The protective effects of nanocurcumin on liver toxicity induced by salinomycin in broiler chickens

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SUMMARY

Curcumin, the active principle of turmeric, has been shown to exhibit a wide range of pharmacological activities and has been reported to reduce the toxic effects of certain toxic agents. However, its low absorption, poor solubility and low stability in the body, has curbed its pharmaceutical application. The aim of the present study was to evaluate the possible protective effects of nanocurcumin against salinomycin-induced liver toxicity in broilers. Sixty broiler chicks were randomly divided into 5 groups. Group 1 served as the control while the second group received salinomycin with a daily dosage of 300 mg/kg body weight during a 14-day diet. Groups 3, 4 and 5 were also treated with salinomycin; however, they were orally administered nanocurcumin with daily dosages of 50, 100 and 200 mg/kg body weight, respectively over the same period. At the end of the experiment, blood and tissue samples were collected for biochemical and histological examination. Based on the results, salinomycin significantly (P<0.05) reduced the body weight from 840±43.21 (in control group) to 626±64.31 g, while decreasing the liver weight from 44.52±20.74 (in control group) to 29.42±27.87 g. Also, salinomycin increased the activities of serum aspartate aminotransferase (AST) from 44.52±20.74 (in control group) to 537.8±40.64 U/L and caused histological alterations in the liver of chicks. Nanocurcumin decreased significantly (P<0.05) the mean serum levels of AST in the 200 mg/kg group (421.5±30.08 U/L) compared to the salinomycin group (537.8±40.64 U/L). In addition, nanocurcumin decreased the incidence and severity of histopathological hepatic lesions, especially in the group 200 mg/kg. The findings of this research indicated that nanocurcumin could protect liver against the toxicity induced by salinomycin.

Keywords: Nanocurcumin, ionophore, hepatoprotection, histopathology, broilers

RéSUMÉ

Effets protecteurs de la nanocurcumine sur la toxicité induite par la salinomycine chez les poulets de chair

La curcumine, principe actif du curcuma, a démontré qu’elle avait une large gamme d’activités pharmacologiques, notamment en termes de réduction de certains effets toxiques. Cependant, sa faible absorption, sa faible solubilité et une faible stabilité dans le corps, freinent son application pharmaceutique. L’objectif de cette étude était d’évaluer les effets protecteurs de la nanocurcumine sur la toxicité hépatique induite par la salinomycine chez les poulets de chair. Soixante poussins ont été divisés au hasard en 5 groupes. Le groupe 1 a servi de contrôle alors que le deuxième groupe a reçu de la salinomycine à une dose quotidienne de 300 mg/kg de poids corporel pendant 14 jours. Les groupes 3, 4 et 5 étaient également traités avec de la salinomycine et on en sus reçu de la nanocurcumine par voie orale aux doses quotidiennes de 50, 100 et 200 mg/kg de poids corporel, au cours de la même période. À la fin de l’expérience, le sang et des échantillons de tissus ont été recueillis pour un examen biochimique et histologique. La salinomycine a significativement (P<0.05) le poids corporel de 840 ± 43,21 (dans le groupe de contrôle) à 626 ± 64,31 g, tout en diminuant le poids du foie de 44,52 ± 20,74 (dans le groupe témoin) à 29,42 ± 27,87 g. Ces effets étaient accompagnés d’une augmentation des activités de l’aspartate aminotransférase sérique (AST) de 364,80 ± 10,93 à 537,80 ± 40,64 U/L et de lésions histologiques. La nanocurcumine a considérablement diminué (P<0,05) les taux sériques moyens de l’AST dans le groupe recevant 200 mg/kg (421,5 ± 30,08 U/L) par rapport au groupe recevant de la salinomycine seule (537,8 ± 40,64 U/L). En outre, la nanocurcumine a diminué l’incidence et la gravité des lésions hépatiques.

