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# Protective and detoxificating effects of selenium nanoparticles and thyme extract on the aflatoxin B1 in rabbits

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## ABSTRACT

Nanotechnology's application in biomedicine has piqued the curiosity of all authorities throughout the world. Different fungus species and aflatoxins were recovered from rabbit feed at significant quantities up to 300 ppb in this investigation. Selenium nanoparticles (Se-NPs) were generated using a chemical process, described briefly, and utilized to reduce the harmful effects of aflatoxicosis in rabbits. Biochemical parameters in tissues and sera of all aflatoxicated rabbits revealed that it caused the development of reactive oxygen species (ROS), an increase in nitric oxide levels, and malonaldehyde levels. While Total Antioxidant Capacity (TAC) activities are reduced, additional antioxidants in rabbit homogenate liver tissues will be found. In aflatoxicosis, decreased serum retinol and a-tocopherol were also found. For aflatoxicated rabbits, oral treatment of SeNPs and thyme restored these alterations to normal levels. Furthermore, SeNPs and thyme triggered the breakdown of AFB1 residues in liver tissues, suggesting that SeNPs and thyme can be introduced to animal feeds as antioxidants with good effects on animal health and mycotoxins removal.

**Keywords:** Nanotechnology, Se-NPs, Aflatoxicosis, Thyme, Antioxidant, fungi.

## INTRODUCTION

Recently, the biomedical application of nanomaterials gained large attention of all authorities related to human and animal health (Khalaf *et al.,* 2019). Several diseases cause serious public health problems on a human resulting in carcinogenic potential and liver cells necrosis (Tiew *et al.,* 2020). Whereas, the majority of used traditional drugs in the control of these diseases are not effective due to elaboration of microbial resistance during their frequent use. This problem gained more attention in finding novel effective agents from metallic nanomaterial for treatments of these infections diseases (Moghimi *et al.,* 2005). The aflatoxin B1(AFB1) is detected in cereals and feed and produced by mycotoxigenic *Aspergillus flavus and A. parasiticus* that resulted in several significant health hazard effects (El Nahass *et al.,* 2019). In developing countries such as Egypt, liver cancers are associated with aflatoxicosis in humans and animals and it can be reacted with DNA and produced reactive oxygen species (ROS), resulting in damage and destruction of lipids, DNA, and proteins (Benkerroum, 2020). These adverse effects have potentiated the occurrence of genetic toxicity and apoptosis. Moreover, the high worldwide morbidity and mortality of mycotoxicosis in humans and animal resulted in serious economic losses in their health activities and production (El-Nahass *et al.,* 2019). Hence, several studies have been evaluated for the degradation and elimination of the toxic effects of aflatoxicosis. The majority of used traditional drugs in the control of mycotoxicosis are not effective during their frequent use. This problem gained more attention in finding novel effective agents from metallic nanomaterial for treatments of toxicities (El-Nahass *et al.,* 2019). In addition, the valuable uses of metals nanoparticles are due to their low doses toxicity and help in drugs delivery to target organs (Youssef *et al.,* 2019) and explore novel effective vaccines. Hence, the synthesis of nanomaterial by simple and safe methods and easy application has been taken all attention. They have significant properties such as large surface area, high bioavailability, and decrease toxicity than bulk materials such as SeNPs (Zhang *et al.,* 2012). The selenium nanoparticles can be produced by the green method and the concentration of (60 µg/ml) caused destruction of cells (Menon and Shanmugam, 2019). Other benefits of SeNPs include activation of the thyroid gland function, antioxidant activity, and preventing harmful products of metabolism (Ulaiwi, 2018). Moreover, the recent strategies directed to the elimination and treatments of aflatoxicosis via the use of natural products in the environment that are available and ecofriendly such as the Thyme plant (a native herb) (Swayeh *et al.,* 2014). It has several potentials as antioxidants and antimicrobial and can be used instead of chemical drugs (Rasooli *et al.,* 2006). In addition, thyme is a potent natural antioxidant agent and can be reduced oxidative stress of toxins (Grigore *et al.,* 2010 and Placha *et al.,* 2014) and have an effective reduction of tumor necrotic factor (TNF- α) (Abdel-Aziem *et al.,* 2014). Hence, the present work was undertaken for evaluation the prevalence of fungi and aflatoxins in rabbits' feeds and drinking water in farms at El Minofya governorate. The induction of aflatoxicosis and their degradation by SeNPs and thyme extracts for improving the biochemical and pathological adverse changes due to aflatoxicosis in rabbits was also investigated.

