Effect of Thyme and Rosemary on Liver Functions in Toxicated Rats by Aflatoxins
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ABSTRACT

Thyme and rosemary assume a part in hepatoprotective and go about as anti-aflatoxicosis. This examination has endeavored to explore impact of subacute measurements of aflatoxin in rats and conceivably to foresee any advantages or disadvantages of thyme and rosemary. Thirty adult male albino rats were partitioned into five groups. Rats were isolated into two main groups, the first main group (n=6 rats), negative control, was fed on basal diet. The second main group was inebriated with Aflatoxin then was divided into four subgroups as follow, the first subgroup was kept as positive control group, sub groups (2 and 3) were fed on basal diet and supplemented with thyme and rosemary at of 5% individually. The fourth subgroup was fed on basal diet and supplemented with a combination of 2.5% thyme and 2.5% rosemary. The results indicated that, supplementation with Thyme, Rosemary and their mixes altogether significantly (P<0.05) enhanced liver functions and also serum protein parameters (albumin, globulin and total protein), serum CAT, SOD and decrease significantly MDA compared to the positive control. Furthermore Thyme, Rosemary and their blend significantly decreased (P<0.05) the mean level of serum TNF-α and IL-1β contrasted with the positive control gathering. The present examination prescribed utilizing Thyme, Rosemary and their blends to shield the body from aflatoxin danger.

Keywords: Thyme, Rosemary, Aflatoxin, Liver functions, Phenolic, antioxidant Rats.

INTRODUCTION

Aflatoxins are the most imperative mycotoxins, perceived as universal contaminants of sustenance all through developing world (Kamkar et al., 2013). Samuel et al., (2013) demonstrated that, the significant aflatoxins are AFB1, AFB2, AFG1, AFG2. Among them, aflatoxin B1 (AFB1) is the most strong reason for human cancer-causing agent (Reddy et al., 2009b and Tavakoli et al., 2013).

Many studies by (Peters and Teel, 2003; Gong et al., 2004; Williams et al., 2004 and Abnet, 2007) revealed that aflatoxins are created by a few types of the fungus Aspergillus. Aflatoxicosis can cause intermittent genuine medical issues and important economic losses. No less than 100 nations have directions to control significant mycotoxins, particularly aflatoxins, in products and nourishment, with the goal that the most extreme bearable mycotoxins levels fluctuate incredibly among the the countries (Reddy et al., 2009a).

Oxidative pressure, including lipid peroxidation, has the significant job in the pathogenesis of aflatoxicosis (Abdel-Wahhab et al., 2006 and Umarani et al., 2008). Chemoprevention of toxicates utilizing supplements is the subject of exceptional investigation. Antioxidants have the ability to reduce disease formation by either enlistment or restraint of key catalyst frameworks (Guarisco et al., 2008).

Herbs are normally wealthy in bioactive plant items with nourishment esteem as considerable restorative incentive in a few illnesses (Sharma, 2010). Thyme (Thymus vulgaris) was having a place with the Lamiaceae family a sweet-smelling local herb in the Mediterranean area. Thyme was presently broadly developed as herbal medicine (Domaracky et al., 2007).Thymus vulgaris possess various beneficial effects, like antiseptic, antimicrobial, bactericidal and antioxidant properties. Also, it has recommended as a natural replacement for synthetic antioxidant (Rasooli et al., 2006). Höferl et al., (2006) found that, thyme functions as a liver decontamination tonic, promotes blood circulation and functions as an exciting stimulant for the entire system. The therapeutic potential of thymus is due to its contents of flavonoids, thymol, carvacrol, eugenol and aliphatic phenols in addition to luteolin and saponins (Amarowicz et al., 2009). These flavonoids advance ideal wellbeing through their antioxidant capacity and rummaging free radicals (El-Nekeety, 2011). Aqueous extract of thyme was wealthy in the phenolic content and have free radical searching movement (Hamzawy et al., 2012). Thymol and carvacrol are recognizable antioxidants found in the concentrate of thyme species plants (Beena and Rawat, 2013).

Rosemary (Rosmarinus officinalis L.) an evergreen perpetual fragrant bush having a place with the family Labiatae (Al-Sereiti et al., 1999). It is normally utilized as a zest and enhancing operator (Saito et al., 2004). It is made out of dried leaves and blooms contain some antioxidant (flavonoids and phenols) which thought about having antioxidant properties (Nabavi et al., 2015). These polyphenols have demonstrated natural activities in vitro as hostile to tumor, chemopreventive (Razavi-AzarKhiavi et al., 2014) and anti-inflammatory agents and may assume a job by directing the movement and additionally articulation of certain enzymatic frameworks ensnared in important physiological procedures in the liver (Del Bano et al., 2006).

