Study of antioxidant effect of *Curcuma longa* L. phenolic extract, sodium selenite and vit. E on some physiological and biochemical criteria of white male rats treated with chromium picolinate

Haider salih jaffat and Afyaa sabah nasir  
Faculty of science/University of kufa

**Abstract**  
The current study was designed to determine the antioxidant effects of phenolic extract of *Curcuma longa* L., sodium selenite and vitamin E against oxidative stress induced by chromium picolinate in some physiological and biochemical criteria for the blood, some antioxidants and neurotransmitters of the brain on the adult male rats.

The study was conducted in the animal house of the Faculty of Science/University of Kufa on 70 animals of adult male rats aged 2.5-3 months and the weight of 200-250 gm. The results showed no significant change (p>0.05) in the average of body weight, body organ weight and liver enzymes in the rats treated with chromium picolinate only for the two periods of the experiment as well as the same results recorded in the animals treated with chromium picolinate with vitamin E and sodium selenite and chromium picolinate with the phenolic extract of *Curcuma longa* for six and eight weeks compared with control group. As it has been noted a significant decrease (p<0.05) in the antioxidants include superoxide dismutase, glutathione peroxidase and significant increase (p<0.05) in the malondialdehyde in the animals treated with chromium picolinate for the two period of administration compared with the control group also a significant decrease (p<0.05) in dopamine (DA) and a significant increase (p<0.05) on serotonin (5-HT) in the animals treated with chromium picolinate only in compared with control group. Moreover, the significant increase occurred (p<0.05) in the antioxidants superoxide dismutase and glutathione peroxidase levels and significant decrease(p<0.05) in malondialdehyde level in the animals treated by chromium picolinate separately with the phenolic extract of *Curcuma longa*, chromium picolinate with vitamin E and sodium selenite compared with the control group and the results showed a significant increase (p<0.05) in dopamine (DA) and significant decrease (p <0.05) in the serotonin (5-HT) in the groups mentioned above for a periods of six and eight weeks compared with control group.

The study conclude from that the phenolic extract of *Curcuma longa*, sodium selenite and vitamin E have effective antioxidants against oxidative stress induced by oral administration of chromium picolinate led to an improvement of the above criteria studied, compared with control group and the best results are recorded after treatment by the phenolic extract of *Curcuma longa* .

Key words : oxidative stress, *Curcuma longa*, sodium selenite, vitamin E and chromium picolinate.

**Introduction :**
Oxidative anxiety mirrors an irregularity between the systemic indication of receptive oxygen species and an organic framework's capacity to promptly detoxify the responsive intermediates or to repair the subsequent harm [1]. Aggravations in the typical redox condition of cells can bring about poisonous impacts through the creation of peroxides and free radicals that harm all parts of the phone, including proteins, lipids, and DNA [2]. Oxidative anxiety from oxidative digestion system causes base harm, and additionally strand softens up DNA. Base harm is for the most part circuitous and brought about by receptive oxygen species (ROS) created, e.g. O2•-(superoxide radical), HO• (hydroxyl radical) and H2O2 (hydrogen peroxide) [3].
Chromium (Cr) is a follow component found in the earth generally in trivalent, Cr (III), and hexavalent, Cr (VI), shapes [4]. Be that as it may, Cr (III) is the most stable structure in the sustenance supply and in vivo, its capacities as a cofactor for the hormone insulin and upgrades the capacity of insulin to manage glucose, protein, and fat digestion system [5]. Different trivalent chromate mixes have been utilized as nutritious supplements as a part of people and sustain added substances in residential creatures, dietary chromium supplementation can modify body synthesis, yet the capacity of various types of chromium may fluctuate in doing this [6]. It is proposed that the retention and use of chromium might be reliant on its status in intestinal tract. Size, nature of the polymer, zeta potential and vehicle have been resolved as basic elements impacting molecule uptake [7].