Mots-clés: curcumin, ionophore, foie, histologie, poulets de chair

Introduction

Curcumin, which is the main component of turmeric, has antioxidant [7, 18], anti-inflammatory [21], anti-tumor, anti-cancer [1, 15] and immune-enhancing functions [38] in biological systems, all of which render it popular for research. The protective effects of curcumin against liver damage have also been demonstrated in numerous studies [5, 9, 37]. Curcumin retention time is short, an issue constraining the therapeutic effects of curcumin. In rats, for instance, the oral bioavailability of curcumin is merely 1% [36]. Moreover, the solubility of curcumin is low in water and zero in neutral or acidic pH waters [33]. The absorption rate of curcumin in intestinal lumen is only 25% of the administered dose, which is very low [14, 34]. Owing to such limitations, a various methods have been employed so as to augment the effectiveness of curcumin, one of which being nanotechnology. Utilizing nanoparticles of 10-100 nm size as the carriers of curcumin enhances its bioavailability in the body, increasing solubility in water while improving the utilization of curcumin properties [2, 14, 35]. Such nanoparticle formulations entail a 10–14-fold higher absorption rate compared with the same oral dose of free curcumin [35]. Salinomycin is a polyether drug belonging to ionophore group obtained as a result of Streptomyces albus fermentation [3]. This drug has been widely used for several years as anticoccidial in different species such as birds [22]. Ionophore drugs must be administered with great precaution as they have a low therapeutic index [8] and any overdose or wrong usage leads to irreversible toxicity [25]. Even a recommended dosage of salinomycin entails mild pathological effects on the body’s tissues, as is reported on
several occasions. The use of salinomycin more than the recommended dosage can significantly reduce growth and induce toxicity [12, 4]. Ionophores such as salinomycin and monencin could be potentially related to the oxidative damage caused by free radicals; using antioxidant supplements, in this regard, can prevent such damages [17, 19]. Ionophore toxicity is deemed to cause oxidative damage, yet the mechanisms of toxicity caused by salinomycin have not been fully described. Besides, there are limitations as to the use of salinomycin, rendering it necessary to conduct research regarding the mechanism of toxicity which ultimately conduces to preempting and treating salinomycin toxicity [17]. The current research, accordingly, was designed in order to study the protective effects of nanocurcumin compound against liver toxicity induced by salinomycin in chicken animal models.

Material and Methods

RAISING CHICKENS

Chickens were raised in an industrial poultry farm from day 0-28. The experimental design adhered to the guidelines of the Animal Ethics Committee of Amol University of Special Modern Technologies, Amol, Iran. Experimental diets were formulated according to Ross 308 Management Manual (2012). Chickens were fed with a diet based on corn and soybean meal. The basic food ration lacked any such additives as growth promoters, antioxidants or coccidiostats. All groups were fed with the same basic food ration until the fourteenth day of the raising period. From day 14 to 28, salinomycin and nanocurcumin drugs, with determined dosages, were daily added to the diet. Salinomycin was obtained from Kimiafaam Company (Iran) for veterinary products under a trade name (Kimiasalino 12®). The employed nanocurcumin drug was a trade drug (SinaCurcumin®, Exir Nano Sina Company, Iran) in the form of gelatin capsules containing curcumin nanomicelles which were removed through the use of a syringe. The calculated dosages were then dissolved in one ml of distilled water and orally administered to the chicks.

EXPERIMENTAL DESIGN

The current study is an experimental study and study population consisted of 60 Ross 308 broiler chickens randomly divided into 5 groups with 12 members.

Chicks were randomly divided into five groups according to the following protocols:
- Group 1: normal control (no treatment), given a basal diet.
- Group 2: salinomycin (300 mg/kg).
- Group 3: salinomycin + nanocurcumin (50 mg/kg).
- Group 4: salinomycin + nanocurcumin (100 mg/kg).
- Group 5: salinomycin + nanocurcumin (200 mg/kg).

STUDIED PARAMETERS

All chickens were weighed on the first, tenth and final (28th) day of the experiment and their mean weight was calculated. At the end of the treatment period, liver weights were recorded, and blood samples were taken from the wing vein, put into serum tubes, placed in the room temperature for several minutes and centrifuged so as to ascertain the enzyme levels of ALP and AST. Blood parameters were analyzed by spectrophotometer making use of diagnostic kits of Pars Azmoon Company.