The antioxidant activity movement of rosemary think can be attributed basically to two sections, carnosic destructive and carnoso Kadri et al., 2011and Machado et al., 2013).

Aim of study: The purpose behind the present work was to survey the effect of Thyme and Rosemary supplementation on liver function and oxidative worry in toxicated rats by Aflatoxins.

MATERIALS AND METHODS

Materials:
Rats: Thirty adult male albino rats were obtained from Helwan Farm, Ministry of Health and Population, Cairo, Egypt. Diet: Casein, vitamins, cellulose, minerals, methionine and choline were obtained from Morgan Company for Chemicals, Cairo, Egypt.
Plants: Leaves of Rosemary (Rosmarinus officinalis)
and thyme (*Thymus vulgaris* L.) were obtained from the Agriculture Faculty, Cairo University and was identified by competent botanist at the herbarium of Agriculture Research Center, Giza, Egypt. Purified AFB1 from *Aspergillus flavus* was purchased from Sigma Company, Cairo, Egypt.

**Methods:**

Experimental design: In this study 30 rats, weighing (180 ± 10 g) were kept under normal healthy condition and fed on basal diet for one week for adaptation (Reeves et al., 1993). Rats were divided into two main groups, the first main group (n=6 rats), negative control, was fed on basal diet and supplemented with thyme and rosemary. The second main group was intoxicated with Aflatoxin, (Aflatoxin B1) though intragastrically route at concentrations 2 mg/kg b.w., for 7 days) according to (Wójtowicz-Dchomicz et al., 2011) then were divided into four subgroups as follow, the first subgroup was kept as positive control group, subgroups (2 and 3) were fed on basal diet and supplemented with thyme and rosemary at the level of 5% respectively. The fourth subgroup was fed on basal diet and supplemented with a combination of 2.5% thyme and 2.5% rosemary. After 8 weeks rats were sacrificed after overnight fasting and blood of each rat was taken from the vein of the eye. The serum was separated at 3000 rpm for 20 minutes by centrifuge, then kept in plastic vials at -20°C until analysis.

**Biochemical analysis:**

Serum liver enzymes including aspartat aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphatase (ALP) were determined (Reitman and Frankel, 1957). Serum total protein, albumin and globulin were estimated according to Weissman et al., (1950). Oxidative stress markers: serum Catalase (CAT), Superoxide Dismutase (SOD) and malondialdehyde (MDA) were determined according to (Beutler et al., 1963; Kakkar et al., 1984 and Draper and Hadly, 1990) respectively.

**Statistical analysis:**

The obtained data was statistically analyzed using the Statistical Package for Social Science (SPSS) version 18.0. Values are represented as means with their standard errors (SE). P value of < 0.05 was considered to indicate statistical significance (Snedecor and Cochran, 1980).

**RESULTS**

Table (1) showed the effect of Thyme, Rosemary and their mixture on serum liver functions in rats with induced toxicity by Aflatoxins. Results indicated that the positive control group had a significant increase (P<0.05) in serum levels of AST, ALT and ALP compared to the healthy group. Supplementation with Thyme, Rosemary and their mixture at the tested level significantly decreased (P<0.05) the elevated levels of serum ALT, AST and ALP compared to the positive control group. Moreover, there was significant difference in serum ALT, AST and ALP between thyme and rosemary treated groups. The best results of liver functions were recorded at the group fed on 2.5% of both Thyme and Rosemary.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AST (µ/L)</th>
<th>ALT (µ/L)</th>
<th>ALP (µ/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>27.40±1.07</td>
<td>105.63±3.53</td>
<td></td>
</tr>
<tr>
<td>Control (-ve)</td>
<td>41.64±1.81</td>
<td>199.36±3.27</td>
<td></td>
</tr>
<tr>
<td>Thyme (5%)</td>
<td>34.13±1.69</td>
<td>147.16±1.81</td>
<td></td>
</tr>
<tr>
<td>Rosemary (5%)</td>
<td>39.67±1.45</td>
<td>161.93±3.12</td>
<td></td>
</tr>
<tr>
<td>Mix. (2.5% Thyme + 2.5% Rosemary)</td>
<td>32.73±0.89</td>
<td>133.50±2.56</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Values at the same column with different letters are significantly different at P<0.05.