Curcuma longa L., on the other hand turmeric (Family: Zingiberaceae) contains more than 80 types of rhizomatous herbs. They happen in wild and developed structures and are generally circulated all through the tropics of Asia, Africa and Australia. The most well-known species is Curcuma Longa L. which is utilized as a characteristic sustenance colorant, fragrance, cancer prevention agent property and as a fixing in different restorative definitions [8]. The therapeutic properties of C. Longa L. have been ascribed to the nearness of curcumin, key oils and phenolics [9]. these enzymatic and non-enzymatic cancer prevention agent frameworks are important for managing life by keeping up a sensitive intracellular redox adjust and minimizing undesirable cell harm brought about by ROS [10].

Selenium is a crucial follow component for people and numerous different types of life, and a lack of this component initiates some neurotic conditions, for example, growth, coronary illness, and liver rot [11]. Selenium is a key follow mineral that is a segment of real cancer prevention agent which has essential part in human wellbeing, secured, the cells from the hurtful impacts of free radicals [12].

Vitamin E is a critical part of human eating routine which ensures the body's organic frameworks by checking lipid peroxidation [13 , 14]. In view of the wellbeing dangers prompted by numerous ecological contaminations, a few studies assessed the relative cell reinforcement power of vitamins E [15, 16].

Materials and Methods

Experimental animals:
Using 70 adult male rats (Rattus norvegicus ) weighting 200-250 gm were obtained from the animals house in high institutes of fertility/University of Nahrain. The animals were housed in the animal house of Faculty of Science, University of Kufa, under standard environment condition (temperature 25-28 C° and 12 hr light-dark cycle) and allowed access to standard laboratory diet and water.

Experimental protocol
The rats were kept in animal house for acclimation to the laboratory condition for two weeks before they were used for the experiment. each group was formed 10 rats and the rats administrated chromium picolinate by intra gastric intubation :

**Group (1)** rats were administrated of chromium picolinate at dose 3 mg/kg for six and eight weeks (as positive control).
**Group (2)** rats were administrated of chromium picolinate at dose 3 mg/kg and phenolic extract of Curcuma longa for six and eight weeks.
Group (3) rats were administrated of chromium picolinate at dose 3 mg/kg and solution contained sodium selenite and vitamin E for six and eight weeks for six and eight weeks.

Group (4) rats were administrated of chromium picolinate at dose 6 mg/kg for six and eight weeks (as positive control).

Group (5) rats were administrated of chromium picolinate at dose 6 mg/kg and phenolic extract of Curcuma longa for six and eight weeks.

Group (6) rats were administrated of chromium picolinate at dose 6 mg/kg and solution contained sodium selenite and vitamin E for six and eight weeks.

Group (7) rats were administrated of normal saline for six and eight weeks (as negative control).

Total content of phenols (Ethanol extract of phenols (curcuminoids))

Twenty grams of turmeric rhizome powder extracted with ethanol (80%) for 24 h in a continuous extraction by Soxhlet apparatus 250 ml volume. The extract was evaporated on a rotary evaporator under vacuum at a temperature of 60 C° until the solution reached to 10 ml. Then, the solution was transferred to a separating funnel and (2 N) HCl was added gradually to get pH 2 then, washed with 10 ml chloroform three times. The solution was separated into two levels, the down level contained the phenols (curcuminoids) which were resided, weighted and kept in a refrigerated until using it [17].

Blood Collection

At the end of experiments. Each animal was anaesthetized by the mixture of xylazine 0.1 ml and ketamine 0.5 ml and they were scarified [18]. Heart cut was finished with a 5 ml expendable syringe and 2-5 ml blood was drawn delicately and gradually. Every blood test was separated into 2 sections. The initial segment (around 0.5 ml) was set in a tube containing EDTA (22mg/ml) as anticoagulant and blended altogether, then utilized for the assurance of hematological investigation by a programmed analyzer. The rest of the blood was put in test tube containing gel and left for 30 minutes in room temperature and used to get serum through centrifugation at 3000 rpm for 15 minutes to separate serum and put in epindroff tubes which kept at (-20) in a cooler for assurance biochemical examination and the belly was opened to get the liver and kidney for weight estimation.

