The Effect of Supplementation with Quercetin, Catechin and Rich Food of Them on Immune System in Experimental Rats

Usama El-S Mostafa, Walaa I.M. Anuess, Hala R. Ataya
Mahmoud R. M. Rashwan

Abstract

Aim of the study: to assess the effect of Quercetin (Q), catechin (CT) and rich food of them supplementation on immune system in rats.

Materials and Methods: 36 rats were allocated in 6 groups, first group (6 rats) served as (-ve) negative control group and fed on basal diet, while main (30 rats) drank polluted water with cadmium chloride (CD) 40mg/k.g for 40 days (to induced immunity inhibition) and the second group was set as a positive control group (+ve) that was fed the basic diet only. Then rats were divided equally to five groups (2, 3, 4, 5, 6) and fed on basal diet only (positive control) or with Q commercial at 45mg swallowed orally, red dried onions as a natural source Q by 12 g, CT commercial at 7.5mg swallowed orally and green tea (GT) powder as a natural source CT by 5.5 g respectively, treatments were for 21 days as mentioned.

Results: In all treatment groups there were an improvement in IgG, RBC, HGB, HCH and MCHC to approach the negative control level (Healthy group). While increased in the SOD, GPX and CAT compared with the control positive group. Also decreased in the MCV, urea, u.acid, creatinine, GOT, GPT and T.P compared with the control positive group. While the better result was in group 4 Q natural as it improvement in Urea, creatinine, GPX, CAT, SOD and IgG compared with all treatment groups.

Conclusion: Consumption Q and CT from natural and commercial sources had important role in Strengthened the immune system, and reduced deleterious effects of CD in rats.

Keywords: quercetin, catechins, immune system, cadmium chloride, food intake

Introduction

The immune system is a systemically mobile network of cells with emergent properties derived from dynamic cellular
interactions (Calder, 2020). Immunosuppression is defined as a reduction in immune function as evaluated by cellular, humoral, or non-specific immunological markers. Although a wide range of modest effects have been observed, the primary immunological response (e.g. macrophage phagocytic activity) is more sensitive to inhibition. Heavy metals, polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), CD toxic effects, air pollution, pesticides, and medications can all have long-term immunosuppressive effects. Increased incidence of infectious illnesses and neoplasia are possible clinical outcomes of immunosuppression (Sullivan et al., 2001 and Bendall et al., 2012).

CD immunotoxic effects on immune organ development, immune cell differentiation, and specific and non-specific immunological responses have been documented (El-Boshy et al., 2015). As a result of this, humans are exposed to high levels of Cd, which has harmful effects on the immune system, immunosuppression, kidney disease, the skeleton, cardiovascular system, and central nervous system, as well as being classed as a human carcinogen (Nair et al., 2013).

Many potential plant-based drugs have been studied in the Ayurvedic system of medicine to combat toxic effects because they are high in antioxidants, act as immune stimulants, and are capable of terminating free radical reactions that protect our bodies from oxidative stress, have fewer side effects, and are compatible with body physiology (Hsu, 2006).

Q has great importance, where have found that Q has great therapeutic potential in the prevention and treatment of various chronic diseases, immune system diseases, immunosuppression, cardiovascular and neurodegenerative diseases, as well as cancer (Boots et al., 2008, Dajas, 2012 and D’Andrea., 2015). Q has been proven to improve health in a variety of cellular and animal models, as well as in people, through regulating signaling pathways and gene expression involved in these processes (Russo et al., 2012, Serban et al., 2016 and Wang et al., 2016). As a
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result, consuming a Q-rich diet has been linked to improved health (D'Andrea, 2015).

Q is a member of the class of flavonoids called flavonoles and forms the backbone for many other flavonoids including the citrus flavonoids like rutin, hesperidin's, naringenin and tangeritin. It is widely distributed in the plant kingdom in rinds and barks. Q itself is an aglycon or aglucone that does not possess a carbohydrate moiety in its structure (Lakhanpal and Rai., 2007). Studies suggest that Q supplementation may promote antioxidant, anti-inflammatory, antiviral and immune protective effects (Nair et al., 2002, Robaszkiewicz et al., 2007 and Uchide and Toyoda., 2011).

Flavonoids, polyphenols, glycosides, anthocyanins, allicin, and Q are abundant in onions (Ye et al., 2013). They're utilised as a natural antioxidant to keep meat from oxidising. This antioxidant activity is owing to their ability to scavenge free radicals and release electrons or hydrogen atoms in the process (Rajani et al., 2011 and Sharma and Goyal., 2015).

Onion (Allium cepa L.) is a biennial plant belonging to the Liliaceae family, one of the most important vegetable crops with a world production of about 55 million tons (Y. Shokoohinia et al., 2016, Griffiths et al., 2002, Teena et al., 2016 and Raja et al., 2016).

Total phenol content, which is high in red onion (RON), and flavonoids, primarily Q, which is high in yellow onions (347 mg/kg Q), have been linked to antioxidant activity (Nemeth and Piskula., 2007). Q contains strong antioxidant properties that can help prevent diseases related by oxidative stress (McDermott, 2000 and Arabbi et al., 2004).

