Enzymatic effect of flavonoids extracted from the leaves of *Camellia sinensis* on liver enzymes in mice

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ABSTRACT

This study was done at AL-Nahran Research Center / University of AL-Nahran on 2009, follow-up enzymatic effects of the flavonoids extracted from *Camellia sinensis* leaves by measuring the effectiveness of the liver enzymes GOT, GPT and ALP in the blood serum of white laboratory mice treated by flavonoids extracte and compared the result with mice treated with a methotrexate (MTX) drug. This study showed that methotrexate (MTX) agent own a toxic effect by raising the effectiveness of quality of liver enzymes in the blood serum of mice treated (GOT:65, GPT:69, ALP:67) while the flavonoids extract showed the efficiency of reducing the effectiveness of specific liver enzymes in the blood serum of mice treated by 4,40,400 mg/kg (GOT: 46, 40.5, 29), (GPT: 46, 39.5, 27) and (ALP: 45.2, 40, 23).

INTRODUCTION

The researchs showed many of the preventive action of materials and elements in the human food and the most important is flavonoids present in *Camellia sinensis*. *Camellia sinensis* as it includes portions of "flavonoids" which is a powerful anti-oxidants. Divided flavonoids into several types depending on their chemical composition which include Anthocyanides responsible for the pigments of red and blue fruits and flowers, Catechines which is concentrated in the subject of tea, Flavonone, and Glycoside flavonones also found in lemon, orange and honey [1].

Many studies have focused on the impact of chemical compounds and drugs to follow up on the level of liver enzymes as a key member of the body which is the metabolism of different compounds and eliminate toxicity [2]. The methotrexate (MTX) drug example of chemicals that cause mutations as it is widely used in the treatment of early tumors as well as addressing psoriasis and chronic arthritis. As it produces its effects by facilitating the taking it by the cell, causing rein in the
metabolic processes that occur inside the cell, whether normal or infected [3].

**MATERIALS AND METHODS**

Extract flavonoids was followed by the procedures described by [4], 50 gm of the dried plant powder was soaked in 70% methanol for 24 hours and the mixture filtered through the filter paper (Whatman NO.1) alcohol evaporated after leaving in the incubator at temp. (37c) for a period of time, then suspended from a certain size and sterility of the distilled water by filtration and preservation in dark bottles at deep freeze (-20) before it was used. Then attended the detection solution with adding 10 ml ethanol (concentration %50) with 10ml solution of potassium hydroxide (concentration %50), mixed equal sizes from each of this solution and extract, when see the yellow color returned a positive result due to the presence of flavonoids.

**Study of enzymatic effects:** - White laboratory mice (weight 25 g and age 5-8 weeks) were divided into following groups:

**First group:** - a positive control group of mice that were intraperetional (IP) injection of MTX concentration 30 mg / kg then it has been anatomy after 24 hours[5].

**Second group:** - a group of mice that were injected by MTX drug and after 24 hours they given orally by flavonoids with three doses (4,40 and 400) mg /kg for six days.

**Third group:** - a negative control group of mice they not given any things, and used for comparison with above groups.

**Enzymatic-study:**

Include examination of GOT, GPT and ALP enzymes, the attending models serum as the collection of blood by cardiac puncture as in [6] in the small test tube and placed then in a water bath at 37 ° C for 30 min for the purpose of the clot (or left vertically on the table for 30 min) and followed by centrifugation at 2000 rpm / min at 37 ° C for 10 minutes. Then collect the serum in the tubes and kept it in deep freeze (-20 C).

**Assess the effectiveness of the GOT enzyme:**

Used in the colorimetric method [7] to estimate the effectiveness of this enzyme in serum. The measured absorption spectrum wavelength 546 nm, was used a kit to measure the effectiveness of this enzyme from Randox company / England that rely on a single Phenyl hydrazone derivatives resulting from the interaction of Oxaloacetate formed by the chemical equation below with 2,4-dinitrophenyl hydrazine:-

\[
\text{Alpha-oxoglutarate + L-aspartate} \rightarrow \text{L-glutamate + Oxaloacetate}
\]

As the unit of measurement is the enzyme international unit (IU).