Determination of liver enzymes

A- Determination of Serum Transaminase Activity Transaminases – Kit
ALT & AST activity were determine by colorimetric method according to the biolabo kit, france [19].

B- Determination of Serum Alkaline Phosphatase Activity
Colorimetric determination of ALP according to biomerieux kit [20].

Determination of antioxidant parameters and neurotransmitters

A- Determination of Super Oxide Dismutase level in serum (SOD)

B- Determination of Serum Glutathione peroxidase Activity (GPX)
The quantitative determination of GPX concentration in serum through the enzyme linked immunosorbant assay using ELISA kit (Elabscience, U.S.A.) (www.elabscience.com, 2016).

C- Determination of Malondialdehyde Activity (MDA)
Measurement of MDA was determine by ELISA kit (Elabscience,U.S.A.) (www.elabscience.com, 2016).

E- Measurement of dopamine (DA)
This laboratory test was determined by ELISA kit (Elabscience, U.S.A.) (www.elabscience.com, 2016).

**F- Assessment of serotonin (5-HT)**

The levels of serotonin in serum were evaluate by ELISA kit (Elabscience, U.S.A.) (www.elabscience.com, 2016).

**Statistical Analysis**

Data were presented as means ± S.E. and statistically analyzed using (ANOVA) test followed by least significant difference (L.S.D.) analyses at 0.05% probability of levels. Using computerized SPSS program [21].

**Results :**

The results in table (1) show no significant decrease (p>0.05) in the average of body weight, liver weight and kidney weight in the groups of rats treated with chromium picolinate at doses 3 mg/kg and 6 mg/kg for six and eight weeks in compare with control group. Also, the results in the same table show no significant increase (p>0.05) in the average of body weight, liver weight and kidney weight in the groups of rats treated with chromium picolinate at doses 3 mg/kg and 6 mg/kg with phenolic extract of *Curcuma longa* for six and eight weeks in compare with control group and chromium picolinate at two doses with antioxidant solution contain sodium selenite and vitamin E for six and eight weeks in compare with control group.
Table (1) Effect of the interaction between the duration, extracts and doses in the average of body weight, liver weight and kidney weight in the treated rats by chromium picolinate for six and eight weeks.

<table>
<thead>
<tr>
<th>Week</th>
<th>Extracts</th>
<th>Dose (mg/kg)</th>
<th>Initial body weight (gm)</th>
<th>Final body weight (gm)</th>
<th>Liver weight (%)</th>
<th>Kidney weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>CrP</td>
<td>3</td>
<td>225±3.26</td>
<td>213±3.33</td>
<td>7.13±0.05</td>
<td>0.98±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>223±5.58</td>
<td>216±6.83</td>
<td>7.52±0.31</td>
<td>1.03±0.02</td>
</tr>
<tr>
<td>6</td>
<td>CrP +S</td>
<td>3</td>
<td>244±8.54</td>
<td>210±1.01</td>
<td>7.20±0.02</td>
<td>1.03±0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>234±1.39</td>
<td>215±5.48</td>
<td>7.57±0.37</td>
<td>0.97±0.05</td>
</tr>
<tr>
<td>6</td>
<td>CrP +C</td>
<td>3</td>
<td>226±5.21</td>
<td>241±7.26</td>
<td>7.49±0.23</td>
<td>1.03±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>236±1.04</td>
<td>238±1.66</td>
<td>7.27±0.04</td>
<td>1.03±0.09</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>235±1.02</td>
<td>245±0.21</td>
<td>7.23±0.25</td>
<td>1.02±0.02</td>
</tr>
<tr>
<td></td>
<td>L.S.D. 0.05</td>
<td></td>
<td>8.215</td>
<td>7.126</td>
<td>0.321</td>
<td>0.253</td>
</tr>
<tr>
<td>8</td>
<td>CrP</td>
<td>3</td>
<td>223±1.23</td>
<td>219±1.34</td>
<td>7.30±0.08</td>
<td>0.97±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>212±6.25</td>
<td>222±3.92</td>
<td>7.62±0.34</td>
<td>0.99±0.06</td>
</tr>
<tr>
<td>8</td>
<td>CrP +S</td>
<td>3</td>
<td>250±35.4</td>
<td>213±8.81</td>
<td>7.65±0.29</td>
<td>1.01±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>238±4.15</td>
<td>221±8.41</td>
<td>7.48±0.16</td>
<td>0.97±0.09</td>
</tr>
<tr>
<td>8</td>
<td>CrP +C</td>
<td>3</td>
<td>225±37.2</td>
<td>247±7.88</td>
<td>7.79±0.21</td>
<td>1.05±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>245±1.65</td>
<td>225±9.83</td>
<td>7.56±0.29</td>
<td>1.09±0.09</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>234±1.23</td>
<td>234±0.15</td>
<td>7.89±0.24</td>
<td>1.06±0.04</td>
</tr>
<tr>
<td></td>
<td>L.S.D. 0.05</td>
<td></td>
<td>6.250</td>
<td>8.254</td>
<td>0.159</td>
<td>0.357</td>
</tr>
</tbody>
</table>

