or intoxicated rats. Rosemary increased testosterone and estradiol levels either in intoxicated rats or not. Rosemary supplemented to the diet of intoxicated rats did not exhibit any improvement in TAC and LPO. It is interesting to report here that the aflatoxicated rats that treated with rosemary and thyme showed a significant diminution the levels of aflatoxin residues in testis, seminal vesicle and prostate gland. Though doses were referenced, more studies, different doses and pure extracts of thyme and rosemary are recommended.

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1. Introduction

In the recent times, there has been a great concern about the increasing trend of male infertility in men **(Krausz and Forti 2000)**. There is also equal concern about the declining semen quality and decrease in sperm counts in otherwise normal men **(Auger et al., 1995)**, domesticated **(Multigner et al., 2000)** and wild animals **(Donohoe et al., 2000)**. The causative factors are essentially environmental, occupational and/or dietary. Dietary toxins such as mycotoxins are among the major contributors to deterioration of male reproductive health **(Hussein and Brasel 2001)**. These toxic compounds, aflatoxins (AFs), ochratoxins, zearelenone, fumonisins, tremorgenic toxins, etc., are produced by certain strains of fungi which grow on moist cereals, nuts, seeds, herbs, medicinal plants, dried vegetables and food preparations **(Sarin et al., 2001)**. AFs are mycotoxins produced by *Aspergillus flavus* and *A. parasiticus*. They occur in a wide range of food and feed commodities **(Carlson et al., 2001)**. The most potent mutagens and hepatocarcinogens **(Groopman et al., 1996)**. At smaller doses during subchronic toxicity, as would usually happen during dietary exposure, AFs produce a milder effect known as aflatoxicosis, which is reflected as feed refusal, decreased feed efficiency, stunted growth, decreased milk production and impaired reproductive efficiency **(Oguz and Kurtoglu 2000)**.

Herbs are naturally rich in bioactive plant products with food value to keep energy balance in the body and substantial therapeutic value in several diseases **(Sharma, 2010).** Natural herbs are widely consumed by humans on a daily basis; these natural products have many biologic and pharmacologic properties **(Hosseinimehr, 2014)**.

Thyme (*Thymus vulgaris L*.) is belonging to the Lamiacea family and is aromatic native herbs in the Mediterranean region. The leafy parts of thyme and its essential oil have been used in foods for the flavour, aroma and preservation so added to meat, fish and food products and also used as herbal medicinal products. Thymol and carvacrol displayed a concentration dependent antioxidant capacity **(Undeger. et al., 2009)**. The main constituents of essential oil extracted from thyme were borneol, thymol,carvacrol methyl ether, camphene, α-humulene and carvacrol **(Bounatirou et al., 2007** and **Amarowicz et al., 2008)**. Thyme functions as a liver decontamination tonic, promotes blood circulation and functions as an exciting stimulant for the entire system. The stimulating action on the nervous system makes the herb a brilliant remedy for physical as well as mental fatigue, alleviating tension, anxiety and sleeplessness. The herb is also effective in treating depression or mood changes (**Höferlet al., 2006**).

Rosemary (*Rosemarinus officinalis*), which is one of household herbs, used as spices in foods, and employed in traditional medicine. Extracts of rosemary contains flavonoids and phenols which showed antioxidant properties **(Nabavi et al., 2015)**. These polyphenols have shown biological activities in vitro as anti-tumor, chemopreventive **(Razavi-Azarkhiavi et al., 2014)** and anti-inflammatory agents and may play a role by regulating the activity and/or expression of certain enzymatic systems implicated in relevant physiological processes like apoptosis, tumor promotion, intracellular signal transduction or xenobiotic-metabolizing enzymes in the liver **(Del Bano et al., 2006)**. It is useful for memory **(Moss et al., 2003)**, a hair growth stimulator **(Murata et al., 2013)**, and acts as antispasmodic, smooth muscles relaxant, memory booster **(Machado et al., 2012).**

Thyme and rosemary play a role in hepatoprotectivity and act as antiaflatoxicosis. This study has attempted to investigate effect of subacute dose of aflatoxin AFB1 on fertility in albino rats and possibly to predict any benefits or harms of rosemary and thyme leaves powder.

