Histopathological and Biochemical study of the effect of Citrullus colocynthis on the heart and liver of mature male rabbits: as a model for mammals

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Summary

The aim of the present work is to found out if there are histopathological and enzymatic effects on the heart and liver after oral administration of Citrullus colocynthis (CC) fruit (bitter apple) for mature male rabbits, as a model for mammals. Two experiments were performed for sixty mature male rabbits that equally and randomly divided into six groups each containing five animals. In the two experiments, independently, three groups in each one were considered as treated groups and represented as T1, T2 and T3 groups. These rabbits groups were orally administrated with low dosage of CC (4.8mg/kg/day, experiment No.1) and double dosage of CC extract (9.6mg/kg/day, experiment No2) for three intervals 2, 4 and 8 weeks, respectively. The other three groups in each experiment were considered control and represented as C1, C2 and C3 groups, respectively. All the rabbits in control groups were given orally distilled water (DW) at the same periods of treated groups. Histopathological changes of the heart and liver with related enzymes namely; serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and creatinine kinase (SCK) were studied. The histopathological study revealed no changes in rabbit’s heart tissue particularly after treatment with 4.8mg/kg/day of CC for 2 and 4 weeks. While no histopathological changes were observed in the heart of rabbit groups treated with 9.6mg/kg/day of CC extract after 2 weeks. The results of the treatment with the two dosages of the CC for 8 weeks indicated a mild degenerative changes and mild necrosis of the myocardial cells. There was swelling of the hepatocytes and perivascular cuffing of mononuclear inflammatory cells after two weeks of daily treatment with 4.8mg/kg/day of CC exposure. After four weeks with the low dosage of the CC extract, caused initiation of necrosis, more inflammation picture of liver portal tract with sinusoid All rabbit groups showed statistically a significant gradual increase (P<0.05) in the value of serum enzyme GOT, GPT and CK levels after treatment with the two dosages of CC at the end of each different period compared with before treatment and control group. Concluded from this study that the dosage 4.8mg/Kg/day of CC plant resulted in simple histopathological effects on the heart and mild histopathological changes on the liver during the entire period of the study, instead of serum enzymatic elevation of SGOT, SGPT and SCK. Further studies are recommended to found out the possibility to use and effects of CC on animal hygiene and reproduction.

Key words: Citrullus colocynthis, histopathological, enzymes, male rabbits
Introduction

Many health centers believed in herbal medicine as effective medicine for different diseases. One of these herbal medicines is Citrullus colocynthis (CC). Physician desk reference (PDR) for herbal medicine observed that the most important parts of CC are the seed and bitter apples removed from the harder outer layer of the plant. Citrullus colocynthis is a well-known medicinal plant (1). The bitter apple of CC has been recommended for indigestion and diabetic people in traditional medicine (2). In United Arab Emirates many traditional plants were used as anti-diabetic remedies, such as the Citrullus colocynthis (3). This plant has an insulinotropic effect which could at least partially account for the antidiabetic activation of the fruits (4).

Citrullus colocynthis is used as a purge for man and animals in Mauritania. However, there are very few studies regarding the therapeutic effect of CC on several body systems. Therefore, in addition to our previous study regarding its effects on some physiological aspects in mice (5 Esawe et al.,2011), the present work is aimed to examine the medical effect of the oral administration of crude extract of Citrullus colocynthis fruit on some histopathological and enzymatic aspects of the heart and liver.

Materials and Methods

Sixty adult healthy local breed of male rabbits, their ages ranged between 6 – 8 months old and weighing 1 – 1.5 Kg, were included in this study from 2008 to 2009 at the College of Veterinary Medicine-Al-Sulaimanyia University. The rabbits were reared at an optimal room temperature ranged between 18 – 24 °C and kept for two weeks for adaptation and physical and laboratory examination to exclude the sick one. (6). They were fed on compressed prepared ration consisted of wheat, barley, protein and corn given twice daily, in addition to green vegetable food that was added to the ration (7).