A research conducted by Hertog et al., (1992) who reported that onions ranked the highest in Q content of 28 vegetables and 9 fruits. Claimed that the levels of Q in RON were 14-fold higher than that of garlic, whereas the levels were twofold higher than white onions. In addition, Q amounts in the
onion peel were 48-fold more than the flesh (Gorinstein et al., 2008).

CT is a type of polyphenol found in a variety of foods and medicinal plants, including legumes, Rubiaceae, stem bark of fabaceae species, including the Mimosoideae subfamily's Abarema cochliacarpos, teas (GT, pu-erh tea, pu-erh GT), Mouriri pusam Gardn, buckwheat, grapes, cocoa beans, litchis (Sánchez-Fidalgo et al., 2013 and Fathima and Rao., 2016). For thousands of years throughout Asia, particularly in China and Japan, CT has been considered as the characteristic chemicals in GT for daily beverage and crude medicine (Saleem et al., 2015). CT comprises CT, epicatechin, epicatechin gallate, epigallocatechin and its stereoisomer gallocatechin, EGCG and its stereoisomer gallocatechin gallate, all of which have comparable components (Muzolf-Panek et al., 2008).

GT, a beverage produced from the leaves and buds of the Camellia sinensis (C. sinensis) plant, is the world's second most popular beverage after water. Tea polyphenols, vitamins, nitrogenous chemicals, caffeine, inorganic elements, lipids, and carbs are the main components of GT (Chu and Juneja, 1997). Several studies in humans and laboratory animals suggest that GT, or 'non-fermented' tea, has a positive impact on bone density, cognitive function, dental cavities, and kidney stones and improving the efficiency of the immune system (Crespy and Williamson., 2004, Cabrera et al., 2006 and Sheikhzadeh et al., 2011).

Through the above this study aimed to study the effect of Quercetin from natural, commercial source and catechin from natural, commercial source on rise the efficiency of the immune system.
MATERIALS AND METHODS

Materials:

1-Animals: 36 male albino rats (weight from 180: 230 g) were obtained from the National Research Center - Dokki Giza, Egypt. Animals were clinically healthy and they randomized and housed in stainless steel wire bottom cages (6 rats / cage) and maintained in air conditioned room on a 12h light/dark cycle at 22 °C. They were acclimatized under the test conditions for one week before treatments. Rats were fed basal diet (table 1) with known chemical composition according to Hegsted et al., (1941). The composition of the basal diet ingredients, the vitamin and salt mixtures Q, CT and CD was given from Middle East Company Cairo, are, CT commercial was purchased from international, RON and dried GT were obtained from the local market in Cairo, Egypt.

Table (1) composition of the basal diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>15%</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10%</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5%</td>
</tr>
<tr>
<td>Vit. Mixture</td>
<td>1%</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>4%</td>
</tr>
<tr>
<td>Corn total</td>
<td>65%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100%</td>
</tr>
</tbody>
</table>

The RON was purchased from the local market in Cairo, Egypt then it was cut into rings and placed in an under vacuum dryer at a temperature of 45 °C for 48 hours, then the dried onions were crushed until it became powder and mixed with the main meal (Gorreapiti et al., 2017).
GT powder was purchased from the local market in Cairo, Egypt and then mixed with the main meal.

During the feeding experiments, animals were daily inspected and food intake was recorded, while body weights were recorded twice a week. The feeding experiment lasted for 9 weeks.

At the end of the experimental period (9 weeks), rats were fasted over night before sacrificing. Blood samples collected from each rats and centrifuged at 3000 r.p.m. to separate the serum. Serum was carefully separated and transferred into dry clean Ebendorf tubes and kept frozen at – 20°C till analysis.

Liver and Spleen were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were washed with cold saline and dried between two filter papers, then weighed and kept in formalin solution (10%), according to Drury and Wallington., (1980).

2-Experimental design:

Experimental diet and water were offered ad labium all over the experimental period. 36 rats were divided into 6 groups (6 rats each) classified as follows:

Based on the previous study (Abnosi and Golami., 2017), control positive group and the four groups treated received 40 mg/L of CD in drinking water for 40 day then 21 day to induce immunity inhibition intervention for group 3,4,5,6.

The first group was served as negative control (-ve) and fed basal diet. Then divided into 5 groups (6 rats each). The second group was served as positive control (+ve) and fed basal diet only. The third group was fed the basic diet, in addition to Q commercial at 45 mg swallowed orally the fourth group is fed on the basic diet, in addition to red dried onions as a natural source Q by 12 g it equal 45mg Q, Fifth group was fed the basic diet, in addition to CT commercial at 7.5 mg swallowed orally. Sixth
group is fed on the basic diet, in addition to GT powder as a natural source CT by 5.5 g it equal 7.5 mg CT.

3-Biochemical analysis:

Hemoglobin concentration:

The concentration of hemoglobin (HGB) (g/dl) was determined using a colorimetric technique using HGB kits. For laboratory services, the Egyptian American Company provided the kits. The determination was carried out using a spectrophotometer in accordance with the manufacturer's instructions.

