RESEARCH ARTICLE

STUDY OF USING KOJIC ACID AS ANTICANCER IN DIFFERENT CONCENTRATION TO TREATMENT CANCERED RATS Rattus norvegicus BY CYCLOPHOSPHAMIDE

Jasim A. Abdullah

Clinical Laboratories Department, Applied Medical Sciences College, Karbala University, Karbala, Iraq.

ARTICLE INFO

Objective: This study was conduct to investigate effect of kojic acid to treatment ofcancered rate.

Methods: Different concentration 0.5, 1, 2 mg/kg used as treatment through 30 days in some histological and functional criteria in cancered rats Rattus norvegicus by cyclophosphamide and the parameters that studied were WBC, PCV, HB, platelets, AST, ALT, and Bilirubin.

Results: The result showed there is significant difference in blood parameters (WBC, platelets) at P≤ 0.01 and there is no significant difference in (HB, PCV) when compared with control group in addition T3, T4, TSH levels showed no significant difference in all group of rats that treated with kojic acid when compared with control group at P≤ 0.01 also showed significant difference in ALT, AST, Bilirubin when compared with control group at P≤ 0.01 that lead to increase of ALT, AST, Bilirubin related to increase with kojic acid and then we showed significant change in the liver and kidney tissues in concentration 2 mg/kg of kojic acid when compared with control group and finally.

Conclusion: We can consider the kojic acid is very good substance may be useful as antibiotic or antioxidant in some researches because it is very safe on the body.

INTRODUCTION

Kojic acid is an organic component and consider secondary metabolite secreted by several microorganism of aspergillus genus such as Aspergillus oryzae, Aspergillus tamnorri, Aspergillus parasiticus, and Aspergillus flavus. The chemical structure was determined as 5-hydroxy-2-hydroxymethy-4-pyrone. acute and sub chronic toxicity resulting from oral dose has not been reported and chronic feeding studies in mice and rats indicated the development of thyroid follicular cell adenomas primarily. Kojic acid investigated the three endpoints of genotoxicity, gene mutations, structural chromosome alterations and aneuploidy. Kojic acid is not significant acute oral toxicant in mice and rats with LD\textsubscript{50} value greater than 1g/kg, at large doses lethargy, piloerection, ataxia, depressed respiratory rate, loss of righting reflex, increased salivation, body tremors were observed and convulsions prior to death were reported. Kojic acid is multifunctional and having weak acidic property and the natural origin of kojic acid confirms it is non-hazardous bio-degradation, it is also used as chelating agent and activator in insecticide production. Recently, a new application of kojic acid is found in the cosmetic industry, it is also used as skin whitening agent and ultraviolet filter in skin care products widely available in cosmetic market, also used as food additive for preventing enzymatic browning and kojic acid can be used as antioxidant iron chelator for topical treatment of wound healing. Kojic acid loaded nanotechnology...
based drug delivery system can modulate drug permeation through the skin and improve the drug activity\textsuperscript{11}, in our experiment we used a kojic acid as drug or anticancer to treat the cancered rats by cyclophosphamide and explain the activity of kojic acid in some histological and functional criteria.

**MATERIALS AND METHODS**

In our experiments used 12 rats and divided it into 1 rat control positive and 2 rats control negative and the other rats divided into three groups each group contain three rats and then give the rats carcinogenic substance cyclophosphamide 50µl/kg by oral administration 1ml one time only.

Then treatment the rats that taking carcinogenic substance by kojic acid every day for 30 days in different concentration for each group.

After 30 days killing the rats by using chloroform and draw the blood for measurements blood components by using Ruby instrument and measurementsT3,T4 and TSH by ELISA instrument , but GPT ,GOT , Bilirubin by using strip method (manual).

After that anatomist all the rats and extracted organs (liver, spleen, lung and kidney) for are preparation histological section, cutting the organs into small pieces and placed in solution 10% formalin for fixation, washing the organ with tap water for 24 hour to remove the formalin and then pass the organs in progressive concentration of ethanol for 30 minute to remove water from them (dehydration) and then liking samples in Xylene for 20 minutes to remove the alcohol from the tissue (clearing ) and then placed the samples in the soft wax and xylene for one hour and place the sample twice in soft wax only for 1.5 – 2 hour in temperature 56-60\degree C to remove the xylene from the sample (embedding ) . buried sample in special molds container on the molten paraffin wax and leave harden in the refrigerator to be cut , then the sample cut in 5\mu m in thickness using Microtome instrument and proven models on glass slide , then put the slide at room temperature for the next day to dry (sectioning).