## MATERIAL AND METHODS

**Ethical approval:**

The current work was done and accepted as recommended local laws and regulations.

**Feed samples:**

A total 100 of rabbits' rations and consumed water samples (50 of each) were collected from rabbits farms El- Menofia governorate, Egypt, where, animals sustained signs of poisoning, diarrhea, and deaths in some cases. The mycological and aflatoxins residues in samples were detected.

**Thyme and chemicals:** They were purchased from ALDRIK Sigma chemical company.

**Prevalence of mold and yeasts in samples**

Samples were processed and examined for recovering and characterization of molds as recommended by(ISO 2008; Pitt and Hocking , 2009)

**Synthesis and detection of aflatoxins:** The recovered *A. flavus* in the present study were used for the synthesis of aflatoxins on yellow corn as recommended by (Smith, 1997). In addition, the toxin content of rabbits' feeds and water samples were determined and quantified as the method as (AOAC, 2000; Refai and Hassan, 2013).

**Chemical production and identification of SeNPs** (Verma and Maheshwari, 2018). The SeNPs were produced by the chemical reduction method. Briefly, in sterile flask contained 9ml of double-distilled water, 3 ml ( 25 mM) sodium selenite, 3 ml (100 Mm) glutathione (reduced form), and 0.15gm bovine serum albumin as stabilizer were added. Continuous stirring the flask for 1 hour using a magnetic stirrer and Sodium hydroxide ( 1M) poured till pH reach to 9 . The color of the solution changed to a red color indicating the formation of Se-NPs. The prepared Se-NPs were identified and their shape, particles size, and morphology were measured by UV-visible spectra, XRD patterns (Atul *et al.,* 2010).

**Experimental Design** (Cam, 2008; Zhou and Wang, 2011; Abdel-Azeim *et al.,* 2013; Hassan *et al.,* 2016)

The Se-NPs and Thyme were evaluated for improving the adverse effects of aflatoxicosis in rabbits. A 35 healthy New Zealand male rabbits (aged 6-8 weeks) were divided into 5 groups as observed in Table 1:

| Groups | Feeding | Treatment |
|---|---|---|
| **G1 (Negative control)** | Healthy Feed and water ad libitum | No treatment |
| **G2 (Positive control)** | Orally given AFB1 at a dose of 50 ug / animal/ day for 4 weeks | No treatment |
| **G3** | Orally given AFB1 at a dose of 50 ug / animal/ day for 4 weeks | Orally administration of Aquas extract of thyme (500mg/Kg body weight) once daily |
| **G4** | Orally given AFB1 at a dose of 50 ug / animal/ day for 4 weeks | Orally administration of Se-NPs (0.3mg/kg body weight/ 0.2 ml of buffer/ day (low dose) |
| **G5** | Orally given AFB1 at a dose of 50 ug / animal/ day for 4 weeks | Orally administration of Se-NPs (0.5mg/kg body weight/ 0.2 ml of buffer/ day (high dose) |

**Biochemical study:**

The blood samples we collected from all bird after experimental work and sera were separated for biochemical analysis. After scarification of animals, liver tissues were obtained for detection the antioxidant assays (Chitra *et al.,* 1999). Also, other antioxidant were determined as catalase; lipid peroxidation as malonaldehyde (MDA) and reduced glutathione (GSH) in liver tissues homogenate ( Aebi**,** 1974; Ohkawa *et al.,* 1979; Ellman, 1959), respectively. The measurements of total antioxidant capacity (TAC) (Koracevic *et al.,* 2001), Superoxide Dismutase (SOD) (Nishikimi *et al.,* 1972), and GSH-px (Paglia and Valentine, 1967) were undertaken, respectively. The protein content of tissues samples were measured by the method of (Bradford, 1976). The levels of vitamins were determined as method as (Henry *et al.,* 1974), and nitric oxide (NO) levels (Burgner *et al.,* 1999).

**Pathological study:**

After the end of the experimental study, liver and kidney tissues of all animals were collected and subjected for histopathological study as recommended by (Suvarna *et al.,* 2012).

**Statistical Analysis**

The statistical interpretation of the results was performed with One-Way ANOVA test. The results were given as mean ± standard error using SPSS 14 (2006). The value of $p<0.05$ was considered significant.