Number of animals = 5 for each group
Each value represents mean ± S.E.
CrP : Chromium picolinate
S : Solution contain sodium selenite and vitamin E

URL: http://www.uokufa.edu.iq/journals/index.php/ajb/index
http://iasj.net/iasj?func=issues&jId=129&uiLanguage=en
Email: biomgzn.sci@uokufa.edu.iq
C : Phenolic extract of *Curcuma longa* (Curcuminoids)

The results in table (2) show no significant increase (p>0.05) in the liver enzyme levels include (AST, ALT and ALP) in the groups of rats treated with chromium picolinate at doses 3 mg/kg and 6 mg/kg for six and eight weeks in compare with control group.

Also, the results in the same table show no significant decrease (p>0.05) in AST, ALT and ALP in the groups of rats treated with chromium picolinate at doses 3 mg/kg and 6 mg/kg with phenolic extract of *Curcuma longa* for six and eight weeks in compare with control group and chromium picolinate at two doses with antioxidant solution contain sodium selenite and vitamin E for six and eight weeks in compare with control group.

**Table (2) Effect of the interaction between the duration, extracts and concentrations in the liver enzyme levels in the rats treated with chromium picolinate for six and eight weeks.**
Number of animals = 5 for each group
Each value represents mean ± S.E.
CrP : Chromium picolinate
S : Solution contain sodium selenite and vitamin E
C : Phenolic extract of *Curcuma longa* (Curcuminoids)

The results in table (3) show significant increase (p<0.05) in malondialdehyde and significant decrease (p<0.05) in Superoxide dismutase and Glutathione peroxidase in the groups of rats treated with chromium picolinate at doses 3 mg/kg and 6 mg/kg for six and eight weeks in compare with control group.
Also, the results in the same table show significant decrease (p<0.05) in malondialdehyde and significant increase (p<0.05) in Superoxide dismutase and Glutathione peroxidase in the groups of rats treated with chromium picolinate at doses 3 mg/kg and 6 mg/kg with phenolic extract of *Curcuma longa* for six and eight weeks in compare with control group and chromium picolinate at two doses with antioxidant solution contain sodium selenite and vitamin E for six and eight weeks in compare with control group.

### Table (3) Effect of the interaction between the duration, extracts and concentrations in the antioxidant levels in the rats treated with chromium picolinate for six and eight weeks.