Blood was extracted using hematocrit capillary tubs and centrifuged for 10 minutes at 3000 r.p.m. using a micro hematocrit centrifuge. The hematocrit (HCT) was measured and recorded as stated by (Nemi, 1986).

Calculation of win Trobe erythrocyte and platelets indices:

Automated blood cell counting was used to measure mean cell volume (MCV), mean cell HGB (MCH), mean cell HGB concentration (MCHC), red cell distribution width (RDW), and blood platelets (PLT), platelets volume (MPV), platelets distribution width (PDW), and PCT, as well as blood platelets (PLT), platelets volume (MPV), platelets distribution width (PDW), and PCT, according to the method of Beckman Coulter and System (Graig, 1998).

Differential of leukocyte:

Blood films were produced, air dried, and stained for 5-7 minutes using Fleishman's stain [0.2g of dye in 100 ml of methanol in a conical flask 200-250 ml and warmed at 50 C for 15 minutes, with periodic shaking, then the solution is filtered]. Slides with blood films were washed in a stream of buffered water until it has acquired a pinkish tinge (up to 2 min), and examined according to Graig., (1998).
Determination of serum Urea:

Serum urea was determined using kits of bio Meraux according to the method of (Fawcett, 1960). Following the enzymatic breakdown of urea by the urease enzyme, ammonium ions were generated. In an alkaline medium, the generated ammonium ions were subsequently reacted with salicylate and hypochlorite, resulting in a green color (2,2-dicarboxyl indophenol), at 580 nm, the colours prod (Dacia and Lewis., 1975).

Determination of serum creatinine:

Serum creatinine was measured by the method of (Fawcett, 1960), using bio Meraux reagent deprotenization, the color developed after the addition of fresh alkaline picrate, was measured calorimetrically at 250 nm.

Determination of serum Uric acid:

Method

Urease – POD enzymatic colorimetric method with 4-Amin- antipyrine.

Assay principle:

The assay is based upon the methods of modified tinder peroxides assay using 3.5- dichloro 2- hydroxyl benzene sulfonic acid (BCHB) Tietz, (1990).

Calculation:

Serum uric acid concentration (mg/dl) = A specimen/ A standard × 6.

Concentration of uric acid in urine = A specimen/ A standard ×6×10.

Determination of serum aspartate aminotransferase (GOT):

Method

AST- (Colorimetric method) according to (Young, 1990).
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**Determination of serum total protein:**

Serum total protein was determined by colorimetric method (biuret reagent) described by Tietz, (1990).

**Determination of serum superoxide dismutase (SOD):**

**Principle:**

This assay relies on the ability of the enzyme to inhibit the phenazine met hosalphate- mediated reduction of nitro blue tetrazolium dye (Young, 1990).

**Calculation:**

Percent inhibition = A control – A sample/ A control ×100.

**Determination of serum Glutathione Peroxidase (GPX):**

**Principle:**

The assay is an indirect measure of the activity of GPX. Oxidized glutathione (GSSG), produced upon reduction of an organic peroxide by C-GPX is recycled to its reduced state by the enzyme glutathione reeducates (GR) (Nishikimi and Yogi., 1972).

**Calculation:**

\[ \text{MU/mL} = \frac{1 \text{ nmol NADPH/min/mL}}{A_{340} \div \text{min} \div 0.00622} \]

**Determination of serum superoxide dismutase (CAT):**

**Principle:**

Catalase reacts with a known quantity of H2O2. The reaction is stopped after exactly one minute with catalase inhibitor (Paglia and Valentine., 1967).

**Calculation:**

\[ \text{In plasma (U/L)} = \frac{A \text{ standard- A sample}}{A \text{ standard}} \times 100 \]
Determination of IgG concentration:

Total IgG levels were measured with ELISA according to the method of (Wiedermann et al., 1993). For measurement of total IgG, 50 µl of diluted serum sample was placed in each well of microliter plates (falcon japan, Tokyo). Samples were kept overnight at 4°C. After washing twice with phosphate buffer saline –T (PBS-T) (phosphate buffer saline with 0.05% tween-20), 200 µl of ELISA buffer per well were added, and the plate was incubated for 2 h at 37°C. after washing four times with PBS-T 0.2 µg of a rat anti-mouse IgG monoclonal antibody (vector lab., Burlingame, CA) in 100 µl of PBS was added for 1h at 37°C bound antibodies were detected with a three-stage indirect immunoperoxidase kit (vectastain, ABC kit, vector lab., burkingame, CA), using 2, 2-azino-d (3-ethyl-benzthiazoline sulfate) (ABTS) as a substrate (Lavanchy et al., 1990).

Thirty minutes later, the plates were placed in a spectrophotometer (Immunomini NJ2300; Nalge Nunc international Japan Tokyo), and absorbance was measured at 405 nm. The amount of IgG was calculated using mouse IgG, purified from mouse myeloma cells (zymed, South San Francisco, CA), as a standard and expressed as g/ L serum. The lower detection limit in this assay was 50 µg/ L.

Histopathological examinations:

Animals were first anesthetized by ether them scarified dissected and spleen, liver, and thymus gland were dissected out the specimens were treated for light microscopy, haematoxylin and eosin methods according to (Disbray and Roch., 1970).