Data in table (2) illustrated the effect of Thyme, Rosemary and their mixture on serum (albumin, globulin and total protein) of intoxicated rats by Aflatoxins. Injection with Aflatoxins could be observed that, the positive control rats had significant reduction (P<0.05) in the mean value of serum albumin, globulin and total protein compared to the negative control group. The supplementation with Thyme, Rosemary and their mixture significantly increased (P<0.05) the mean level of serum protein parameters compared to the positive control group. There was significant difference in serum protein parameters between thyme and rosemary treated groups. The concentration of serum protein parameters significantly increased (P<0.05) as a result of supplementation with 2.5% of both Thyme and Rosemary. Moreover, there was no significant change in serum albumin and globulin between the two treated groups (Thyme and their mixture).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>Total Protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>5.76±0.24</td>
<td>3.13±0.14</td>
<td>9.80±0.23</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>2.72±0.17</td>
<td>1.40±0.09</td>
<td>4.30±0.15</td>
</tr>
<tr>
<td>Thyme 5%</td>
<td>4.63±0.08</td>
<td>2.38±0.12</td>
<td>6.73±0.27</td>
</tr>
<tr>
<td>Rosemary 5%</td>
<td>3.93±0.17</td>
<td>1.94±0.04</td>
<td>5.80±0.41</td>
</tr>
<tr>
<td>Mix. (2.5% Thyme + 2.5% Rosemary)</td>
<td>5.03±0.20</td>
<td>2.65±0.05</td>
<td>7.90±0.26</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Values at the same column with different letters are significantly different at P<0.05.

Table (3) revealed the effect of Thyme, Rosemary and their mixture on serum CAT, SOD and MDA of intoxicated rats by Aflatoxins. Injection with Aflatoxins significantly decreased (P<0.05) the mean value of serum CAT and SOD but caused an increase in the level of MDA compared to the negative control group. The supplementation with Thyme, Rosemary and their mixture significantly increased (P<0.05) the mean level of serum CAT and SOD and decrease significantly serum MDA compared to the positive control group. There was no significant difference in serum CAT and SOD between thyme and rosemary tested groups. The best results for the concentrations of CAT, SOD and MDA were recorded at the group fed on basal diet supplemented with 2.5% combination of (Thyme and Rosemary).
Table 3. Effects of Thyme, Rosemary and their mixture on serum Catalase, Superoxide Dismutase and malondialdehyde of intoxicated rats by Aflatoxins

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAT (µ/L)</th>
<th>SOD (µ/dl)</th>
<th>MDA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>100.10±2.62</td>
<td>95.13±2.67</td>
<td>8.06±0.79</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>24.20±2.95</td>
<td>48.10±2.12</td>
<td>36.83±2.47</td>
</tr>
<tr>
<td>Thyme 5%</td>
<td>60.23±3.09</td>
<td>75.20±1.56</td>
<td>23.46±1.89</td>
</tr>
<tr>
<td>Rosemary 5%</td>
<td>47.10±1.81</td>
<td>63.76±2.47</td>
<td>28.40±2.21</td>
</tr>
<tr>
<td>Mix. (2.5% Thyme + 2.5% Rosemary)</td>
<td>76.00±2.64</td>
<td>83.83±2.34</td>
<td>15.20±2.16</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

DISCUSSION

Liver is the main organ to experience ingested supplements, drugs and ecological toxicants that enter the hepatic entry vein from the stomach related framework and liver capacity can be changed by damage coming about because of intense or endless presentation to toxicants. Aflatoxin B1 is a hepatotoxic and hepatocarcinogenic compound delivered by the growth, *Aspergillus (A.) flavus* (Eaton and Gallagher, 1994). An assortment of nourishments, for example, grains, millets and oil seeds, are powerless to contamination by *A. flavus*, which produces aflatoxins amid its development, reaps, transport and capacity. AFB1 is likewise bio changed by P450 catalysts to yield an electrophilic epoxide (Kodama et al., 1990), which assaults the DNA to start hepatotoxicity (Shen et al., 1996).

Low patient satisfaction from the consumption of synthetic drugs, due to high costs and side effects of these medications caused an increased tendency to traditional treatments (Al-Atar and Shawush, 2015). Extraordinary consideration has been paid to the defensive impacts of antioxidants by regular root mixes against harming caused by concoction operators (Abdel-Wahhab et al., 2011). Phenolic mixes with antioxidant activities have been appeared to have defensive impacts on different organs (Amarowicz et al., 2009).

Fresh Thyme herb has a standout amongst the most antioxidant levels among herbs. It is squeezed with minerals and vitamins that are fundamental for perfect prosperity. Its leaves are affluent in potassium, squeeze, calcium, manganese, magnesium and selenium (Sharangi and Guha, 2013). Rosemary and its constituents especially caffeic corrosive subordinates, for example, rosmarinic corrosive have a remedial potential in anticipation of inflammatory diseases and hepatotoxicity (Al-Sereiti et al., 1999).