## RESULTS

**Table 2.** Prevalence of fungi in rabbits feeds and water samples.

| | No. | % | No. | % |
|---|---|---|---|---|
| **Total fungi** | 40 | 80 | 30 | 60 |
| ***A. flaves*** | 35 | 70 | 26 | 52 |
| ***A. niger*** | 9 | 18 | 6 | 12 |
| ***A. Ochraceus*** | 5 | 10 | 3 | 6 |
| ***A.fumigatus*** | 1 | 2 | 2 | 4 |
| ***Penicillum species*** | 22 | 44 | 12 | 24 |
| ***Fusariumspecies*** | 12 | 24 | - | - |
| ***Cladosporium species*** | 3 | 6 | 5 | 10 |
| ***Scopulariopsis species*** | 2 | 4 | - | - |
| ***Mucor Species*** | - | - | - | - |
| ***Candida species*** | 3 | 6 | 10 | 20 |

The tabulated data in Table (2), illustrated that out of 100 rabbits feed and water samples (50 of each), from private rabbits farms at El- Menofia governorate, Egypt. Forty samples of rabbit feed and thirty samples of consumed water were contaminated with fungi (80% and 60%), respectively**.** The highest incidence of *A.flavus* was recovered from 35 feed samples (70%) and 26 samples of water (52%), respectively. While, other *Aspergillus species* were isolated at relatively lower rates as *A. niger* recovered from(18% and 12%) and *A. ochraceus* from (10% and 6%), respectively.

**Table 3.** Levels of aflatoxins in rabbits feed and water (ppb).

| Samples | Total Aflatoxins in samples (ug/kg)(ppb) | | | | |
|---|---|---|---|---|---|
| | +ve | % | Max. levels | Min. levels | Mean levels |
| **Feed(50)** | 30 | 60 | 42.5±2.63 | 12±0.1 | 35.0±1.5 |
| **Water (50)** | 0 | 0 | ND | ND | ND |

ND: not detected during measurement of toxins by TLC method

In the presented data in Table (3), we detected that the incidence of AFB1 residues in rabbits' feeds were reached 60% of feeds samples. However, the mean values of AFB1residues in feeds was (35.0±1.5µg/kg) whereas, Afs residues did not detect in all water samples. Currently, most of the recovered *A. flavus* from the present rabbits' feed was aflatoxigenic.

**Table 4.** Levels of aflatoxins $B_1$ produced by *A. flavus* isolated from Rabbits rations.

| Source of tested *A. flavus* | No. of tested isolates | Aflatoxigenic isolates | | Levels of $AFB_1$ ppb | | |
|---|---|---|---|---|---|---|
| | | No. | % | Max | Min | Mean ± SE |
| Rabbits feed | 20 | 15 | 75 | 300.0 | 50.0 | 100.5 ± 7.1 |

The recorded data in Table (4) illustrated that 15 of 20 tested isolates (75%) were produced significant levels of AFs on yellow. The mean levels of produced AFB1 were (100.5 ± 7.1 ppb) and the maximum value was (300 ppb) and the minimum was (50 ppb).

In recent years, nanotechnology has been used employed in several biomedical fields (Bai *et al.,* 2018; Hassan *et al.,* 2020, 2021). Herein, production and identification of Se-NPs proceeded and the obtained nanoparticles were spherical in forms and of (60 nm) in size Fig., 1(A). While, the UV-VIS detected that the maximum absorption occurred at a wavelength of 400 nm Figure, 1(B).