2. Materials and methods

Production of aflatoxin

Investigation of fungal contamination and detection of aflatoxin. One hundred samples of poultry rations and processed animal feeds (50 of each) were collected from markets at Cairo governorate for investigation of fungal contamination and detection of aflatoxin. The collected samples were screened for fungal contamination**.** The toxigenic strains of *A. flavus* were obtained continuously colonies for repeated sub-culturing according to **(Smalla et al., 1998).** After incubation of plates for 3 days at 30°C, from the grown fungi, hyphal tips or single inoculums were transferred to test tubes containing slant PDA medium. The purified fungi were identified by the author according to **Pitt and Hocking, (2009).**

Cultivation, extraction and estimation of aflatoxins: Isolated strains of *Aspergillus flavus* from the collected samples were inoculated into flasks containing 50 ml of sterile yeast extract solution 2% containing 20% Sucrose (YES). The inoculated flasks were incubated at 25ºC for 7-10 days. At the end of the incubation period, extraction and purification of produced aflatoxins using immunoaffinity column and quantitatively estimated by fluorometric method according to **(AOAC, 1990) and (Hansen, 1993).** Standard Aflatoxin: standard aflatoxins B_1, was purchased from sigma chemical company (USA).

Experimental animals

Forty eight apparently health male albino rats weighed (150-170 g) were housed under hygienic conventional conditions in suspended stainless steel cages. Rats were acclimatized to laboratory conditions for 1 week prior to experiment; rats fed on healthy commercial pelleted basal diet free from any cause of disease. Drinking water was supplied in glass bottles, ad libitum.

Experimental Design

Rats were divided randomly into 6 equal groups. Animals of the first group were given healthy commercial pelleted basal diet and kept as a negative control. The animals of groups 2, 4 and 6 were injected intraperitoneal with a single dose AFB1, 1.5 mg/kg body weight freshly prepared in dimethyl sulphoxide **(Bao, 2002)**. Then on the second day the diet of rats were supplemented with 5% commercial thyme leaves powder **(Al Badr, 2011)** for groups 3&4 and 2.5 % commercial rosemary leaves powder **(Abd El-Ghany et al., 2012)** for groups 5 & 6,. The second group was left without any treatment and kept as positive control. The period of feeding was continued for 28 days.

Experimental Procedures

Rats were weighed at the beginning and at the end of the experiment. After completion of the experimental period, the rats were fasted overnight, blood samples were collected from the retro-orbital venous plexus from each animal under ether anesthesia. Blood samples were left to clot and the sera were separated using cooling centrifugation at 3000 rpm for 15 min and stored at -20ºC until analysis. After collection of blood samples, animals were killed by cervical dislocation. Testis, epididymis, prostate gland, and seminal vesicles

were quickly removed, cleared of the adhering tissues, and weighed wet to the nearest milligram and used for the determination of tissue somatic indices (TSIs).

TSI = [Weight of the tissue (g)/ Body weight of the animal (g)] X100.

Testis were used for determination of total antioxidant capacity and lipid peroxidase.

Analysis of Epididymal Sperm

Epididymal sperm were obtained by chopping cauda epididymis in physiological saline (0.9% NaCl in distilled water). The sperm density was determined using a Neubauer chamber (Rohem, India) as described by **Belsey et al. (1980)**. The data were expressed as millions/mL. Progressive sperm motility was evaluated by the method described by **Belsey et al. (1980)** within 5 minutes following their isolation from cauda epididymis at 37^{o}C, and the data were expressed as percentage of motility.

Hormones and biochemical assays

Enzyme immunoassay test for serum testosterone, estradiol and progesterone were performed according to manufacturer instructions. Testosterone and estradiol were determined by using kits purchased from Diagnostic Biochem Canda Inc. The sensitivity of the assay was (0.022 ng/ml) for testosterone and (10 pg/ml) for estradiol. Progesterone was determined by using kit purchased from Immunospec Corporation with minimum detectable limit of (0.2 ng/ml).