HISTOPATHOLOGY

The livers of chicks in all the groups were fixed in 10% formaldehyde solution, dehydrated in an ascending graded series of ethanol solutions, cleared in xylene and embedded in paraffin. Fine sections were obtained, mounted on glass slides and stained with hematoxylin and eosin (H&E) for light microscopic analyses. Histopathological changes based on scoring were recorded according to Rasgele et al. [27]. From each chick liver, three slides were prepared with six to

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
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<tbody>
<tr>
<td>Body weight in day 1 (gr)</td>
<td>374±15.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>375±13.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>375±16.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>374±17.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>374±11.74&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Body weight in day 10 (gr)</td>
<td>612±135.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>524±150.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>558±156.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>563±166.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>577.8±153.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body weight in day 15 (gr)</td>
<td>840±136.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>626±203.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>663±182.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>662±185.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>679±165.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver weight (gr)</td>
<td>44.52±6.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.42±8.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.84±7.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.13±6.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.98±8.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST</td>
<td>364.80±10.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>537.80±40.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>470.3±23.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>460.4±28.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>421.5±30.08&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>ALP</td>
<td>4125.3±118.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5293.8±219.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5151.9±113.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5143.2±191.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4988.9±125.67&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
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I: control; II: salinomycin; III: salinomycin + 50 mg/kg nanocurcumin; IV: salinomycin + 100 mg/kg nanocurcumin; V: salinomycin + 200 mg/kg nanocurcumin; The data are expressed as mean ± SD (P<0.05).
seven tissue sections, all of which were evaluated under a microscope for the degree of necrosis, bile ducts hyperplasia, hyperemia, infiltration, vacuolar degeneration and fibrosis. Each slide was examined and assigned a severity of change using scores on a scale of none (0), mild (1), moderate (2) and severe (3) damage. (Table II)

**Statistical analysis**

Results are expressed as mean ± SEM for the number of observations indicated. Mean values were compared using one-way analysis of variance (ANOVA) followed by Duncan’s test. Differences were considered significant at P<0.05 unless otherwise stated. In addition, histopathologic parameters were compared between groups by the Kruskal-Wallis test.

**Results**

**THE EFFECT OF NANOCURCUMIN ON BODY WEIGHT AND LIVER WEIGHT**

The data recorded on the body weight showed that on days 10 (D10) and 15 (D15), the body weight of the salinomycin group significantly decreased compared to the control group (P<0.05). By adding nanocurcumin to the food ration, the weight gain underwent a non-significant (P>0.05) increase compared to the salinomycin group (Table I). At the end of the experiment, the liver weight of the salinomycin group was significantly decreased compared to the control group (P<0.05), a loss not compensated for by adding nanocurcumin to the food ration (Table I).

**BIOCHEMICAL FINDINGS**

Table I illustrates the biochemical results obtained from the measurement of serum AST enzyme. With the addition of salinomycin to the rations, the amount of serum AST enzyme significantly increased compared to the control group (P<0.05). With the addition of nanocurcumin to the rations, the AST enzyme level was significantly reduced only in the nanocurcumin group administered a dose of 200 mg/kg compared with salinomycin group (P<0.05). The measurement of the amount of ALP enzyme showed that there is no significant change among the different groups (Table I).

**HISTOPATHOLOGICAL EXAMINATION**

Based on the histological slides, in salinomycin group, the loss of arrangement was observed in the parenchymal structure of the liver, along with vacuolar degeneration, hepatocytes necrosis, hyperemia, infiltration of inflammatory cells and bile duct hyperplasia. Furthermore, the damaged liver tissue was replaced with fibrous tissue (Figures 1-3 and table II). By adding 50 mg/kg nanocurcumin to the food ration, even though parenchymal irregularities, hepatocytes necrosis and infiltration of inflammatory cells were seen in the liver, the severity of vacuole degeneration, hyperemia and bile duct hyperplasia was much more reduced compared with the salinomycin group, and no replacement of fibrous tissue was observed in the liver parenchyma (Figure 4 and table II). By adding 100 mg/kg nanocurcumin to the food ration, compared with the 50 kg/mg dosage, hepatocytes necrosis and infiltration of inflammatory cells were relatively reduced along with the severity of bile duct hyperplasia. Moreover, we did not observe the fibrous tissue replacement of liver parenchyma; the liver tissue, on the other hand, was still replete with blood (Figure 5 and Table II). In the fifth group (administered 200 mg/kg nanocurcumin), vacuole degeneration and fibrotic liver tissue were totally absent while other damages were significantly decreased (Figure 6 and Table II).