-Plant and preparation techniques: The fresh fruits of Citrullus colocynthis were purchased from the village of Bakrbaef- Darbandekhan district in Kurdistan of Iraq. The crude powder extract was prepared as follows. The shade-dried fruit was crushed into small pieces and then grinded until it became a powder by special electric grinder. The crude powder extract (1gm) was mixed with distilled water (1Litter) in order to prepare stock solution (1%) (8).

Two experiments were performed for sixty mature male rabbits that equally and randomly divided into six groups (five animals per group) according to the dose of CC (in each experiment, thirty adult male rabbits were used). In Experiment no 1, three treated
groups(T1 ,T2, and T3 ) were orally administered with low medical dose (4.8mg/kg/day) of CC crude fruit powder extract(8) once a day in intervals (2,4 and 8weeks) by using stainless steel gavages needle .The other three groups were considered as control groups (C1,C2 and,C3 ) and were gavages distilled water(DW) for the same periods of treated groups .In the other experiment, six rabbits groups (T1,T2, andT3) were treated with double medical dose(9.6mg/kg/day) of CC once a day for 2,4 and 8weeks . The control groups in the experiment no 2 (C1,C2 and,C3 ) were divided into similar groups of treated rabbits and were gavages DW through the same periods too .

- Dosage Preparation: The concentration was prepared for each two experiment groups by preparing the required amount of CC stock solution (1mg /ml) with the appropriate amount of vehicle (distilled water) in a manner that:

1- Each 4.8 ml of distilled water contained 4.8mg of CC crude powder for experiment No.1.
2- Each 9.6 ml of distilled water contained 9.6mg of CC crude powder for experiment No.2.
up to 5 ml of blood were safely being collected from the auricular marginal vein before the time of sacrificed of animal at the periods of treatment , using 23- to 25-gauge needle. Blood samples for serum separation were collected in plastic test tubes without anticoagulant

-Enzymes Measurement:
Different serum clinical enzymes including: glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and creatinine kinase (CK) were measured and recorded by standard assays on a biochemical analyzer (Lisa-200 ,Pejohesh-Co., France) at the beginning of experiment and at the end of each different periods of treatment for all the rabbits of the two experiments involved (9).

-Histopathological Study:
Histological sections were performed for heart and liver to study their microscopic changes to find out the effect of plants on the tissues. Tissue samples (Heart and Liver) from rabbits were removed. The organs were fixed in 10% NBF (100 ml formalin (40%), 4 g sodium phosphate monobasic, 6.5 g sodium phosphate dibasic and 900 ml D.W) and processed for paraffin embedding. The histopathological sections (4-6 µm) were stained with hematoxylin and eosin. The slides were coded and examined as described by Luna,( 10).

Statistical Analysis: Data were shown as the mean ± SE. Analysis of variance (ANOVA) test was used to distinguish the significance in enzymatic levels in different periods and dosages used .Then Duncan's new multiple range test was used post-ANOVA to identify significant differences between mean values. The a probability level of 5% (P < 0.05) was taken as significant (11)

Results

1-. Histopathological Changes:
1.1-Experiment No 1.
- The Heart: Results in the present study showed no histopathological changes in the heart of rabbits of control group and after gavages of the rabbits with low medical dosage (4.8mg/kg/day)of CC extract for two weeks and four weeks(figures1,3,5 ,respectively) . While results of the treatment after eight weeks with the same dose of the CC indicated a mild degenerative change and mild necrosis of the myocardial cells, figure (7).
- The Liver: The results of histopathological changes in the present study showed that there is swelling of the hepatocytes and perivascular coughing of leukocytes after two weeks of daily treatment with 4.8mg/kg/day of CC exposure(figure 4) compared to control (figure 2). After treatment of the rabbits for four weeks with the low dosage of the CC extract, caused initiation of necrosis, more inflammation picture of liver portal tract with sinusoid, figure (6). After eight weeks of treatment ,there is inflammatory reaction within hepatic lobule with kupffer cell hyperplasia (figure8).

1.2. Experiment No2
- The Heart: Sections of the heart showed that the cardiac muscles were normal after two weeks of treatment with oral double dose(9.6mg/kg/day of CC) of CC extract, figure (9). The
histopathological changes observed in the heart after four and eight weeks of treatment with 9.6mg/kg/day of CC extract. There is a mild degeneration and mild necrosis changes of the myocardial cells.