Lipid peroxidation (LPO) as malonaldehyde (MDA) level in homogenate testis tissue was determined according to **Aebi (1974) and Ohkawa et al., (1979)** respectively. Total antioxidant capacity (TAC) in testis homogenate waestimated according to kit instructions. Kit was purchased from Biodiagnostic Co. (Cairo, Egypt).

The extraction, purification and measurement of aflatoxins residues in Testis, Seminal vesicles and Prostate glands of rats after experimental work was monitored according to the method described by **Mazzani et al. (2001)** and **Ozaslan et al. (2011).**

Statistical analysis

Data obtained were statistically analyzed using analysis of variance (ANOVA) using F- test according to **SPSS-18 (2009).**

3. Results and Discussion

Incidence of members of *Aspergillus* species isolated from poultry rations and processed animal feeds was showed in table (1). *Aspergillus flavus* was the most frequent mould of *Aspergillus* species isolated from all tested samples of Poultry rations and Processed animal feeds (50% with mean count of 7.6 x 10 ± 0.2x10) and (38% with mean count of 5.0 x 10^2 ± 2x10) respectively. Similar results were previously reported by **Krnjaja et al., (2008); and kana et al., (2013).** Other members of *Aspergillus* were isolated in various frequencies (Table, 1). The isolation of large numbers of fungi in collected samples may be due to the exposure to environmental factors as high temperatures and humidity during preparation, and/or storage. Direct contamination for samples itself may be occur during handling, processing and transportation which help in all ways to fungal pollution by different genera of fungi. Significant levels of aflatoxin were produced by *A. flavus* isolated from collected samples (Table, 2), where, the maximum levels of toxin were obtained from *A. flavus* isolated from Processed animal feeds (42% of isolates produced mean level of 260 ± 0.1 ppb) followed by those isolated from Poultry rations (40% with the mean level of 10.0 ± 0.71 ppb). **Andrew and Christopher, (1994) and Smith et al., (1994).**

Table (3) represented the effect of thyme and rosemary as antiaflatoxicosis on weight gain, testis, seminal vesicles and prostate gland weights of male albino rats. Weight gain of aflatoxin injected rats showed unexpectedly significant increase comparing to all tested groups. There was a significant decrease in somatic index of testis, though non-significant difference in weight of testis between aflatoxicated rats and control. No significant difference in seminal vesicles and prostate gland. These findings was completely disagreed with **EL-shewy and Ebrahem 2004**, **Tas et al., 2010, Hamzawy et al., 2012** and **Supriya et al., 2014** who reported Significant decrease in body weights were found in AFB1-treated rats when compared to controls. Significant decrease in the indices of testis and accessory sex organs were also observed. The authors added that this loss of body weight may be due to improper assimilation or metabolism of feed due to hepatotoxic effect of AFB1. The reverse obtained results in the current study may be due to differences in dosage, route of administration and accumulative effect of AFB1 comparable to most previous studies. In current study the significant increase in weight gain in intoxicated rats may act as a clue for all obtained results, so studying the effect of subacute dose of AFB1 on leptin hormone is recommended.

Thyme leaves powder fed to healthy or aflatoxin injected rats resulted in significant decrease in weight gain compared to controls and aflatoxins respectively. Concerning effect of thyme leaves powder supplemented to the diet on weight of sexual organs or its tissue somatic indices (TSIs) table (3) showed no significant in testis weight or TSI. Seminal vesicles weights decreased significantly. Prostate gland though insignificant in weight but there was a significant decrease in TSI. Intoxicated rats were fed on diet supplemented with thyme leaves powder affected weight of testes insignificantly, but significantly decreased TSI in comparable to aflatoxin group. Also there were a significant decrease in seminal vesicles

and prostate gland weights with no significant in TSI of prostate gland. Reported data were agreed with **Al Badr 2011**, **Hamzawy et al., 2012** and **Neven et al., 2012**.

Rosemary leaves powder supplemented to apparently health rats increased the weight gain significantly in comparable to control group, but reverse effect on weight gain occurred in intoxicated rats as the weight gain decreased significantly comparing to aflatoxin group. No significant effect on testis and prostate gland weights due to rosemary supplemented diet either in healthy or intoxicated rats in comparable to control and aflatoxicated rats respectively, whereas a significant decrease in seminal vesicles weights and its TSI were occurred. Obtained data were the same as that stated by **Nusier et al., 2007, Abd El-Ghany et al., 2012** and **Vala et al., 2013**.