Regents:

a) 10% formalin solution
b) Harris haematoxylin.

The haematoxylin (lg) was dissolved in 10 ml ethanol, mixed with 10 ml contains 20 g potassium alum (dissolved by heat). The mixture was boiled and 0.5g mercuric oxide was
added. The solution was cooled rapidly by immersing the flask in ice cold water then 6 ml glacial acetic acid were added.

c) Hydrochloric acid in 70% alcohol.

d) Eosin (yellowish), 1g of eosin was dissolved in about 80 ml glass distilled water then 7g crystals of thymol were added and completed to 100 ml.

**Procedure:**

1) specimens were fixed in 10% formalin for 3 days and paraffin, 10 micrometers thickness were then prepared from the paraffin, blocks according to bancraft technique *(Bancraft, 1975).*

2) Sections were deparaffinized and brought down to water then stained in Harris's hematoxylin for 15 minute.

3) The sections were blued in running tap water for 10 minutes and differentiated in HCL / alcohol for 15 seconds.

4) Sections were put in eosin for 15 second to 2 minutes and washed in running tap water 5 minutes then dehydrated in ascending grades of alcohol, starting with 50% for 3 minutes.

5) Sections were cleared in xylem then mounted in Canada balsam.

**4-Statistical analysis:**

The statistical analysis was carried out by using SPSS, PC statistical software (version 10.0; SPSS Inc, Chicago, USA).

The results were expressed as mean ± SD. Data were analyzed by one way analysis of variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at (P<0.05) *Steel and Torri., (1980).*
RESULTS AND DISCUSSION:

Hematological analysis:

The effect of feeding Quercetin, Catechin from different source on RBC count, HGB concentration and hematocrit:

The data showed in Table (2) demonstrated that drinking CD for 40 days lowered RBCs, HGB and HCT level in plasma, as seen in positive control compared with negative one (p<0.01), while treatment with Q or CT Ameliorated these deficiencies even natural or commercial source to approaches the negative control level (p<0.01) except RBC in CT from natural and commercial source supplementation group, HGB in Q from commercial and CT from natural source supplementation group and HCT in Q from commercial group. This result agreed with (Donmez et al., 2019) who discovered that in the Cd group, the number of RBCs, Hb levels, Htc values, and the number of PLT reduced significantly (p 0.05) when compared to the other three groups. Q in the Cd Q group alleviated these losses; the values were substantially lower than in the C and Q groups (p 0.05). When compared to the other three groups, the Cd group had a significantly higher number of WBC (p 0.05). The percentage of neutrophils in the Cd group increased significantly (p 0.05). Although the percentage of lymphocytes declined dramatically in the Cd group (p 0.05), it was significantly improved in the Cd+ Q group (p 0.05).

And in the line with El-Boshy et al., (2015) who showed that Chronic Cd poisoning produced anemia in Cd-treated rats. While Cd-induced anemia is caused by toxic metal deposition in the kidney, liver, and spleen, which leads to hemolysis due to a malformation of peripheral RBCs (Kunimoto et al., 1985). Iron deficiency by competition with duodenal iron absorption, and renal anemia caused decreased production of erythropoietin (Horiguchi et al., 1994 and El-Demerdash et al., 2004). In rats with acute Cd toxicity, the number of RBC, Htc value, Hb concentration, and number of PLT were all drastically reduced, different dosages of Cd for different durations have been
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shown to cause detrimental effects in rats, which match our findings (Karmakar et al., 2000 and Ognjanović et al., 2003). RBC, Hb, Hct, and PLT measures were found to be significantly lower in the Cd group compared to the other three groups, C, Q and Cd+ Q, which is consistent with previous results (Kunimoto et al., 1985 and Kocak and Akcil., 2006 and Nazima et al., 2016). Cd exposure results in microcytic hypochromic anemia as well as alterations in RBC membrane permeability, resulting in a lower hematocrit. Due to mucosal lesions, Cd lowers iron absorption in the intestine, resulting in a lower Hct content (Kunimoto et al., 1985 and El-Boshy et al., 2015). The C and Q groups had equal RBC, Hb, Hct, and PLT values, but the Cd+ Q group had considerably higher RBC, Hb, Hct, and PLT values than the Cd group. Some antioxidants have been shown to protect RBC from Cd destruction (Nazima et al., 2016). The observed modifications can be attributed to the protective effect of Q.

Table (2) Effect of Supplementation with Quercetin and Catechin (commercial and natural) on RBC, count, HGB, and HCT in rats