<table>
<thead>
<tr>
<th>Week</th>
<th>Extracts</th>
<th>Con. (mg/kg)</th>
<th>MDA (ng/ml)</th>
<th>SOD (ng/ml)</th>
<th>GPX (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>CrP</td>
<td>3</td>
<td>45.67 ±26.11</td>
<td>0.15 ±0.04</td>
<td>64.28 ±1.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>47.92 ±5.53</td>
<td>0.10 ±0.03</td>
<td>77.05 ±1.99</td>
</tr>
</tbody>
</table>
The results in table (4) show significant decrease (p<0.05) in Dopamine level and significant increase (p<0.05) in Serotonin level in the groups of rats treated with chromium picolinate at doses 3 mg/kg and 6 mg/kg for six and eight weeks in compare with control group.

Also, the results in the same table show significant decrease (p<0.05) in Dopamine level and significant increase (p<0.05) in Serotonin level in the groups of rats treated with chromium picolinate at doses 3 mg/kg and 6 mg/kg with phenolic extract of *Curcuma longa* for six and eight weeks in compare with control group and lithium carbonate at two doses with antioxidant solution contain sodium selenite and vitamin E for six and eight weeks in compare with control group.

Number of animals = 5 for each group
Each value represents mean ± S.E.
CrP : Chromium picolinate
S : Solution contain sodium selenite and vitamin E
C : Phenolic extract of *Curcuma longa* (Curcuminoids)
Different litters mean significant difference (0.05) between the chromium picolinate groups and control group
Table (4) Effect of the interaction between the duration, extracts and concentrations in the neurotransmitter levels in the rats treated with chromium picolinate for six and eight weeks.

<table>
<thead>
<tr>
<th>Week</th>
<th>Extracts</th>
<th>Dose (mg/kg)</th>
<th>DA (ng/ml)</th>
<th>5-HT (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>CrP</td>
<td>0.422 a ±0.236</td>
<td>0.621 b ±0.245</td>
<td>29.09 a ±6.38</td>
</tr>
<tr>
<td></td>
<td>CrP +S</td>
<td>0.554±0.249</td>
<td>0.633±0.480</td>
<td>21.59±1.29</td>
</tr>
<tr>
<td></td>
<td>CrP +C</td>
<td>0.664±0.402</td>
<td>0.560±0.319</td>
<td>21.08±0.79</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.521±0.815</td>
<td>0.124</td>
<td>17.09±1.30</td>
</tr>
<tr>
<td></td>
<td>L.S.D. 0.05</td>
<td>0.124</td>
<td></td>
<td>2.163</td>
</tr>
</tbody>
</table>

| 8    | CrP      | 0.909 a ±0.201 | 0.838 b ±0.141 | 33.01 a ±2.857 | 41.73 b ±2.465 |
|      | CrP +S   | 0.837±0.336   | 0.774±0.365 | 25.16±4.938   | 37.73±3.564   |
|      | CrP +C   | 0.815±0.501   | 0.949±0.219 | 28.40±1.659   | 32.40±4.761   |
|      | Control  | 1.429±0.366   | 0.356      | 18.34±0.317   | 3.308        |
|      | L.S.D. 0.05 | 0.356       |            | 3.308        |

Number of animals = 5 for each group
Each value represents mean ± S.E.
CrP : Chromium picolinate
S : Solution contain sodium selenite and vitamin E
C : Phenolic extract of Curcuma longa (Curcuminoids)
Different litters mean significant difference (0.05) between the chromium picolinate groups and control group
Discussion:

The results in the current study are recorded no significant decrease (p>0.05) in the average of body weight, Liver weight and Kidney weight in the groups of rats treated with chromium picolinate for six and eight weeks in compare with control group. These results agreed with Long-ying et al. [22] recorded are chromium assumes a part in the direction of body mass (BM), percent muscle to fat ratio ratios, and weight lessening. But the perspective is still very questionable, in swine, dietary expansion of chromium is accounted for to expand cadaver leanness and abatement body bloatedness.

Page et al. [23] reported that longissimus muscle region and rate of muscling are expanded and fat is diminished by chromium picolinate (CrP) added to the weight control plans of developing completing pigs.