**Assess the effectiveness of the GPT enzyme:**

Effectiveness of this enzyme was estimated using the colorimetric method contained in the [7] using a kit from Randox company / England, which depends on the concentration account Pyruvate hydrazone formed from 2,4-dinitrophenyl hydrazine, according to the chemical equation:
Alpha-oxoglutarate + L-alanine → Pyruvate + L-glutamate
And read the result in a spectrophotometer optical wavelength 546 nm. The unit of measurement is the international unit of enzyme (IU).

**Assess the effectiveness of the ALP enzyme:**
Estimated the effectiveness of this enzyme in serum using a kit from Biomerieux company / French, according to the (8) and by the following chemical equation:
Phenyl phosphate → Phenol + Phosphate
The measured absorption spectrum wavelength 510 nm. The unit of measurement is the international unit of enzyme (IU).

**Statistical analysis:**
Results were analyzed statistically using the statistical packages for Social Sciences (SPSS). It was the moral test of differences between the averages, the application of a one-way analysis of variance (ANOVA) and Duncan test [9].

**RESULTS AND DISCUSSION**
The interaction effect of flavonoids and MTX in the effectiveness of quality for the GOT enzyme: -
Table (1) shows that mutagen MTX, which represents the positive control was the latest clear increase in effective qualitative GOT enzyme, this is a moral elevation difference at the level of (P> 0.05) compared with negative control. When an overlap between MTX and flavonoids extracted from the leaves of *Camellia sinensis*, showed a decrease in effective qualitative of the GOT enzyme and moral difference at the level of (P> 0.05) compared with positive control.

<table>
<thead>
<tr>
<th>Concentrations mg / kg</th>
<th>control</th>
<th>treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>20.0±0.31 a</td>
<td>65.0±0.14 c</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>46.0±0.42 b</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>40.5±0.23 b</td>
</tr>
<tr>
<td>400</td>
<td></td>
<td>29.0±0.7 a</td>
</tr>
</tbody>
</table>

*a :- Indicate no significant differences at the level of probability P <0.05
b :- Indicate little significant differences at the level of probability P <0.05
c :-Indicate that presence of significant differences at the level of probability P <0.05

The interaction effect of flavonoids and MTX in the effectiveness of quality for the GPT enzyme: -
The results shown in table (2) that the treatment MTX (positive control) led to an increase in the specific effectiveness of GPT enzyme and therefore an significant difference at the level of (P> 0.05) compared with negative control.
When an overlap between MTX and flavonoids, it was observed a decrease in the effectiveness of quality of GPT enzyme when dosing animals (4.40 and
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400) mg / kg and thus showed a significant difference at the level of (P> 0.05) compared with positive control.

Table -2: Specific effectiveness of the GPT enzyme between the flavonoids and MTX drug.

<table>
<thead>
<tr>
<th>Concentrations mg / kg</th>
<th>control</th>
<th>treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>25.0±0.11 a</td>
<td>69.0±0.44 c</td>
</tr>
<tr>
<td>40</td>
<td>46.0±0.49 b</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>39.5±0.23 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.0±0.5 a</td>
<td></td>
</tr>
</tbody>
</table>

a :- Indicate no significant differences at the level of probability P <0.05
b :- Indicate little significant differences at the level of probability P <0.05
c :-Indicate that presence of significant differences at the level of probability P <0.05

The interaction effect of flavonoids and MTX in the effectiveness of quality for the ALP enzyme: -

The results of this experiment are shown in table (3) showed that the treatment MTX (positive control) led to an increase in the effective quality of the ALP enzyme and this is a significant difference at the level of (P> 0.05) compared with negative control.