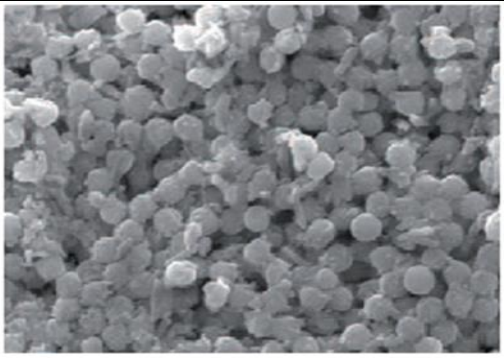

**Fig. 1(A):** SEM analysis of the SeNPs showed the size and morphology (60 nm) at 9,500 magnifications.

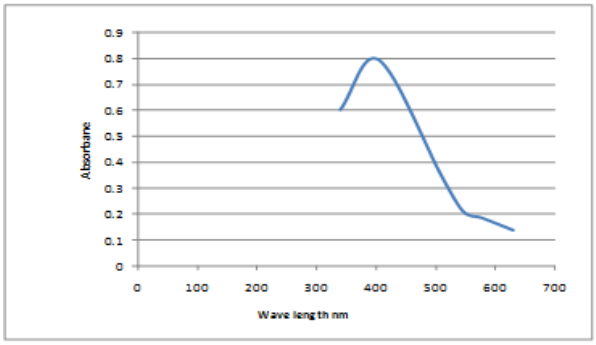


**Fig. 1(B):** The UV-VIS absorbance spectra of Se-NPs (60nm) at 400 nm wave length.

**Table 5.** Amelioration of AFB1-induced hepatocarcinogenesis by nano selenium and thyme (n = 5 for each group).

| Groups | MDA nmol/g tissue | TAC Mmol/l | Nitric Oxid | GSH µmol/mg protein | CAT IU/mg protein | Gpx u/mg protein | Sod u/mg protein |
|---|---|---|---|---|---|---|---|
| **Control** | $7.40^{d}$ ±0.36 | $6.40^{a}$ ±0.13 | $0.52^{d}$ ±0.01 | $411.60^{a}$ ±2.04 | $9.62^{a}$ ±0.07 | $17.12^{a}$ ±0.18 | $247.64^{b}$ ±8.70 |
| **AFB1** | $13.16^{a}$ ±0.48 | $3.63^{d}$ 0.08 | $0.76^{a}$ ±0.01 | $329.00^{c}$ ±9.66 | $5.10^{d}$ ±0.15 | $10.34^{d}$ ±0.41 | $224.38^{c}$ ±3.84 |
| **AFB1+low dose of SeNPs** | $9.16^{c}$ ±0.24 | $6.16^{a}$ ±0.06 | $0.63^{c}$ ±0.01 | $396.40^{ab}$ ±4.37 | $7.36^{b}$ ±0.10 | $14.21^{b}$ ±0.11 | $265.09^{a}$ ±4.87 |
| **AFB1+high doseofSeNPs** | $10.46^{b}$ ±0.28 | $5.58^{b}$ ±0.12 | $0.66^{b}$ ±0.01 | $355.2^{b}$ ±5.17 | $609^{c}$ ±0.17 | $13.69^{b}$ ±0.19 | $241.94^{b}$ ±6.06 |
| **AFB1+ Thyme** | $10.42^{b}$ ±0.07 | $4.74^{c}$ ±0.07 | $0.65^{bc}$ ±0.01 | $355.60^{b}$ ±8.06 | $5.83^{c}$ ±0.18 | $12.91^{c}$ ±0.21 | $257.29^{ab}$ ±0.59 |

Significantly different ($p<0.05$) according to Duncan´s test.

Our biochemical findings in the Table (5) illustrated that AFB1 increased the concentration of NO and MDA but reduced values of GSH and TAC. Also, the values of SOD, CAT, and GSH-PX in the homogenate of liver tissues of rabbits have been decreased.

**Table 6.** Amelioraton of AFB1-induced hepatocarcinogenesis by nano selenium and thyme extract (n= 5 for each group).

| | Vit c (µg/dl) | Vit e (µg/dl) | Vit a (µg/dl) | β carotene (µg/dl) |
|---|---|---|---|---|
| Control | 0.70[a] ±0.01 | 508.58[a] ±2.30 | 43.54[a] ±0.92 | 24.61[a] ±1.20 |
| AFB1 | 0.39[c] ±0.01 | 364.52[c] ±3.32 | 32.26[d] ±0.51 | 13.46[c] ±0.21 |
| AFB1+ nanoselenim | 0.57[b] ±0.02 | 446.19[b] ±10.91 | 40.04[b] ±0.28 | 19.29[b] ±0.32 |
| AFB1+ nanoselenim | 0.58[b] ±0.01 | 438.55[b] ±6.01 | 37.01[c] ±0.52 | 18.35[b] ±0.76 |
| AFB1+ Thyme | 0.57[b] ±0.02 | 443.15[b] ±7.40 | 37.60[c] ±0.45 | 18.59[b] ±0.60 |

Herein, results in Table (6) showed the low serum retinol, and a-tocopherol in aflatoxicated rabbits compared to control animals. Currently, data observed. In Table (5 and 6) regarded that feed supplementation with SeNPs and Thyme extract in aflatoxicated rabbits resulted in ameliorating their toxic effects on biochemical functions of vital organs and increased the liver antioxidant activity.