Some semen traits of male albino rats in healthy and aflatoxicated rats with or without thyme or rosemary supplemented diets showed in table (4). No significant difference in sperm count and motility between intoxicated rats and controls. It is worthy to focus that intoxicated rats exhibited the lowest sperm motility though insignificancy. The obtained results were disagreed by **EL-shewy and Ebrahem 2004** who reported a highly significant reduction in the percentage of morphologically normal sperm and epididymal sperm count in relation to control rats after three weeks of dosing with AFB1.Also, **Tas et al., 2010** and **Supriya et al., 2014** recorded significant decrease in sperm count and motility.

Thyme leaves powder supplemented to the diet of male albino rats improved sperm count and motility, with a significant increase in motility incomparable to aflatoxin group. Previous studies showed no significant effect of *Thymus vulgaris* alcoholic extract on sperm parameters in mice **(Alasadiy 2014**) and a significant increase in sperm count and decrease in motility in male broiler which might be a product of both potent antioxidant properties and androgenic activities of thyme **(Shanoon and Mahdi 2012).**

No significant effect of rosemary supplemented diet on sperm count and motility either in healthy or intoxicated rats. These findings were the same as that of **Vala et al., 2013.** On the other hand **Nusier et al., 2007** reported a significant decrease in sperm count and motility in rosemary treated rats.

Sexual hormone levels presented in table (5) showed a significant increase in testosterone and estradiol levels in intoxicated rats compared to the control group, whereas, no significant difference in testosterone estradiol ratio T/E_2 was observed. Progesterone exhibited a significant decrease. Concerning obtained results of the effect of intrapritoneal injection of AFB1 (1.5 mg/kg body weight) on testosterone and estradiol levels in male rats may it looks in a reverse direction with **(EL-shewy and Ebrahem 2004 & Adedara et al., 2014)** as they reported serum testosterone level was significantly decreased after dosing AFB1**.**Also oral administration of the aflatoxin B 1 strictly alters the concentrations of FSH, LH, prolactin, and testosterone in male Wistar rats (**Hasanzadeh et al., 2011).** T/E_2 ratio showed no alteration in sexual hormones and keeping the balance of this ratio saved male fertility though AFB1 injection. This finding may be related to significant increase in weight gain, dose and route of administration. AFB1 suppressed testosterone secretion in a dose-dependent manner **(Adedara et al., 2014).**

Thyme powder supplemented to the diet of either injected or not rats resulted in no significant difference in testosterone and progesterone levels comparing to aflatoxicated rats, while a significant increase in testosterone and decrease in progesterone levels comparable to control and intoxicated rats, respectively. Though a significant increase in estradiol level in male rats that fed thyme leaves powder supplemented to diet of either intoxicated rats or not, there is a significant decrease in T/E_2ratio. These findings in a large extent are in concomitant with **(Zava et al., 1998)** who found that thyme has demonstrated estradiol and progesterone receptor-binding activity in vivo. Based on laboratory research, thymol may inhibit testosterone-induced transcriptional activity **(Chen et al., 2007).**

Rosemary increases testosterone and estradiol levels either in intoxicated rats or not comparing to both control and aflatoxicated rats. Progesterone level decreased significantly in injected rats fed diet supplemented with rosemary accompanied by significant increase in T/E_2 in comparable to all tested groups, whereas a significant increase in progesterone level and decrease in T/E_2 ratio in rats fed diet supplemented with rosemary leaves powder. These results disagreed by **Nusier et al., 2007** who recorded a significant decrease in testosterone due to Ingestion of rosemary (*R. officinalis* L.) at levels of 250 and 500 mg/kg body wt for 63 days**.** Also **(Vala et. al., 2013)** *Rosmarinus officinalis* may have antiandrogenic effect potentially indicating the possibility of developing herbal male contraceptive. In the current study rosemary used as a leaves powder, this may be a cause of significant increase in testosterone.