<table>
<thead>
<tr>
<th>Variables groups</th>
<th>RBC (ML/U)</th>
<th>HGB (ML/U)</th>
<th>HCT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Group(1) Negative control</td>
<td>8.67 ± 0.61</td>
<td>15.98 ± 1.46</td>
<td>54.92 ± 0.50</td>
</tr>
<tr>
<td>Group(2) Positive control</td>
<td>5.00 ± 0.28</td>
<td>10.17 ± 0.30</td>
<td>34.86 ± 0.52</td>
</tr>
<tr>
<td>Group(3) Quercetin commercial</td>
<td>6.12 ± 0.69</td>
<td>16.28 ± 0.37</td>
<td>53.63 ± 0.67</td>
</tr>
<tr>
<td>Group(4) Quercetin natural</td>
<td>7.03 ± 0.26</td>
<td>12.22 ± 0.57</td>
<td>43.65 ± 1.32</td>
</tr>
<tr>
<td>Group(5) Catechin commercial</td>
<td>8.113 ± 0.49</td>
<td>14.27 ± 1.36</td>
<td>39.6 ± 3.71</td>
</tr>
<tr>
<td>Group(6) Catechin natural</td>
<td>8.13 ± 0.39</td>
<td>15.45 ± 0.53</td>
<td>50.85 ± 4.50</td>
</tr>
<tr>
<td>F</td>
<td>51.81</td>
<td>43.60</td>
<td>65.00</td>
</tr>
</tbody>
</table>

The data presented as mean ±SD.

(**) =highly significant at p<0.01, (*) = significant at p<0.05, (-) = Non significant p>0.05. Values which have different letters in each column differ significantly, while those with have similar or partially are not significant.

The effect of feeding Quercetin, Catechin from different source on MCV, MCH and MCHC:

Data in Table (3) showed that there were significant difference between all groups in Mean Corpuscular Hemoglobin (MCH), Mean corpuscular volume (MCV), and Mean corpuscular hemoglobin concentration (MCHC). It showed the highest level
of MCH was in favor of the Group Q commercial and negative control group, among all investigated samples was (25.13 g/dl and 23.28 g/dl) respectively. and group CT commercial was at the closest level with the negative control group (the healthy group). While the lowest level of MCH was in favor of group (6) CT natural and the positive control group was (19.85 g/dl and 20.03 g/dl) respectively, Control positive group tended to have the highest level of MCV, among all investigated samples. All treated groups have MCV lower than control positive group. Even statistical significant difference was observed between the best results for MCV was observed in group ingested and treat with natural Q. Even there were no statistical difference observed between this group and control negative group (healthy group). From the data illustrated in table (3) it was observed that there were many variation for MCHC score detected in investigated groups. The worst MCHC were detected in control positive group tended to have the lowest MCHC among all investigated groups, on contrast, groups in CD water Ural and treated with commercial CT have MCHC comparable with control negative group.

This result agreed with Patil et al., (2018) who observed that MCH, MCV, and platelet count were all affected, in the Carbendazim- treated groups, Q treatment led in a substantial increase (p 0.05) in MCV, MCH, and platelet count Hb percent, and RBC count are used to calculate MCH, MCV, and MCHC percentages Kao et al., (2000). On the other hand GT group showed increased in MCV AND Mchc compared to control negative group according to Shoshin et al., (2020).
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Table (3): Effect of Supplementation with Quercetin and Catechin (commercial and natural) on MCH, MCV and MCHC in rats

<table>
<thead>
<tr>
<th>Variables groups</th>
<th>MCH (g/dl)</th>
<th>MCV (g/dl)</th>
<th>MCHC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Group(1) Negative control</td>
<td>23.28 ab ± 3.38</td>
<td>56.25 c ± 3.11</td>
<td>34.27b ± 4.06</td>
</tr>
<tr>
<td>Group(2) Positive control</td>
<td>20.03 b ± 1.60</td>
<td>70.85 * ± 4.32</td>
<td>27.58 * ± 2.66</td>
</tr>
<tr>
<td>Group(3) Quercetin commercial</td>
<td>25.13 a ± 4.23</td>
<td>65.10 b ± 2.81</td>
<td>30.37 c ± 2.19</td>
</tr>
<tr>
<td>Group(4) Quercetin natural</td>
<td>20.30 b ± 2.18</td>
<td>56.73 c ± 3.26</td>
<td>33.05 b ± 1.70</td>
</tr>
<tr>
<td>Group(5) Catechin commercial</td>
<td>22.50 a ± 3.21</td>
<td>58.50 c ± 3.31</td>
<td>35.58 a ± 2.16</td>
</tr>
<tr>
<td>Group(6) Catechin natural</td>
<td>19.85 b ± 0.91</td>
<td>67.07 ab ± 3.41</td>
<td>37.58 a ± 1.69</td>
</tr>
<tr>
<td>F</td>
<td>19.26</td>
<td>3.46</td>
<td>12.17</td>
</tr>
<tr>
<td>Sig.</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

The data presented as mean ±SD.

(**) = highly significant at p<0.01, (*) = significant at p<0.05, (-) = Non significant p>0.05.

Values which have different letters in each column differ significantly, while those with have similar or partially are not significant.

The effect of feeding Quercetin, Catechin from different source on kidney function, urea, uric acid and Creatinine:

The data showed in table (4) demonstrated that drinking CD for 40 days raised urea, uric acid and creatinine level in plasma as seen in positive control compared with negative one p<0.01 while treatment with Q or CT ameliorated these increment's even natural or commercial source to approaches the negative control level p<0.01 except creatinine in quercetien from commercial source supplementation group, It was noticed that the significantly improvement in urea, uric acid and urea response were detected in animals. This result agreed with the finding of (Liu et al., 2019) who showed that Q effect on renal function in injured kidneys after injection we found that the obstructed group's blood urea nitrogen (BUN) and serum creatinine (SCr) values in whole blood were significantly higher than the sham group. The levels of SCr and BUN in whole blood did not exhibit a significant drop with Q low-dose and intermediate-dose treatment, but SCr and BUN levels were significantly.