**Discussion**

In the current study, consumption of toxic dose of salinomycin reduced the body weight in chickens. In accordance with this result, the study of Demirulus et al. (2006) showed that the body weight was decreased after increasing the five different doses of salinomycin (1, 3, 5, 7 or

<table>
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<tr>
<th>Lesions</th>
<th>I</th>
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<th>P value</th>
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<td>Hepatocytes necrosis</td>
<td>0</td>
<td>3±.21</td>
<td>2.9±.23</td>
<td>1.9±.18</td>
<td>1.2±.2</td>
<td>P&lt;0.05</td>
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<tr>
<td>Bile ducts hyperplasia</td>
<td>0</td>
<td>3.1±.23</td>
<td>1.5±.31</td>
<td>1.1±.23</td>
<td>1.1±.23</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>0</td>
<td>2.9±.28</td>
<td>2.1±.23</td>
<td>2.1±.23</td>
<td>1.1±.23</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Infiltration of inflammatory cells</td>
<td>0</td>
<td>3.1±.23</td>
<td>3±.26</td>
<td>2.1±.23</td>
<td>1.3±.15</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Vacuolar degeneration</td>
<td>0</td>
<td>3.1±.28</td>
<td>2.1±.23</td>
<td>2.1±.23</td>
<td>0</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0</td>
<td>2±.21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

I: control; II: salinomycin; III: salinomycin + 50 mg/kg nanocurcumin; IV: salinomycin + 100 mg/kg nanocurcumin; V: salinomycin + 200 mg/kg nanocurcumin; 0: absence of the lesion; 1: mild; 2: moderate; 3: severe.

Table II: Histopathological scoring of liver sections from all groups.
HEPATOPROTECTIVE ROLE OF NANOCURCUMIN IN BROILERS

Figure 1: A&B: Photomicrographs of liver sections from the control group, normal liver tissue. H&E (40X).

Figure 2: Photomicrographs of liver sections from the salinomycin group. A: Hepatocyte necrosis (1); Bile duct hyperplasia (2); inflammatory cells Infiltration (3); vacuolar degeneration (4); B: Fibrosis (1). H&E (40X).

Figure 3: Photomicrographs of liver sections from the salinomycin group. A: Hepatocyte necrosis (1); Vacuolar degeneration (2); B: Hyperemia (1); Infiltration of inflammatory cells (2). H&E (40X).

Figure 4: Photomicrographs of liver sections from the group 3 (salinomycin + 50 mg/kg nanocurcumin). A: Hepatocyte necrosis (1); Vacuolar degeneration (2); inflammatory cells infiltration (3); B: Hepatocyte necrosis (1); Hyperemia (2); Bile duct hyperplasia (3). H&E (40X).
9 mg/kg feed over 49 days) administered to boiler chickens [6]. In another study, by adding salinomycin (180 mg/kg feed up to the age of 12 weeks) to the food ration of laying hens, it was shown that at dose more than the recommended therapeutic dose, the body weight was lost alongside liver and kidney weights [30]. Another study also demonstrated that the use of toxic dose of salinomycin (120 mg/kg feed for 4 weeks) reduced the body weight of chickens [17]. Low dose of salinomycin is considered as coccidiostat and growth promoting; however, the reduction in growth is not merely related to its high and toxic doses. The recommended therapeutic doses of salinomycin may also cause growth reduction that can be attributed to the low therapeutic index of this drug [29]. In the present research, although adding nanocurcumin to food ration led to weight gain, but this increase was nonsignificant. Hassan et al. (2014) studied the effects of nanocurcumin in rats with liver cell carcinoma caused by poisoning with diethyl nitrosamine and it was showed that there were no changes in the liver weight of all groups compared to the control group [11]. However, in a number of studies, turmeric (administered 2 and 4 weeks via a feeding needle at 50 mg/kg/d) or curcumin (administered for 7 days by oral route at 100 mg/kg) was able to amend the changes in liver weight in mice [10, 20]. In the present study, nanocurcumin could not make up for the liver weight loss resulted by the toxicity of salinomycin. On the other hand, no compensation of body or liver weight reduction in the treatment groups can indicate the severity pertaining to the toxic effect of salinomycin on organs and finally the weight of the birds.