Table (6) showed a significant decrease in total antioxidant capacity (TAC) and a significant increase in lipid peroxides (LPO) of testis tissues in intoxicated rats comparable to the control. Pro-oxidant and antioxidant balance is vital for normal biological functioning of the cells and tissues **(Velmurugan et al., 2004).** Increases of the lipid peroxidation product (MDA) can be used as sensitive indicator of the toxicity of AFB1 **(Evans and Maxwell 1987)**. In our study, significant increase was observed in the level of LPO in testis tissue of rats treated with a single dose of AFB1. Similar

observations have been reported by different researchers **Shen et al., (1994) & Towner et al., (2002)**. The increase of MDA concentration during aflatoxicosis may confirm the impaired immunomodulation is resulting in underlining mechanisms for AFB1 induced cell injury and DNA damage **(Tas et al., 2010)**.

Thyme supplemented to the diet of intoxicated rats did not exhibit any improvement in total antioxidant capacity and lipid peroxides.Thyme leaves powder in the diet of healthy rats showed no significant difference in TAC&LPO compared to controls. These findings were disagreed by **(El-Nekeety et al. 2011, Abd El Kader & Mohamed 2012 and Hamzawy et. al., 2012)** who reported a significant improvement in oxidative stress in liver due to thyme extracts in control and hepatic injured rats. Extract or oil essence may be considered as a must to realize antioxidant property of *Thymus vulgaris*.

Rosemary supplemented to the diet of intoxicated rats did not exhibit any improvement in total antioxidant capacity and lipid peroxides, moreover; it had a bad effect on both TAC and LPO comparable to control. The obtained results seemed to be parallel with **(Galobart et al., 2001)** they found that, the dietary supplementation with 500 or 1000 mg/kg of a commercial rosemary extract had no effect on the lipid oxidative stability of eggs enriched with omega-3 fatty acids.On the other hand, **Abd El-Ghany et al. (2012), Labban et al., 2014 and El-Morsy et al., 2015** concluded the antioxidant property of rosemary extract. The antioxidant activity of polar extracts of rosemary is related to the content of phenolic compounds (i.e. carnosol, carnosic acid) which have shown a variety of pharmacological activities or cancer chemoprevention **(Shabtay et al., 2008**) and therapy in vitro and, in vivo models **(Shabty et al., 2008)**. Extract or oil essence may be considered as a must to realize antioxidant property of *Rosemarinus officinalis*.

It is interesting to report here that the aflatoxicated rats that treated with rosemary and thyme showed (Table 7) a significant diminution the levels of aflatoxin residues in testis, seminal vesicle and prostate gland. Whereas, nearly degradation of aflatoxin residues were more detected in rosmary than thyme. Similar results were obtained by **Verma et al., (2008) and Awad et al., 2011** who detected that the treatment of aflatoxicated rats with herbal extracts resulted in a significant degradation of aflatoxins from vital organs particularly from testis, seminal vesicle and prostate gland.

It is worthy to report that intraperitoneal injection with 1.5 mg/kg body weight AFB1 increased weight gain significantly that may consequently led to significant increase in both testosterone and estradiol but insignificant effect on T/E_2 ratio, sperm count and sperm motility. Also, there were a significant decrease in TAC and decrease in LPO accompanied by high level of aflatoxin residue in testis tissues. Thyme and rosemary leaves powder added to ration in (5 % and 2.5%, respectively) were insufficient to shed a light on sharp effects on male fertility. Though doses were referenced, more studies, different doses and pure extracts of thyme and rosemary are recommended.