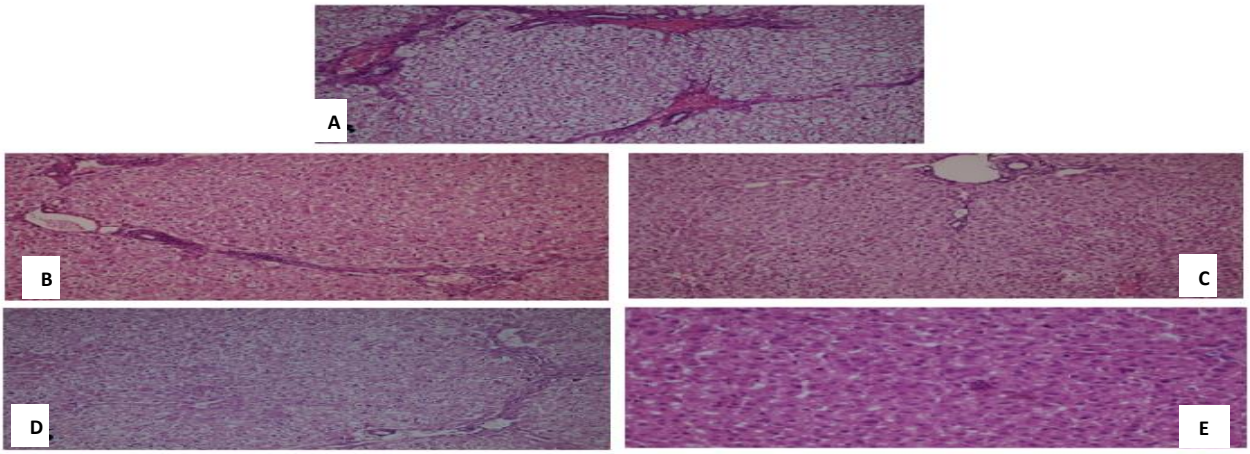


**Fig.(2):**Aflatoxicated control group revealed thickening of portal traids' areas, portal-portal bridge formation associated with sever hydropic degeneration of hepatocytes and congestion of hepatic blood vessel. H&E X 200 (A). High dose selenium-treated group showed improvement of hepatocytes status, reduction in the incidence of portal- portal bridge formation and less thickening of portal traids' areas. Multiple foci of neutrophil infiltration were demonstrated (B). Low dose selenium-treated group had healthy hepatocyte, absence portal- portal bridge formation and normal portal traids' areas H&E X 200 (C). Thyme- treated group showed mild portal bridge formation H &E x200 (D). Thyme treated group exhibited minute foci of neutrophils. H &EX 200 (E).

The histopathological status of renal tissues revealed that the aflatoxicated group showed severely swollen distal tubular lining epithelial and reduction of Bowman,s space. Fig. 3 (A). Whereas the supplementation of thyme, Se-NPs in feeds of aflatoxicated rabbits resulted in a mild degree of tubular degeneration, and Bowman,s spaces appeared normal. Fig. 3 (B, C, D).