Staining section by hematoxeline and eosin stain as passed the slide in xylene twice for 30 minutes each time an then passed in concentration regressing of ethanol for 3-5 minutes for each concentration , pass in hematoxeline for 15-20 minutes , wash the sample by distal water for 5 minutes , pass in eosin dye for 20-30 second , and then passed up ward concentration of ethanol for 3-5 minutes for each concentration and the pass in xylene for 15 minutes (staining) in the end placed cover slide using canda balsam (mounting) and after being diagnosis histological section by Microscope.

**Statistical Analysis**

We used (SPSS) program in 18\textsuperscript{th} version and enter the data to get the statistical analysis process then find the relationship between the coefficients dealing through complete random testing and extracted LSD values and compared with differences between medians and find standard error values\textsuperscript{14}.

**RESULTS**

**Effect of kojic acid on T3,T4 and TSH**

From the results of this study we showed there is no significant differences of T3,T4 and TSH in all groups of rats that treated with kojic acid for each concentration when compared with control group and we show this result in Table 1 So we showed no high different in the concentration of T3,T4 and TSH that affected by kojic acid and cyclophosphamide , also we showed there is increase in the weight of thyroid gland of rats that will be treated with kojic acid when compared with control group.

Table 1. Effect of kojic acid on T3,T4 and TSH in rats.

<table>
<thead>
<tr>
<th>Conc. mg/Kg</th>
<th>Parameter</th>
<th>T3 (pg/m)</th>
<th>T4 (ng/l)</th>
<th>TSH (µu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont. (n=1)</td>
<td></td>
<td>0.0441±0.0013</td>
<td>0.0625±0.005</td>
<td>0.037±0.002</td>
</tr>
<tr>
<td>Cont. (n=2)</td>
<td></td>
<td>0.0442±0.0013</td>
<td>0.0655±0.005</td>
<td>0.039±0.002</td>
</tr>
<tr>
<td>0.5 (n=3)</td>
<td></td>
<td>0.0448±0.0014</td>
<td>0.0593±0.00219</td>
<td>0.037±0.0031</td>
</tr>
<tr>
<td>1 (n=3)</td>
<td></td>
<td>0.0466±0.0016</td>
<td>0.0583±0.0014</td>
<td>0.039±0.041</td>
</tr>
<tr>
<td>2 (n=3)</td>
<td></td>
<td>0.047±0.0032</td>
<td>0.0593±0.0018</td>
<td>0.038±0.0023</td>
</tr>
</tbody>
</table>

* The number refer to mean ± Standard error.
* Similar vertically capital letters refer to no significant difference (P>0.01) between hormones for each concentration.

**Effect of Kojic Acid on Biochemical Criteria**

This study will be indication there are significant difference at P≤ 0.01 in the concentration of both GPT, GOT enzymes and in the concentration of bilirubin in all groups of rats that will be treated with kojic acid for each concentration when compared with control groups .we showed there are significant increase at P≤ 0.01 in the level of GPT enzyme in all groups of rats will be treated with kojic acid in concentration 0.5 , 1 , 2 mg/kg that mean an increase in the concentration of GOT related with increase in the concentration of kojic acid . also the concentration of GPT will be affected with kojic acid in different concentration 0.5 , 1 , 2 mg/kg observed significant increase in each rats will be treated with kojic acid but the highest value recorded at concentration 2mg/kg of kojic acid So GPT concentration will be increase with increase in the concentration of kojic acid,
also the concentration of bilirubin in groups of rats that treated with kojic acid will be increase in both concentration of kojic acid 1, 2 except 0.5 mg/kg when compare with control group that mean there are slightly increase in the concentration of bilirubin in treated rats and these result can be show in Table 2.

Table 2. Effect of kojic acid on some of biochemical criteria in rats compare with control group.

<table>
<thead>
<tr>
<th>Concen. mg/Kg</th>
<th>Parameter</th>
<th>GOT U/L</th>
<th>GPT U/L</th>
<th>Bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.+ (n=1)</td>
<td>7±2.3</td>
<td>D</td>
<td>8.5±4.17</td>
<td>21±4.66</td>
</tr>
<tr>
<td>Cont.- (n=2)</td>
<td>10±2.3</td>
<td>D</td>
<td>14.75±14.17</td>
<td>32±4.66</td>
</tr>
<tr>
<td>0.5 (n=3)</td>
<td>23±1.5</td>
<td>B</td>
<td>25±0.34</td>
<td>18.3±3.33</td>
</tr>
</tbody>
</table>
| 1 (n=3)       | 28±4.88   | A       | 19.6±2.185| 24.3±2.8 | B
| 2 (n=3)       | 22±0.5    | C       | 42.6±12.19| 36.3±0.8   |
| LSD0.01       | 13.10     |         |         | 15.27    |

*The number refer to mean of enzyme concentration (U/l)±stander error
*Various vertically capital letter refers to present significant difference (P≤0.01) between enzymes for each concentration .