In human, there were additionally reports of expanded bulk and diminished body heftiness because of chromium supplementation [24]. Notwithstanding, reports from Clancy et al. [25] and Campbell et al. [26] don’t bolster an impact of supplemental chromium on modifying body organization. The measurements in studies on rats [27], pigs [28] and steers [29], which have discernible changes in body creation because of chromium supplementation extended from 25 to 1 000 ppb.

In addition, the results in the following study appear no changed (p>0.05) of the liver enzymes levels in the laboratory animals after oral administration of chromium picolinate for six and eight weeks. These results agreement with Ernest et al. [30] have reported the normal estimation of AST, ALT and ALP are not influenced by the organization of the chromium picolinate in the male white rats.

Oral organization of 0.45-77ppm of trivalent chromium drinking water don’t deliver any obsessive changes in the liver and kidneys of canines, however these organs had a generally high chromium content [31]. Be that as it may, in one case, 1200 to 2400 mg chromium picolinate for 4-5 months is found to create renal poisonous quality and for another situation 600 mg of chromium day by day for 6 weeks is found to deliver intense interstitial nephritis [32].

Treatment with chromium picolinate don't modify serum AST and ALT levels and in addition liver morphology of typical rats and this shows chromium picolinate does not bring about hepatoxicity in rats under the ordinary conditions [33].

Treatment with chromium picolinate essentially diminished hoisted AST and ALT levels. Treatment additionally diminished the vacuolization and hypertrophy of hepatic cells saw in streptozotocin-diabetic rats. Further, liver being the following organ to kidney in collecting chromium, the likelihood of nephrotoxicity notwithstanding the impact on modified hepatic capacity is examined in the present examination. Treatment with chromium picolinate don't modify serum AST and ALT levels and additionally liver morphology of non-diabetic rats [34]. This demonstrates chromium picolinate does not bring about hepatoxicity in non-diabetic or diabetic rats under the states of the present examination. Since chromium picolinate is found to have noteworthy against diabetic potential in streptozotocin and also streptozotocin-diabetic rats. it is sensible to trust that change in renal and hepatic capacity and also morphology could have come about because of the easing of the diabetic condition of creatures [35].

The results in the present study are reported significant decrease (p<0.05) in the levels of SOD and GPX and significant increase (p<0.05) in the level of MDA in the experimental rats after exposed to chromium picolinate. a few studies are recorded which chromium poisonous quality can be connected with oxidative anxiety [36, 37].
As long haul chromium treatment is utilized as a part of the cure of this infection, the topic of the impact of lithium on oxidative procedures is an issue of incredible significance. The living beings built up a mind boggling arrangement of resistance against oxidative anxiety which incorporates various substances, in addition to other things cancer prevention agent chemicals, to be specific superoxide dismutase, glutathione peroxidase and catalase [38, 39]

The comparative results are recorded in the creatures after oral organization of chromium picolinate for six and eight weeks interestingly with control bunch. Trivalent chromium supplements, for example, chromium picolinate and niacin bound chromium, Cr(III) is devoured by the overall public through its nearness in numerous nourishments [40].

What's more, there is across the board utilization of chromium (III) present in dietary supplements, for example, CrP, that are showcased fundamentally as antidiabetic impact. People ordinarily ingest 20 to 45 μg chromium (III) every day in the eating regimen [41], while run of the mill day by day measurements of supplements may contain 200 to 1000 μg chromium (III). It is realized that chromium (III) can collect in the body. Ingestion of CrP supplements is found to create serum levels of chromium that are proportionate to serum levels measured in laborers occupationally presented to chromium, and to deliver urinary chromium levels higher than those in the general population earth presented to chromium. Chromium has additionally been appeared to gather in rodent liver, kidneys, spleen, lungs, legs, testicles and heart after ingestion of CrP in the eating routine. The structure and coordination science of CrP may make it more lethal than different types of chromium (III) [42, 43].

References