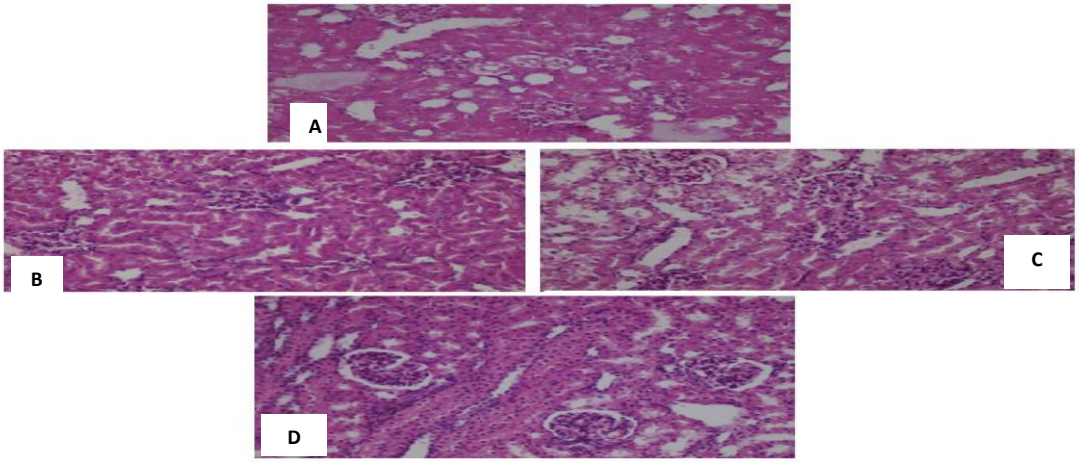


**Fig 3.** Aflatoxicatedgroup revealed highlyesinophilic tubular lining, severely swollen distal tubular lining epithelia. The renal corpuscles showed reduction of Bowman's space H&E X 200 (A).High dose selenium-treated group revealed mild degree oftubularepithelia hydropic degeneration associated with reduced Bowman'spaces (B). Low dose selenium-treated group had healthy hepatocyte, absence portal- portal bridge formation and normal portal traids' areas H&E X 200 (C). Thyme- treated group showed mild portal bridge formation. H&E X 20 (D)

**Table 7.** Levels of AFB1 residues in liver of rabbits in the different experimental groups (ppb).

| Examined tissues | | Trials of experimental design | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 |
| Liver (ug/kg) ppb | 0.0 | 23.30±0.186 | 2.5±0.102 | 1.3±0.124 | 0.010±0.001 |

T1: Negative control - T2: aflatoxicated( Positive control) - T3: afla + Thym Extract
T4: afla + Low dose of SeNPs (0.3 mg/kg) - T5: afla + High dose of SeNPs (0.5 mg/kg obb.w.)

In the present work, the foregoing results in Table (7) significant beneficial effects of SeNPs and thyme supplementation against aflatoxicosis are also accompanied by decreasing AFB1 residues in liver tissues. The positive control rabbits of T2 had a high amount of AFB1 in the liver (23.30±0.186 ppb). Whereas, the aflatoxicated animals of T3 that supplemented with thyme extracts are also showed a reduction in the amount of AFB1 up to (2.5±0.102). The AFB1 residues Se-NPs treated aflatoxicated rabbits in T5 showed marked degradation toxin from liver higher ((0.010±0.001 ppb) than T4 (1.3±0.124 ppb).

## DISCUSSION

The fungal pollution of animal feed is associated with the adverse environmental factors of climates (Monda *et al.,* 2020). (Hassan *et al.,* 2016) isolated *Aspergillus* sp. as predominant molds in animal feeds(68%), while, the other fungi were also recovered as *Mucor* sp. (35%),*Penicillium* sp.(31%), *Fusarium* sp.(23%), *Rhizopus* sp.(21%),*Cladosporium* sp.(6%) and *Scopulariopsis* sp.(4%).(Similarly, Nooh *et al.*, 2014; Hassan *et al.,* 2017,2018), recovered the *Aspergillus* sp. as the most common isolated molds (100%) from feed in comparison to other molds.

Mycotoxins are of significant health hazard, especially in tropical districts and over 80% of the cereals foods have high levels of mycotoxins (Monda *et al.,* 2020). In addition, several adverse effects were detected due to consumption of contaminated food and feeds with fungi and aflatoxins as the occurrence of liver cancers (Nayak and Sashidhar, 2010, El-Nahass *et al.,* 2019). Moreover, various studies recovered AFs in animal and poultry feeds (El-Hamaky *et al.,* 2016) at levels ranging from (170-750 ppb) and (Hassan *et al.,* 201T6) in poultry feed (97.5± 1.63 ppb) and yellow corn (31±1.36 ppb). (However, Hassan *et al.*, 2017) illustrated that he recovered *A. flavus* strains from ration samples produced a significant level of aflatoxin (100%) with the mean levels of (266±4.16).

In recent years, nanotechnology has been used employed in several biomedical fields (Bai *et al.,* 2018; Hassan *et al.,* 2020, 2021). The obtained results were similar to the detected findings of (Atul *et al.,* 2010; Jay and Shafkat 2018; Verma and Maheshwari, 2018). All updated studies detected the useful ability of nanomaterials as antioxidants, antitoxin, and other uses in the improvement of animal health (El-Sayed, 2020; Salem and Fouda, 2021; Hassan *et al.,* 2021).

In the present work, Se NPs were employed to improve the adverse carcinogenic effects 0f aflatoxins in rabbits. Several studies were detected these changes in biochemical parameters in aflatoxicated rabbits (Hassan *et al.,* 2016; El-Nahas *et al.,* 2019). Aflatoxins B1 can also activate the oxidative reactions (ROS) and consequent lipid peroxidation in cells via inducing phospholipase that disrupts the integrity of cell membran**e,** DNA damage, and cancers **(**Lawrence, 2009; Aborehab and Waly, 2019). Moreover, the ability of AFB1 to induce DNA damage plays an important role in AFB1 carcinogenicity (Al-Anati and Petzinger, 2006; Yunus *et al.,* 2011).