Effect of Kojic Acid on Blood Criteria

In this study we showed there are no significant effects of kojic acid in concentration 0.5, 1, 2 mg/kg on Hb and PCV in rats that will be treated with kojic acid at P≥0.01 when compared with control group , but we showed there are significant difference in both platelets and WBC in rats treated with kojic acid when compared with control group also WBC have slightly increase that mean WBC will be increase when the concentration of kojic acid will be increase , whereas platelets at control group =710 but when treated with kojic acid about 0.5mg/kg =786 and in1mg/kg =855.6 and in 2mg/kg =912.3 and that mean platelets will be increase when increase of kojic acid at P≤0.01 , these result show in Table 3.

Table 3. Effect of kojic acid on blood criteria in rats compare with control group.

<table>
<thead>
<tr>
<th>Concen. mg/Kg</th>
<th>Platelet (×10^9/l)</th>
<th>WBC (×10^9/l)</th>
<th>Hb (g/dl)</th>
<th>P.C.V (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.+ (n=1)</td>
<td>710±50.50</td>
<td>7±0.51</td>
<td>13±1.75</td>
<td>36±1.15</td>
</tr>
<tr>
<td>Cont.- (n=2)</td>
<td>786.5±50.50</td>
<td>5.6±0.51</td>
<td>9.3±1.75</td>
<td>33±1.15</td>
</tr>
<tr>
<td>0.5 (n=3)</td>
<td>855.6±34.66</td>
<td>8.066±0.61</td>
<td>11.27±0.69</td>
<td>37.3±1.185</td>
</tr>
<tr>
<td>1 (n=3)</td>
<td>912.3±33.34</td>
<td>11.7±0.70</td>
<td>11.95±0.80</td>
<td>39.9±2.9</td>
</tr>
<tr>
<td>LSD0.01</td>
<td>132.6</td>
<td>2.58</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*The number refer to mean of blood components (g/dl) ± stander error
*Various vertically capital letter refers to present significant difference (P<0.01) between blood parameter for each concentration . NS refer to no significante.

cortex but in medulla occur interstitial bleeding and expansion in loop of Henley . therefore , when treated the rats by kojic in different concentration 0.5 , 1 , 2mg/kg as treatment we showed no change in tissue of liver and kidney that treated by kojic acid 0.5 , 1mg/kg except concentration 2mg/kg of kojic acid we showed change of tissue in both liver and kidney when compared with control group and we can show decrease in effect of cyclophosphamide in this organs respectively, these results can be show from Figure 1-10.

Histological examination of liver and kidney

We showed from this study the cyclophosphamide possess high effects on liver and kidney in rats that treated with cyclophosphamide in concentration 50µl /kg we can observed vacuolization in cytoplasm of hepatocyte and increase size of Kupffer cell because enters much more amounts of water to cell that’s lead to formation vacuoles in same cell, in addition occur congesting in some liver veins , but in kidney we observed some effects like glomerularenhritis and occur alienation in lining tissue of renal tubules of
Fig. 2 T.S in kidney tissue appears normal structure that composed of R (Renal corpuscle), G (Glomerulus), D (Distal convoluted tube), P (Proximal convoluted tube) and GA (Guxtaglomerulappartus) positive control group (E&H 100X).

Fig. 3 T.S in liver tissue treated with cyclophosphamide 50µl/kg appears abnormal structure that composed of A (congesting in hepatic vein) and B (expansion in sinusoid of liver) negative control group (E&H 40X).

Fig. 4 T.S in kidney tissue treated with cyclophosphamide 50µl/kg appears abnormal structure that composed of A (congesting in branch of renal artery), B (shrinking of glomerulus) and C (necrosis in proximal and distal renal tubules) negative control group (E&H 100X).

Fig. 5 T.S in liver tissue treated with kojic acid 0.5 mg/kg appears abnormal structure that composed of A (congesting in hepatic vein) and B (expansion in sinusoid of liver) (E&H 40X).

Fig. 6 T.S in kidney tissue treated with kojic acid 0.5 mg/kg appears abnormal structure that composed of A (shrinking of glomerulus), B (necrosis in proximal and distal renal tubules), and C (Alienation of lining tissue in proximal and distal renal tubules) (E&H 100X).