The liver functions were analyzed through the assurance of ALT and AST activities which known as cytosolic marker catalysts reflecting hepatocellular putrefaction as they are discharged into the blood after cell layer harm damage (Andallu and Vardacharyulu, 2001). The present outcomes demonstrate a significant increase in AST, ALT and ALP because of aflatoxin infusion. Rise of liver catalysts was as per that revealed by many examinations (Salama et al., 2013; Zargar and Kim et al., 2014). Sherif et al., (2009) indicated that liver enzymes increased after liver damage because of increased membrane permeability or because of liver cell necrosis and cytosol leakage into the serum.

The treatment of rats with Thyme, Rosemary and their combination improved the liver functions that decreased by AFB1 intoxication asw seen in table (1). Shanon, (2011) reported that adding aqueous extract of thyme at 10% to drinking water for the mothers of broiler chickens causes decline the level of liver enzymes, this may be due to thyme compounds that enhance the status of antioxidants. Thyme contains flavonoids that due to its antioxidant properties in combating free radicalD...
results were obtained by (Haroun et al., 2002 and Mansour et al., 2002).

Seong et al., (2005) detailed that thyme have a successful defensive instrument because of responsive oxygen species and might be related with diminished oxidative stress. As reported by Abd El Kader and Mohamed, (2012) it is feasible for the thyme extract to be mediated its antioxidant activities by enhancing the antioxidant defense enzymes SOD, CAT and replenishing GSH storage. Furthermore, thyme extract which show antioxidant activity has an inhibitory effect on lipid peroxidation, which could decrease the strength of inflammatory response (Bozin et al., 2006). The same results were obtained by Rubiò et al., (2014).

Rosemary enhanced the toxic impacts of AFB1 on liver. This was showed by decrease of the level of MDA and increment CAT and SOD. The obtained results about oxidative stress are in concurrence with Parmar et al., (2011), who reported that treatment with aqueous extract of rosemary leaves prevents oxidative stress due to carcinogenic corrosive (CA), carnosol and other phenolic acids in rosemary (Abd El-Ghaniy et al., 2012). CA can avert lipid peroxidation and it avoids the disruption of the biological membrane by searching free radicals (Munné-Bosch and Alegre, 2001). Virk et al., (2013) reported also that rosemary aqueous extract given with CdCl2 caused a lessening in MDA, increment in SOD in liver. Similar outcomes were gotten by the finding with (Rasoolijazi et al., 2014 and Saeed et al., 2015).

Liver damage was controlled by surveying serum levels of TNF-α and IL-β. In our examination, hepatotoxicity actuated by AFB1 was reflected by a stamped height of TNF-α and IL-β exercises. These outcomes are in concurrence with the aftereffects of numerous examinations many studies (Marin et al., 2002; Serhan, 2007; Dönmez and Keskin, 2008 and Weaver et al., 2013). The systemic inflammatory reaction is interceded by actuated pro-inflammatory cytokines, for example, tumor pulefaction factor-α (TNF-α), interleukins (IL-1β and IL-6) and oxygen radicals which may sharpen hepatocytes to the toxicity (Li et al., 2010b).

In the current study, TNF-α level was reduced significantly in animals receiving thyme with AFB1, the effect which probably related to the carvacrol that has anti-inflammatory effect, carvacol was a known component presented in the thyme extract (Juhas et al., 2008). Fachini-Queiroz et al., (2012) said that the inhibitory impact of carvacol on leukocyte movement adds to its calming activity, notwithstanding the aggravation impact of thymol. Other study by (Elhazabi et al., 2003) showed that thyme extract increase the quantity of polymorphonuclears and total lymphocytes.

Soosani and Szegar, (2018) reported that Thyme daenensis extracts have cell reinforcement and calming properties and can enhance liver damage in mice by diminishing pro-inflammatory TNF and IL-6 cytokines. Thus, this concentrate may be utilized as anti-inflammatory and hepato-protective agent.

Nafees et al., (2016) reported that extract of Thyme leaves has been shown to reduce oxidative stress and liver inflammation induced by aflatoxine in Wistar rats. Abdel-Aziem et al., (2014) indicated treatment with Thyme led to significant decrease in TNF-α and IL-6 levels compared with the control group. Ahmed et al., (2015) report 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.

**CONCLUSION**

This study suggests the potent role of Thyme and rosemary in management of liver toxicity -induced by Aflatoxin and this effect could be attributed to their antioxidant activity.

**REFERENCES**