While high dose of Q, may play a role in kidney protection in general treatment restored creatinine levels in obese mice
(P=0.01 vs. high-fat diet + Q) and urinary micro albumin levels were no longer higher than in controls (P=0.08 vs. high-fat diet + Q) Kim et al., (2019). When compared to non-supplemented groups, GT supplementation resulted in significant reductions in plasma urea and creatinine levels (Elhalwagy et al., 2008).

Table (4): Effect of Supplementation with Quercetin and Catechin (commercial and natural) on urea, uric acid and creatinine in rats

<table>
<thead>
<tr>
<th>Variables groups</th>
<th>Urea (MG/DL) Mean ± SD</th>
<th>U.acid (MG/DL) Mean ± SD</th>
<th>Creatinine (MG/DL) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group(1) Negative control</td>
<td>33.32 ± 1.19</td>
<td>2.75 a ± 0.46</td>
<td>0.50 e ± 0.13</td>
</tr>
<tr>
<td>Group(2) Positive control</td>
<td>58.63 a ± 4.64</td>
<td>4.67 a ± 0.29</td>
<td>1.13 a ± 0.24</td>
</tr>
<tr>
<td>Group(3) Quercetin commercial</td>
<td>34.48 c ± 5.00</td>
<td>3.28 cd ± 0.17</td>
<td>1.00 ab ± 0.17</td>
</tr>
<tr>
<td>Group(4) Quercetin natural</td>
<td>29.02 d ± 5.56</td>
<td>4.03 b ± 0.59</td>
<td>0.62 de ± 0.15</td>
</tr>
<tr>
<td>Group(5) Catechin commercial</td>
<td>40.67 b ± 2.98</td>
<td>3.73 bc ± 0.51</td>
<td>0.90 bc ± 0.16</td>
</tr>
<tr>
<td>Group(6) Catechin natural</td>
<td>35.68 bc ± 4.67</td>
<td>2.88 bc ± 0.29</td>
<td>0.72 de ± 0.17</td>
</tr>
<tr>
<td>F</td>
<td>36.18</td>
<td>18.73</td>
<td>11.47</td>
</tr>
</tbody>
</table>

The data presented as mean ±SD.

(***) = highly significant at p<0.01, (*) = significant at p<0.05, (-) = Non significant p>0.05.

Values which have different letters in each column differ significantly, while those with have similar or partially are not significant.

The effect of feeding Quercetin, Catechin from different source on GOT, GPT and TP:

Data in Table (5) showed that there were significant difference between all groups in glutamic-oxaloacetic transaminase (GOT), Glutamic-pyruvic Transaminase (GPT) and total protein (TP), the data showed in demonstrated that drinking CD for 40 days raised GOT, GPT and TP level in plasma as seen in positive control compared with negative group at p<0.01, while treatment with Q or CT ameliorated these increment’s even natural or commercial source to approaches the negative control level p<0.01 except GOT in Q from natural source supplementation group and TP in Q from commercial source supplementation group.

This result agreed with (Liu et al., 2020) who showed that rats with hepatic damage, the values of GPT, GOT, and DBIL
The Effect of Supplementation With Quercetin, Catechin and Rich Food of Them on Immune System in Experimental Rats

were significantly lower when pure Q and Nano liposomal Q were given together instead of saline. The hepato protective and therapeutic effects of Nano liposomal Q on liver function in rats were not different (P > 0.05), indicating that Nano liposomal Q had no influence on liver function in rats. ALP decreased in the Q group (from 179 to 172 IU/L) before adjustment for baseline values (P > 0.05), while it increased in the placebo group (from 219 to 227 IU/L) (P > 0.05) (Hezaveh et al., 2019). But disagreed with (Wong and Rabie., 2008) who showed the concentrations of total protein were displayed at either concentration of Q or time interval, there were no statistical differences between the experimental and control groups. While agreed with Jatuworapruk et al and Sakanaka whose showed rats with blood pressure that treated by GT showed decreased in ALT, AST, and ALP compared to control positive group (Jatuworapruk et al., 2014 and Sakanaka et al., 1992).

Table (5): Effect of Supplementation with Quercetin and Catechin (commercial and natural) on GOT, GPT and TP in rats