Our biochemical findings illustrated that AFB1 increased the concentration of NO and MDA but reduced values of GSH and TAC. Also, the values of SOD, CAT, and GSH-PX in the homogenate of liver tissues of rabbits have been decreased. It is suggested that the elevated MDA as an oxidative stress system and lowered CAT and GPx activities as a protective system caused the development of cell damage (Benkerroum, 2020). Other studies detected that AFs caused liver cirrhosis due to decrease in the GSH stores (Mogda *et al.,* 2014, Neeff *et al.,* 2018). The decline in enzyme activities and increased MDA levels produced by AFB1 may be resulted from decreased ability of cells and tissues and also the nitric oxide formation in liver cells (Wang, 2009).

Herein, the low serum retinol, and a-tocopherol in aflatoxicated rabbits compared to control animals was also detected by (Mubarak *et al.,* 2009; Neeff *et al.,*2018; Dana *et al.,* 2018). They illustrated that aflatoxins cause a reduction in the antioxidant defense systems of cells (vitamins and glutathione) and disrupt the cell membrane integrity (Lee and Jacobs 2005; Kanchana *et al.,* 2012). Currently, data observed in our study regarded that feed supplementation with SeNPs and Thyme extract in aflatoxicated rabbits resulted in ameliorating their toxic effects on biochemical functions of vital organs and increased the liver antioxidant activity. Similarly, several studies reported that SeNPs have a significant functional role in GSH-PX activities which has huge importance during normal metabolic activity and stress during aflatoxicosis in rabbits (Jampílek *et al.,* 2020; Hamed and Selim, 2021). It has anti-apoptotic activities on oxidation-reduction, DNA synthesis and initiating benefit functions of carotenoids and vitamin A (Tayeb and Qader, 2012; Hamed and Selim, 2021). Currently, Se-NPs improved the adverse actions which resulted from aflatoxicosis as protein and DNA synthesis in rabbits) and reduced destruction of lymphocyte cells and DNA damages (Bhattacharjee, 2016). Moreover, SeNPs have significant effects in the initiation of the formation of glutathione and cause less oxidative stress, and showed high biological activity and good (Cheng *et al.,* 2003; Peng *et al.,* 2007). Whereas, the thyme extracts are known antioxidant materials which have similar activity as a-tocopherol and other biological antioxidants (Lee and Shibamoto, 2002). Moreover, the administration of thyme compounds improves the adverse effects that resulted from aflatoxicosis, where, they prevent oxidative damage and oxidative stress as (lipid peroxidation which is associated with liver cells cancer (Esmail, 2018; Nazarizadeh *et al.,* 2019).These effects due to their contents of thymol and Phenolic compounds which have antioxidant activity (El-Nekeety *et al.,* 2011; El-Sherbeny *et al.,* 2021).

Herein**,** the observation of treatment effects on the tissues of vital organs was investigated via histopathological studies. The results indicated the large prevalence of liver cells cirrhosis and necrosis in aflatoxicated groups. It is suggested that these adverse effects of Afs are due to the liberation of large amounts of ROS that destroyed cells membrane, DNA, and death of liver cells (El-Nekeetya *et al.,* 2014; Hassan *et al.,* 2016, 2020, 2021; Hamed and Selim, 2021). Currently, the supplementation of aflatoxicated rabbits with high and low doses of SeNPs resulted in significant histological improvement of hepatic cells injury and neutrophil infiltration. These beneficial effects may be due to the SeNPs preventing cell membrane disruption and enhancement of antioxidant capacities ( Shi *et al.,* 2015; Sun *et al.,* 2015) and its sequestration by the mitochondrial membrane and activation and hence no occurrence of cell apoptosis (Wang *et al.,* 2013, Hassan *et al.,* 2020, 2021; Hamed and Selim, 2021).

Similar results in our study were obtained in the thyme extracts treated group, where, the histological hepatocyte necrosis of aflatoxicated rabbits was significantly repaired in form of mild necrosis. These benefits of thyme were detected in several studies as (Lin *et al.,* 2009; Esmail, 2018; Nazarizadeh *et al.,* 2019) who recorded their potentials against inflammations of aflatoxicosis and prevention the liver cells necrosis and cause tissue restoration**.** They added that these effects were dose-dependent, where; the low concentrations prevent cell cirrhosis, while higher levels of thyme extracts resulted in apoptosis and fibrosis.