In the biochemical study of liver function, appraising the amount of liver enzymes in serum such as AST can indicate the rate of damage to liver cells. These enzymes are naturally in liver cells, released into the blood in large amounts when the liver cell membrane is damaged, hence the fact that evaluating the serum level of these enzymes could supplement histopathological studies [26]. In our research, the serum level of ALP enzyme was not affected by salinomycin. However, 200 mg/kg nanocurcumin reduced the serum level of AST enzyme which was increased as a result of consuming salinomycin. In accordance with these results, Hussein et al. (2005), demonstrated that a toxic dose of salinomycin (300 mg/kg feed for 15 days, 1, 2 and 3 weeks) increased the AST enzyme of the chickens' serum after one week, which is accounted for by the injuries in the liver cells [12]. Other studies have reported an increase in this enzyme as a result of employing high doses of salinomycin (120 mg/kg feed for 4 weeks in broilers or 180 mg/kg feed up to the 12 weeks in layer chickens) which was attributed to the changes and complications in liver cells [17, 30]. Mathews et al. (2012) studied the protective effects of curcumin against liver poisoning with arsenic in rats and showed that serum levels of AST and ALP, as well as liver tissue damage decreased in the group treated with curcumin comparisons to the arsenic group [24]. In a study conducted by Son et al. (2013) on mice with acute carbon tetrachloride poisoning, nanocurcumin reduced the serum levels of ALT and AST enzymes along with reducing tissue complications [32]. The changes in serum biochemical enzymes can be a sign of tissue complications and lesions. The increase in AST enzyme

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**Figure 5:** Photomicrographs of liver sections from the group 4 (salinomycin + 100 mg/kg nanocurcumin). A: Hepatocyte necrosis (1), Vacuolar degeneration (2); B: Bile duct hyperplasia (1); Infiltration of inflammatory cells (2). H&E (40X).

**Figure 6:** Photomicrographs of liver sections from the group 5 (salinomycin + 200 mg/kg nanocurcumin). A: Hepatocyte necrosis (1), Inflammatory cells infiltration (2), Hyperemia (3); B: Hepatocyte necrosis (1), Bile duct hyperplasia (2). H&E (40X).
levels caused by toxic dose of salinomycin may be due to the oxidative effects associated with free radicals in liver cells. In this study, the reduction of this enzyme using nanocurcumin can be attributed to its antioxidant properties.

In the present investigation, the histopathological study revealed that tissue complications in the salinomycin group were decreased with nanocurcumin consumption and in the group consuming the highest dose of nanocurcumin, these complications were observed far less than in other treatments. Several studies have demonstrated complications in chicken liver tissue resulting from toxic doses of salinomycin [12, 30]. The effect of curcumin on reducing tissue complications in different liver toxicities has also been investigated in certain studies where the antioxidant and anti-inflammatory effects of curcumin were expressed as the reason for the reduction in the severity of complications [16, 28]. The protective effect of curcumin was associated with its antioxidant properties which inhibit free radical generation. Curcumin’s antioxidant properties have been demonstrated by its ability to inhibit reactive oxygen species, lipid peroxidation and enhance the activity of several antioxidant enzymes in animal models [23]. In line with the results of this study, in a study, the use of nanocurcumin could reduce the microscopic complications in the liver of rats caused by Zearalenone Mycotoxin poisoning. In that study, it was also reported that the levels of biochemical markers (such as AST and ALP) and liver oxidative stress markers, altered as a result of liver toxicity, were modified as a result of taking curcumin nanoparticles [13]. Sankar et al. (2015) compared the effects of curcumin and curcumin nanoparticles on the improvement of complications in liver poisoning with arsenic in rats. They reported that curcumin nanoparticles were more effective than curcumin in reducing the incidence of liver tissue complication and ameliorating the levels of serum biochemical markers and oxidative stress markers [31].

Pharmacokinetic studies on curcumin have shown that, while having the trace plasma concentration, its oral absorption and bioavailability are much too low [14]. For example, a study conducted on rats showed that administration of 500 mg curcumin/kg feed resulted in 1% bioavailable curcumin in plasma [36]. In order to achieve an effective concentration of curcumin in blood, the use of novel formulations, such as synthesizing nanoparticles and liposomes has become the subject in several new studies. The histopathological results of the present are consistent with the findings that indicate the serum enzymes levels and the liver tissue lesions resulting from salinomycin poisoning decrease by augmenting the intake of nanocurcumin. In conclusion, the nanocurcumin can be orally used for its beneficial effects.

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References