When an overlap between MTX and flavonoids, it was observed a decrease in the effective quality of the ALP enzyme when dosing animals (4,40 and 400) mg / kg and thus showed a difference significant at (P> 0.05) when it compared with positive control.

Table -3: Specific effectiveness of the ALP enzyme between the flavonoids and MTX drug

<table>
<thead>
<tr>
<th>Concentrations mg / kg</th>
<th>control</th>
<th>treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>19.1±0.21 a</td>
<td>67.0±0.34 c</td>
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<tr>
<td>40</td>
<td>45.2±0.19 b</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>40.0±0.11 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.0±0.21 a</td>
<td></td>
</tr>
</tbody>
</table>

a :- Indicate no significant differences at the level of probability P <0.05
b :- Indicate little significant differences at the level of probability P <0.05
c :-Indicate that presence of significant differences at the level of probability P <0.05

The present study results showed that the flavonoids extracted from the leaves of *Camellia sinensis* (4,40 and 400) mg / kg had a significant role in reducing the value of the effective quality of enzymes GOT, GPT and ALP in blood serum of mice compared with control treatment rationale for the treatment of drug MTX, as we note the increase of effectiveness of quality for these enzymes in the serum compared with negative control.

The reason for this increase that MTX had a toxic effect on liver cells increases the permeability of membranes to result in permeable of these enzymes and other enzymes to the blood serum, so this is the reason for the high levels of these enzymes in serum and decrease in liver cells [10]. Or presence of such toxic substances lead to a decomposition of self-liver cells
due to increased effectiveness of the lysosomes, leading to the death of liver cells that cause an increase in the enzymes GOT, GPT and ALP in serum [11], as well as inhibition of the process of protein synthesis and to influence the function of the protein factory which affects the production of new molecules of the enzyme [12], and that the entry of mutagen to the body followed by the process of metabolic activation by enzymes Cytochrome P450 in mitochondria lead to chemically generated free radicals involved in the oxidation of fat cell membranes to make them more accessible, as it leads to a decrease in the effectiveness of enzymes such as de-toxic enzyme Glutathion-S-transferases [13].

The effect of plant extracts for different kinds in the removal of toxic chemical compounds and protect liver cells from damage resulting from treatment with such compounds has been found in [14] that the flavonoids extracted from the plant licorice had a protective effect against the toxic effects and destructive to liver cells caused by a chemical compound carbon tetrachloride (CCl4) by reducing the effectiveness of the liver enzymes GOT and GPT in serum.

As noted [15] that treatment of mice exposed to the impact of a real estate Methotrexate (MTX) and Cyclophosphamide (CP) separately in different concentrations of extracts of the seeds of Nigella sativa water and alcohol led to maintain the level of enzymes GOT, GPT and ALP in liver cells heterogeneous compared with untreated mice.

It also found that the compound Thymoquinione (TQ) isolated from the seeds of Nigella sativa important role in protected liver cells isolated from rats and treatment by Tetra butyl hydroperoxide (TBHP) by maintaining the enzyme liver from exuding out of the cell, particularly the enzyme Glutathion reductase (GSH), which has a key role in the removal of toxic compounds [16].

While study of [17] determined the ability of vitamin C in protected liver cells from the toxic effects induced by Aflatoxin-B1 by preventing the leakage of liver enzymes to the blood serum of rabbits treated with poison.

**CONCLUSIONS**

1 - The methotrexate (MTX) agent had toxic effect when tested in laboratory mice.
2 - The flavonoids extracted from the leaves of *Camellia sinensis* inhibition the enzymatic efficiency of the toxic effect of the mutagenic MTX when the overlap between this extract and mutagen.

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5. Intratumoral administration of methotrexate bound to activated carbon particles: antitumor effectiveness against human colon carcinoma xenografts and acute toxicity in mice. [http://jpet.aspetjournals.org/cgi/content/full/311/1/382](http://jpet.aspetjournals.org/cgi/content/full/311/1/382). (2004).