Herein, the histopathological status of renal tissues revealed that the aflatoxicated group showed severely swollen distal tubular lining epithelial and reduction of Bowman,s space. Whereas the supplementation of thyme, Se-NPs in feeds of aflatoxicated rabbits resulted in a mild degree of tubular degeneration, and Bowman,s spaces appeared normal. Similar findings were detected by (Hassan *et al.,* 2016, 2020; El-Nahas *et al.,* 2019; Hamed and Selim, 2021).

In the present work, the foregoing results significant beneficial effects of SeNPs and thyme supplementation against aflatoxicosis are also accompanied by decreasing AFB1 residues in liver tissues Therefore, the feed supplementation with Se-NPs and thyme extracts has significant potential in activating the hepatic antioxidant actions and removing the toxic adverse effects of aflatoxins (Hamed and Selim, 2021).

## CONCLUSION

Animal mycosis and mycotoxicosis caused significant economic losses in animal health and output across the world. As a result, all authorities are concerned about effective management of unfavourable environmental variables that encourage the production of fungus and their toxin. The negative toxic effects of AFB1 on rabbit biological metrics were discovered in this investigation. These included potentially harmful oxidative effects on liver cells, which resulted in a reduction in antioxidant activity, leading to liver cirrhosis and death in toxicities rabbit cells. The toxic alterations of aflatoxicosis were greatly improved by supplementing rabbit diets with Se-NPs or thyme extracts. Metal nanoparticles, such as SeNPs, and natural plants, such as thyme, exhibit hepatoprotective properties due to their antioxidant activity. Furthermore, essential oils like thyme have a minimal toxicity for cattle and are a non-toxic environmental substance. Because the protective benefits of SeNPs are restricted in order to prevent the toxicity risk of nanomaterials by using low safe dosages, more laboratory research is needed to ensure their safe biomedical application in field animals to enhance human and livestock health and production.

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**AUTHOR'S CONTRIBUTION:**

Atef Hassan conceived and designed the experiments; Mahmoud M. Arafa Mogda K. Mansour, Essam M. Ibrahim, Noha H. Oraby, and AlaFoad did the methodology, all authors did the manuscript preparation, analyzed the data and wrote this manuscript. Corresponding author: Noha H. Oraby. All authors read and approved the final manuscript.

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# التاثير الوقائى للجسيمات النانومترية للسيليينيوم ومستخلص الزعتر فى ازالة الاثار السامة للافلاتوكسين ب1 فى الارانب

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## الملخـــص

في هذة الدراسة تم عزل العديد من العترات التي لها القدرة علي افراز السموم في علائق الارانب حيث وصل مستوى الافلاتوكسين في العلائق الى 300جزء من البليون. وقد تم استخدام جزيئات السيليينيوم المحضرة كيميائيا لتخفيف الأثار الضارة للسموم الفطرية في الارانب . كشفت القياسات البيوكيميائية في الانسجة والامصال لجميع الارانب التي تعاني من التسمم الفطري انها تسببت في تكوين الشوارد الحرة و أرتفاع مستويات أكسيد النيتريك والمالون الدهايد وانخفاض في مستوي المستوى الكلي لمضادات الاكسدة. بالاضافة الى انخفاض نسبة الريتنول في الدم والبيتا توكوفيرول . هذة التغيرات تبدلت الى القيم الطبيعية عن طريق الجرعات المختلفة عن طريق الفم لجزيئات السيليينيوم النانومترية (0.3- 0.5 مللجم/كجم) و الزعتر للارانب التى تعاني من التسمم بالافلاتوكسين . كما لوحظ دورهما في تراجع نسبة بقايا سموم الافلاتوكسين في الكبد. وبالتالي، يمكن أضافة جزيئات السليينيوم النانومترية والزعتر الى الاعلاف الحيوانية كعوامل مضادة للاكسدة لها الدور في تنشيط الحالة الصحية للحيوان والقضاء على السموم الفطرية.

**الكلمات المفتاحية** : تقنية النانو ، جزيئات السليينيوم النانومترية ، أفلاتوكسيس ، الزعتر ، مضادات الأكسدة ، الفطريات