Table (1): Incidence of members of Aspergillus species isolated from poultry rations and processed animal feeds

	Poultry rations			Processed animal feeds		
Fungal geanera	+ve	%	Colony count ± SE	+ve	%	Colony count ±SE
A. flavus	25	50	7.6 x 10± 0.2x10	19	38	5 x 10^2±2x10
A. niger	18	36	2.8 x 10±0.3x10	17	34	2 x 10±1x10
A. candidus	15	30	2.5 x10±0.1 x10	5	10	0.5x10^2±0.03
A. fumigatus	10	20	1.0 x 10±0.0	5	10	3x10±0.1x10
A. ochraceus	8	16	0.7 x 10±0.0	3	6	1x10±0.3x10

Table (2): Levels of aflatoxins production by *A. flavus* isolated from Poultry rations and Processed animal feeds

Source of isolates	No. of isolates	+ ve samples		Mean of count	Levels of AF ppb		
		No.	%		Max	Min	Mean ± SE
Poultry rations	25	10	40	16 ± 2.0	13	5.0	10.0± 0.71
Processed animal feeds	19	8	42	10 ± 0.042	1000	150	260 ± 0.1

Table (3): Effect of thyme and rosemary as antiaflatoxicosis on weight gain, testis, seminal vesicles and prostate gland weights of male albino rats

Treatments	Weight gain	Testis	Seminal Vesicles	Prostate gland
Control	71.75+8.17^{c}	2.53±0.07^{a} (1.042±0.03^{a})	1.32±0.09^{a} (0.58±0.018^{a})	0.095±0.006^{ab} (0.04±0.003^{a})
Aflatoxin	91.88±2.98^{a}	2.23±0.18^{a} (0.93±0.04^{b})	1.38±0.006^{a} (0.52±0.026^{ab})	0.105±0.006^{a} (0.040±0.003^{a})
Thyme	66.25+13.22^{d}	2.26±0.07^{a} (1.082±0.042^{a})	0.929±0.04^{bc} (0.46±0.043^{bc})	0.103±0.014^{a} (0.152±0.11^{b})
Thyme & Afla	71.75+15.23^{c}	2.55±0.08^{a} (1.057±0.041^{a})	1.045±0.08^{b} (0.43±0.023^{c})	0.07±0.005^{b} (0.029±0.003^{a})
Rosemary	83.38+6.57^{b}	2.62±0.06^{a} (1.126±0.028^{a})	0.795±0.09^{c} (0.39±0.016^{c})	0.086±0.006^{ab} (0.038±0.113^{a})
Rosemary & Afla	61.75+8.24^{d}	2.49±0.09^{a} (1.092±0.037^{a})	0.787±0.05^{c} (0.39±0.034^{c})	0.078±0.004^{ab} (0.144±0.07^{b})

- Values are expressed as mean ± SE (n=8) within the same column with different superscripts are significantly different ($p< 0.05$)-Data expressed in (g)- Values in the parentheses are (TSI); **Aflatoxin:** A group that injected I.P. with 1.5mg\kg bodyweight aflatoxin B1; **control:** A control group fed on basal diet; **Thyme:** A group fed on basal diet supplemented with 5% thyme**; Thyme & Afla:** A group that injected I.P. with 1.5mg\kg bodyweight aflatoxin B1and fed with basal diet supplemented with 5%thyme; **Rosemary:** A group fed on basal diet supplemented with 2.5% rosemary; **Rosemary & Afla:** A group that injected I.P. with 1.5mg\kg bodyweight aflatoxin B1and fed with basal diet supplemented with 2.5% rosemary.

Table (4): Effect of thyme and rosemary as antiaflatoxicosis on sperm count and motility in male albino rats

Treatments	Sperm Count (10^{6}/ml)	Mass sperm motility (%)
Control	81.0±4.01^{b}	70.12±2.88^{abc}
Aflatoxin	89.0±8.4^{ab}	66.05±1.34^{c}
Thyme	79.83±8.6^{b}	78.68±1.01^{a}
Thyme & Afla	107.50±4.04^{a}	77.88±0.80^{a}
Rosemary	90.17±1.68^{ab}	75.20±2.47^{ab}
Rosemary & Afla	77.33±4.42^{b}	71.33±2.2^{abc}

- Values are expressed as mean ± SE (n=8) within the same column with different superscripts are significantly different ($p< 0.05$); **Aflatoxin:** A group that injected I.P. with 1.5mg\kg body weight aflatoxin B1; **control:** A control group fed on basal diet; **Thyme:** A group fed on basal diet supplemented with 5% thyme**; Thyme & Afla:** A group that injected I.P. with 1.5mg\kg bodyweight aflatoxin B1 and fed with basal diet supplemented with 5% thyme; **Rosemary:** A group fed on basal diet supplemented with 2.5% rosemary; **Rosemary & Afla:** A group that injected I.P. with 1.5mg\kg body weight aflatoxin B1and fed with basal diet supplemented with 2.5% rosemary.