Fig. 7 T.S in liver tissue treated with kojic acid 1mg/kg appears abnormal structure that composed of A (congesting in hepatic vein) and B (expansion in sinusoid of liver) C (enlargement of hepatocyte) and D (necrosis in hepatoplate) (E&H 40X).
Fig. 8 T.S in kidney tissue treated with kojic acid 1 mg/kg appears abnormal structure that composed of A (interstitial bleeding), B (expansion in loop of Henley), and C (Alienation of lining tissue in proximal and distal renal tubules) (E&H 40X).

Fig. 9 T.S in liver tissue treated with kojic acid 2 mg/kg appears normal structure that composed of H (Hepatocyte), K (Kupffer cells), S (Sinusoids), HE (Hepatoplate), and C (Central vein) (E&H 40X).

Fig. 10 T.S in kidney tissue treated with kojic acid 2 mg/kg appears normal structure that composed of R (Renal corpuscle), G (Glomerulus), D (Distal convoluted tube), P (Proximal convoluted tube), and GA (Gustaglomerulappartus) (E&H 100X).

DISCUSSION

Effect of kojic acid on T3, T4 and TSH

This result will be agree with indicate after repeated oral doses of kojic acid in rats the main target organs affected are thyroid gland and pituitary gland as well as liver, an increase in the weight of thyroid gland showed at doses about 0.95 mg/kg of kojic acid in diet given to male and female rats for 28 consecutive days and observed there are significant difference at $P \leq 0.01$ in the number of colloid in thyroid follicles and follicular cell hypertrophy in thyroid gland that’s suffer from enlargement, in addition showed there are decrease in the concentration of T3 and T4 and increase in the concentration of TSH. Also observed the increase in the weight of thyroid gland that caused by kojic acid may be lead to inhibition of iodine up take to organs and lead to decrease in the concentration of T3 and T4 and increase in the concentration of TSH, also indicate the kojic acid do not have any carcinogenic effect on thyroid gland specifically and body generally. While showed kojic acid leads to inhibition of iodine up take by thyroid gland also inhibition of iodine in the organs at high doses of kojic acid and this cause lead to decrease in the concentration of T3,T4 and increase in the concentration of TSH as well as increase in the weight of thyroid gland. Whereas ensure that kojic acid when treated to rats that it is not have any carcinogenic effect on thyroid gland but lead to inhibition of iodine up take that result in decrease in concentration of T4 and T3 and increase in the concentration of TSH. But showed significant decrease in the concentration of T3 and T4 to the male rats when treated with cyclophosphamide, and this decrease will result from precipitation of cyclophosphamide in the tissue of thyroid gland and cause damage to colloid in thyroid follicles and follicular cell of thyroid gland.

Effect of Kojic Acid on Biochemical Criteria

Indicate there are increase in the level of both GOT and GPT enzymes at dose 500-1000mg/kg of kojic acid also level of ALP enzyme may be increase and also there are increase in bilirubin, cholesterol and calcium concentration. Also showed significant increase in the concentration of both GOT and GPT enzyme in male of rats treated with cyclophosphamide and these results caused by inflammation and fibrosis in liver and indicate there are increase in the level of bilirubin and total cholesterol in male of rats treated with cyclophosphamide. Whereas mention that increase in the level of bilirubin result from intoxicating and chronic inflammation in liver when treated with cyclophosphamide in male of rats. While showed that female of rats treated with doxorubicin may cause increase in the level of both GPT and GOT and when infected with hepatitis or other inflammation that will be affected on liver that cause increase in GPT more than increase in GOT. But indicate that the level of GOT may be increase when occur injury or infection to the heart muscle while the level of GPT do not affect because of GPT is more specific for liver, therefore, when occur damage to the heart muscle that cause increase in the activity of GOT and increase in this concentration in heart muscle.
Effect of Kojic Acid on Blood Criteria

Showed the changes in the functioning criteria in rats can be occur depending on the high concentration of kojic acid and the period of dose –time .therefore, showed lymphocytes and WBC decrease in male and female of rats treated with kojic acid at concentration 500-1000mg/kg through 4weeks. While showed in dose 1000mg/kg of kojic acid that will be cause decrease in RBC also decrease in PCV and concentration of HB in rats treated with kojic acid. Also indicate there are significant differences in blood criteria and showed decrease in number of WBC in rats treated with doxorubicin agent also increase in number of neutrophils and decrease in number of lymphocytes when compare with control group and the number of neutrophils will be increase five time compare with control group while lymphocyte will be decrease two time compare with control group and do not show any difference in the RBC and Hb concentration between the groups of rats treated with doxorubicin agent and control group.

CONCLUSION

We can consider the kojic acid is very good substance may be useful as antibiotic or antioxidant in some researches because it is very safe on the body.

REFERENCES