<table>
<thead>
<tr>
<th>Variables groups</th>
<th>GOT (g/dl) Mean ± SD</th>
<th>GPT (g/dl) Mean ± SD</th>
<th>TP (g/dl) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group(1) Negative control</td>
<td>22.82 ± 3.32</td>
<td>8.00 ± 1.18</td>
<td>114.88 ± 2.99</td>
</tr>
<tr>
<td>Group(2) Positive control</td>
<td>36.18 ± 4.72</td>
<td>14.23 ± 1.09</td>
<td>131.18 ± 6.23</td>
</tr>
<tr>
<td>Group(3) Quercetin</td>
<td>25.55 ± 3.72</td>
<td>10.63 ± 1.51</td>
<td>126.10 ± 10.12</td>
</tr>
<tr>
<td>Group(4) Quercetin</td>
<td>33.15 ± 3.06</td>
<td>8.43 ± 0.70</td>
<td>113.75 ± 3.22</td>
</tr>
<tr>
<td>Group(5) Catechin</td>
<td>26.58 ± 1.48</td>
<td>11.23 ± 2.22</td>
<td>113.17 ± 4.65</td>
</tr>
<tr>
<td>Group(6) Catechin</td>
<td>29.00 ± 5.53</td>
<td>9.20 ± 0.77</td>
<td>114.00 ± 4.15</td>
</tr>
<tr>
<td>F</td>
<td>9.64</td>
<td>17.52</td>
<td>10.92</td>
</tr>
<tr>
<td>Sig.</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

The data presented as mean ±SD.

(***) =highly significant at p<0.01, (*) = significant at p<0.05, (-) = Non significant p>0.05.

Values which have different letters in each column differ significantly, while those with have similar or partially are not significant.

The effect of feeding Quercetin, Catechin from different source on SOD, GPX, CAT and IgG

Immunoglobulin analysis: Data in Table (6) noticed that, control positive group tended to have serum SOD much lower than control negative group. Even control positive group have
SOD two fold lower than healthy group 1 (control negative). All treated group have serum SOD level higher than control positive group, but still lower than control negative group. The best result were recorded for SOD, GPX and CAT in group treated with natural Q. Control positive group tended to have the lowest level of serum GPX among all investigated groups and recorded 15.58g/dl. Even, control positive group have GPX threefold lower than healthy group. All treated groups with both natural and commercial sources of Q and CT had GPX higher than the positive control group but still lower than control negative group. Even, there was significant differences were observed between them.

It should be noted that, control positive group tended to have serum CAT much lower than control negative group. All treated group have serum CAT level higher than control positive group, but still lower than control negative group, with the exception of the natural quercetin group 4, which reached a level of GPX even it was higher than the (the healthy group) negative control group. The best result for treated with natural Q group (both natural and commercial).

In experiment data in table (6) showed that serum IgG in group (2) Positive control increased was (857.40 g/dl) when compared to negative control of the experiment, but decreased in group (4) Q natural (681.33 g/dl) when compared to positive negative control and this the better result.

This result agreed with (Mlcek et al., 2016) who showed Q was well-known for its antioxidant and anti-allergic qualities, which include immune system activation, antiviral activity and inhibition of histamine release, reduction of pro-inflammatory cytokines, leukotriene generation, and suppression of interleukin IL-4 production, and improve level of SOD and CAT. And agreed with Magrone et al who showed Q was known to impact on the recruitment of immune cells to the skin and in preventing the development of secondary infections following disruption of the skin barrie and improved level of CAT and GPX (Magrone
The Effect of Supplementation With Quercetin, Catechin and Rich Food of Them on Immune System in Experimental Rats and Jirillo., 2012). And in a line with (Rawangkan et al., 2018) who observed that GT that contain CT works as an immune checkpoint inhibitor, preventing and treating cancer and decrease level of IgG. And agreed with (Jiri et al., 2016) who showed Q is the most prominent member of the polyphenols, flavonoids, and flavone's subgroups, Q is renowned for its antioxidant activity in radical scavenging and anti-allergic qualities defined by immunological activation and improved IgG.

Table (6): Effect of Supplementation with Quercetin and Catechin (commercial and natural) on SOD, GPX, CAT and IgG in rats

<table>
<thead>
<tr>
<th>Variables groups</th>
<th>SOD (g/dl) Mean ± SD</th>
<th>GPX g/dl Mean ± SD</th>
<th>CAT g/dl Mean ± SD</th>
<th>IgG (g/dl) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group(1) Negative control</td>
<td>66.98 a ± 4.65</td>
<td>45.54 a ± 2.91</td>
<td>37.92 ab ± 4.83</td>
<td>682.70 c ± 17.90</td>
</tr>
<tr>
<td>Group(2) Positive control</td>
<td>33.73 e ± 2.78</td>
<td>15.58 d ± 1.04</td>
<td>25.55 d ± 1.43</td>
<td>857.40 a ± 25.44</td>
</tr>
<tr>
<td>Group(3) Quercetin commercial</td>
<td>55.42 c ± 2.79</td>
<td>29.60 c ± 2.61</td>
<td>28.95 cd ± 3.03</td>
<td>755.18 b ± 36.45</td>
</tr>
<tr>
<td>Group(4) Quercetin natural</td>
<td>65.53 ab ± 6.31</td>
<td>40.50 b ± 5.96</td>
<td>40.27 a ± 5.31</td>
<td>681.33 c ± 12.20</td>
</tr>
<tr>
<td>Group(5) Catechin commercial</td>
<td>43.58 d ± 5.77</td>
<td>32.02 c ± 3.63</td>
<td>30.50 cd ± 2.03</td>
<td>773.70 b ± 33.36</td>
</tr>
<tr>
<td>Group(6) Catechin natural</td>
<td>58.97 bc ± 9.80</td>
<td>37.82 b ± 1.78</td>
<td>34.17 bc ± 7.03</td>
<td>705.68 c ± 21.40</td>
</tr>
<tr>
<td>F</td>
<td>29.57</td>
<td>58.15</td>
<td>9.68</td>
<td>41.25</td>
</tr>
<tr>
<td>Sig.</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

The data presented as mean ±SD. (***) =highly significant at p<0.01, (*) = significant at p<0.05, (-) = Non significant p>0.05. Values which have different letters in each column differ significantly, while those with have similar or partially are not significant.