Table (5): Effect of thyme and rosemary as antiaflatoxicosis on some sexual hormones in male albino rats

Treatments	Testosterone[T] (ng\ml)	Estradiol[E_2] (pg\ml)	T/E_2	Progesterone (ng\ml)
Control	2.96±0.61^{c}	39.38±1.99^{e}	0.078±0.005^{b}	2.74±0.14^{b}
Aflatoxin	4.46±0.95^{b}	58.38±7.21^{d}	0.070±0.011^{bc}	2.04±0.14^{cd}
Thyme	5.01±0.17^{b}	96.25±4.7^{b}	0.053±0.002^{c}	1.71±0.01^{d}
Thyme&Afla	5.0 ±0.25^{b}	111.25±8.33^{a}	0.049±0.0 1^{c}	2.07±0.004^{c}
Rosemary	4.25±0.17^{b}	76.25±1.25^{c}	0.055±0.002^{c}	4.0±0.15^{a}
Rosemary&Afla	7.98±0.31^{a}	80.63±3.2^{c}	0.100±0.001^{a}	0.44±0.008^{e}

- Values are expressed as mean ± SE (n=8) within the same column with different superscripts are significantly different ($p< 0.05$.**T/E $_2$**: testosterone /estradiol ratio. **Aflatoxin:** A group that injected I.P. with 1.5mg\kg body weight aflatoxin B1; **control:** A control group fed on basal diet; **Thyme:** A group fed on basal diet supplemented with 5% thyme**; Thyme & Afla:** A group that injected I.P. with 1.5mg\kg body weight aflatoxin B1 and fed with basal diet supplemented with 5% thyme; **Rosemary:** A group fed on basal diet supplemented with 2.5% rosemary; **Rosemary & Afla:** A group that injected I.P. with 1.5mg\kg body weight aflatoxin B1and fed with basal diet supplemented with 2.5% rosemary.

Table (6): Effect of thyme and rosemary as antiaflatoxicosis on total antioxidant capacity and lipid peroxidase in testis tissue of male albino rat

Treatments	TAC (µmol\g)	LPO (nmol\g)
Control	8.80±0.13[a]	0.86±0.003[e]
Aflatoxin	8.34±0.007[bc]	2.14±0.01[d]
Thyme	8.56±0.17[ab]	1.06±0.002[e]
Thyme&Afla	7.59±0.15[d]	2.54±0.12[c]
Rosemary	8.16±0.008[c]	2.94±0.005[b]
Rosemary&Afla	7.52±0.003[d]	3.23±0.008[a]

- Values are expressed as mean ± SE (n=8) within the same column with different superscripts are significantly different ($p< 0.05$**); TAC**: total antioxidant capacity**. LPO:** lipid peroxides**. Aflatoxin:** A group that injected I.P. with 1.5mg\kg bodyweight aflatoxin B1; **control:** A control group fed on basal diet; **Thyme:** A group fed on basal diet supplemented with 5% thyme**; Thyme & Afla:** A group that injected I.P. with 1.5mg\kg body weight aflatoxin B1and fed with basal diet supplemented with 5% thyme; **Rosemary:** A group fed on basal diet supplemented with 2.5% rosemary; **Rosemary & Afla:** A group that injected I.P. with 1.5mg\kg body weight aflatoxin B1 and fed with basal diet supplemented with 2.5% rosemary.

Table (7): Detection of aflatoxin residues in the internal organs of rats after administration of aflatoxin alone or in combination with thyme and rosemary

Organs	Levels of AFB1 residues in organs of treated groups of rats(ppm)			
	Control	Aflatoxin	Thyme&Afla	Rosemary&Afla
Testis	0	1.0	0. 3	0.2
Seminal vesicles	0	1.2	0. 5	0. 3
Prostate gland	0	1.0	0.4	0. 3

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