- The Liver: Oral administration of the rabbits with 9.6mg/kg/day of CC extract for two weeks revealed a cellular swelling, congestion of the central vein and fatty changes of the hepatocytes, figure (10). After four weeks of treatment, the CC treatment caused initiation of necrosis, more inflammation picture of liver portal tract with sinusoid. Moreover, the histopathological study of the liver after eight weeks with the same oral dose of CC shows that there is severe hepatocytes necrosis and fat droplet in hepatocytes with kupffer cell hyperplasia, rash of neutrophils and degeneration.

2-Enzymatic Changes:

There was no significant difference (P>0.05) in the levels of SGOT, SGPT, SCK enzymes between the rabbits treated with a dosage of 4.8 mg/kg/day and control groups prior of treatment. All rabbit groups showed statistically a significant gradual increase (P<0.05) in the value of serum enzyme GOT, GPT and CK levels after treatment with CC at the end of each different period compared with before treatment and control group (Table 1).

Treatment with 9.6 mg/kg/day of CC caused a significant (P<0.05) increase in SGOT, SGPT and SCK, levels in the entire treated rabbit groups after different periods of treatment (Table 2).
Figure 5: Histomicrograph of rabbit heart treated with 4.8mg/kg CC extract for four weeks illustrates no pathological changes (H & E, X40).

Figure 6: Histomicrograph of rabbit liver treated with 4.8mg/kg CC extract for four weeks illustrates presence vacuolar degeneration of Hepatocytes (H & E, X40).

Figure 7: Histomicrograph of rabbit heart treated with 4.8mg/kg CC extract for eight weeks shows the presence of mild degenerative changes (a) and minimal myocardial necrosis (b)(H & E (X40).

Figure 8: Histomicrograph of rabbit liver treated with 4.8mg/kg CC extract for eight weeks shows: vacuolar degeneration of Hepatocytes (a), inflammatory reaction within hepatic lobule (H & E, X40).

Figure 9: Histomicrograph of rabbit heart after two weeks of treatment with 9.6mg/kg CC extract shows: unremarkable pathological changes in the myocardium.

Figure 10: Histomicrograph of rabbit liver treated with 4.8mg/kg CC extract for two weeks reveals the presence of: Cellular swelling of liver (a), perivascular cuffing (infiltration of the mononuclear inflammatory cells) (b) and congestion of the central vein (c). (H&E, X40).
Table 1: Effect of oral low dose (4.5 mg/kg/day) of Crotalaria Cotyledons on SGOT, SGPT and SCK levels (LUL) of rabbits in different periods.

<table>
<thead>
<tr>
<th>Group</th>
<th>Period of treatment</th>
<th>SGOT level (LUL)</th>
<th>SGPT level (LUL)</th>
<th>SCK (LUL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treatment (n=5)</td>
<td>Control (n=5)</td>
<td>Treatment (n=5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>T1</td>
<td>2 weeks</td>
<td>20.36 ±0.65</td>
<td>36.56 ±0.52</td>
<td>20.26 ±0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>B</td>
<td>a</td>
</tr>
<tr>
<td>T2</td>
<td>4 weeks</td>
<td>21.51 ±0.30</td>
<td>88.90 ±0.57</td>
<td>20.52 ±0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>C</td>
<td>a</td>
</tr>
<tr>
<td>T3</td>
<td>8 weeks</td>
<td>20.90 ±0.40</td>
<td>106.06 ±0.82</td>
<td>20.65 ±0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>d</td>
<td>a</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE  * Different letters mean a significant difference at P < 0.05.

SGPT: Serum glutamate pyruvate transaminase  SGOT: Serum glutamate oxaloacetate transaminase  SCK: Serum creatine kinase
Table 2: Effect of orally administering a double dose (9.6mg/kg/day) of *Citrullus colocynthis* on SGOT, SGPT and SCK levels (IU/L) of rabbits in different periods.