Histopathological examination:

A-Liver:

Microscopically, liver of normal rat from control (-), group 1 showed no histopathological alteration and the normal histological structure of the central vein as well as the portal area (portal vein and bile ducts) with the surrounding hepatocytes in both were recorded in (fig.1). Liver of hepatotoxic rat from control (+) group 2, the hepatocytes in the parenchyma showed focal inflammatory cells infiltration, as well as focal necrosis and
suffer cells proliferation in between the hepatocytes (fig. 2). However, liver of rat from Q chemical group 3 showed diffuse suffer cells proliferation was detected in between the hepatocytes (fig. 3). Group 4 Q natural, showed diffuse suffer cells proliferation was observed in between the hepatocytes (fig. 4). And liver of rat from CT chemical group 5, observed that there was no histopathological alteration as recorded in (fig. 5). Meanwhile, liver of rat from CT natural group 6 showed diffuse suffer cells proliferation was detected in between the hepatocytes (fig. 6).

**Protective and therapeutic effect of Quercetin, Catechin and Rich Food of them on inhibited immune system in experimental rat**

Figs. 1-6: section in liver of rats from 6 groups, stained with H & E, X400.
Fig. (1): liver of normal healthy control (-), showing normal histological structure of the central vein and surrounding hepatocytes in the parenchyma.
Fig. (2): liver of normal healthy control (+), showing focal inflammatory cells infiltration in the hepatic parenchyma.
Fig (3): liver of group 3, showing diffuse suffer cell proliferation in between the hepatocytes.
Fig. (4): liver of group 4, showing diffuse suffer cell proliferation in between the hepatocytes.
The Effect of Supplementation With Quercetin, Catechin and Rich Food of Them on Immune System in Experimental Rats

Fig. (5): liver of group 5, showing normal histological structure.
Fig. (6): liver of group 6, showing diffuse suffer cell proliferation in between the hepatocytes.

B- Spleen:

Microscopically, spleen of normal rat from control (-), group 1 showed there was no histopathological alteration and the normal histological structure of the lymphoid cells in the white pulps and the surrounding red pulps with sinusoids were recorded in (fig. 7). While group 2 control (+) showed the white pulps showed lymphoid hyperplasia (fig. 8). Meanwhile spleen of rat from Q chemical group 3, showed the white pulps showed lymphoid depletion (fig. 9). On the other hand rat from Q natural group 4, showed there as congestion in the red pulps and sinusoids (fig. 10). Also, spleen of rat from CT chemical group 5, showed congestion was noticed in the red pulps and sinusoids (fig. 11). As well as spleen of rat from CT natural group 6 showed, there was no histopathological alteration as recorded in (fig. 12).
C- Thymus:

Microscopically, thymus of normal rat from control (-), group 1 showed that there was normal histological structure of the lymphoid cell hassle's body (fig. 13) and in (fig. 14) was the magnification to identify the control portion. While in group 2 control positive showed that necrosis in some lymphoid cell in cortex and medulla (fig. 15). Beside showed in group 3 the magnification to identify the necrosis of some lymphoid cells (fig. 16). And in group 4 showed that congestion in the medullary blood vessels with lymphoid hyperplasia, and magnification to identify the lymphoid hyperplasia (fig. 17). On the other hand (fig. 18) in group 5 showed that congestion in the medullary blood vessels. While thymus in group 6 that have (CT commercial) showed that normal histological structure (fig. 19).
The Effect of Supplementation With Quercetin, Catechin and Rich Food of Them on Immune System in Experimental Rats

Figs. 13-19: section in thymus of rats from 6 groups, stained with H & E, X400.
Figs. (13) and (14): thymus of group1 control negative, showing normal histological Structure of the lymphoid Cells in the cortex and medulla with Hassles' body and showing the magnification to identify the central portion
Fig. (15): thymus of control positive, showing necrobiosis in some lymphoid cells in cortex and medulla.
Fig. (16): thymus of group3, Showing the magnification to identify the necrobiosis of some lymphoid cells.
Fig. (17): thymus of group 4, showing congestion in the medullary blood vessels with lymphoid hyperplasia.
Fig. (18): thymus of group5, showing congestion in medullary blood vessels.
Fig. (19): thymus of group 6, showing normal histological structure.

CONCLUSION

The results of this study summarized that Q and CT from natural and commercial source had a similar effect on improved HGB, liver enzyme activities, kidney function, SOD, GPX, CAT and IgG in level blood. And it strengthened immune system in
experimental rats, and reduced deleterious effects of CD in rats. Beside the dried RON and GT powder that contain natural Q and CT respectively had improved efficiency immune system.

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