<table>
<thead>
<tr>
<th>Group</th>
<th>Period of treatment</th>
<th>SGOT level (IU/L)</th>
<th>SGPT level (IU/L)</th>
<th>SCK level (IU/L)</th>
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<td></td>
<td></td>
<td>Treatment (n=5)</td>
<td>Control (n=5)</td>
<td>Treatment (n=5)</td>
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<tr>
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<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>T1</td>
<td>2 weeks</td>
<td>20.36±0.65</td>
<td>50.26±0.86</td>
<td>20.36±0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
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<td>Before treatment</td>
</tr>
<tr>
<td>T2</td>
<td>4 weeks</td>
<td>21.51±0.38</td>
<td>105.40±0.92</td>
<td>20.42±0.21</td>
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<tr>
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<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>T3</td>
<td>8 weeks</td>
<td>20.90±0.40</td>
<td>190.18±0.87</td>
<td>20.74±0.25</td>
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<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE.
Different letters mean a significant difference at $P < 0.05$

SGPT: Serum glutamate pyruvate transaminase  SGOT: Serum glutamate oxaloacetate transaminase  SCK: Serum creatine kinase
Discussion

The results of the first experiment found no histological changes following 2-4 weeks of treatment but induced spots of hepatotoxic effect at the dose of 4.8mg/kg/day with mild changes on the histology of the myocardial cells particularly after chronic administration of the plant extract (i.e. after 8 weeks). At the same time, incidence of SGOT, SGPT and CK enzymes in rabbit groups treated with low and double dose of CC increased in time and dose dependant manner.

It has been noticed that the CC extract could be free of hepatotoxic effects in concentrations up to 0.1 mg/ml when in vitro investigation on liver toxicity was performed using standardized dried extract of CC in adult rats (12). This observation may be consistent with our work. In the present study the low dose or herbal medical dose of CC as Al-Dujaily, (8) term was 4.8mg/kg/day which is slightly higher than 0.1mg/ml (3mg/kg/day) used for rats in the work of Barth et al., (12). Therefore, this work found a starting of hepatotoxic and enzymatic effects particularly after prolong administration (8 weeks) of low dose of CC extract. Whereas in the use of double dose 9.6mg/kg/day of CC following 2 weeks and 4 weeks of treatment, the levels of SGOT, SGPT enzymes showed the beginning of hepatotoxicity after which it became more prominent during eight weeks of treatment.

The effects of low and high dose of CC extract on the serum enzymatic activity may be attributed to the influence of CC extract on the liver and heart. This effect on tissues and increase in enzymatic levels may be related to the alkaloid and saponin, a substances found in the constituents of CC which may affect some liver and myocardial cells (13). These chemicals may lead to the damage of the hepatic cell (14), cellular leakage and functional integrity of the cell membrane of the liver (15). The elevated serum enzyme levels like GOT and GPT are indicative of cellular leakage and functional integrity of cell membrane in liver (15).

Our results are in comparable with study reported by Bakheit and Adam, (16). They found that high oral dose of CC for 6 weeks causes a significant increase in the level of CK serum enzyme. Therefore, the observed increase in the serum levels of these enzymes indicates the extent of temporary cellular damage on the liver and myocardial cell, more specifically the cell membrane permitting the leakage of the enzymes from the liver and heart.

The spots of histological changes confined to heart may be due to the effect saponin and glycosides component of the CC on the myocardial cells leading to a minor effect following 8 weeks of treatment with low dose (4.8mg/Kg/day) of CC. Whereas, other study administered orally 50mg/Kg/day of CC, and it has been reported that the glycosides at toxic levels produce cardiac irregularities and heart block or it may be a result of a more direct effect on the heart musculature (17). The inconsistent between our study and previous study resulted from the differences of quantity of dosage administrated in each one.

The present research has been noticed that the lesions observed in the liver and heart tissues after giving of Citrullus colocynthis are reversible injury (unpublished data). These tissues can return to normal physiological function after the stoppage of plant feeding (16).

In conclusion, the low medical dosage (4.8mg/Kg/day) of CC have slight histopathological and enzymatic effects after oral administration for 8 weeks. When 9.6mg/Kg/day of CC used, more histopathological and enzymatic changes are recorded. Therefore, the study recommended further researches to found out the effects of use the low medical dosage of CC on animal production and reproduction.

References


