Amelioration of Uranyl Acetate Induced Toxicity on Male Rats by Using Hypericum Perforatum Herb

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ABSTRACT

The present study was performed to estimate the protective effect of Hypericum perforatum (HCP) powder and its oil against uranyl acetate induced toxicity in male rats. Thirty male Albino rats were divided into two groups, group (1) (n=6) normal control. Group (2) (n=24) control positive were daily treated for 28 days with uranyl acetate 75 mg/kg/rats/day by oral intubation then divided into four groups: group (2) control positive (untreated), group (3) HCP powder 5 mg/kg/diet., group (4) HCP oil 5 ml/kg/rats. Group (5) mixture HCP powder mg/kg/diet & oil 5 ml/kg/rats.

The protective groups showed significant decrease in serum total cholesterol, triglycerides, LDL cholesterol, ALT, AST, urea, creatinine, uric acid, and MDA. While body weight gain (BWG), HDL cholesterol, SOD and GSH were significantly increased compared to (+ve) group. The HCP powder & oil are readily available supplier of polyphenols with great antioxidative are considered as functional food ingredients since they affect physiological and biochemical processes in rats and human beings, resulting in better health and improved the symptoms of uranyl acetate toxicity and prevent its complications. So, this research suggest that the anti-inflammatory and antioxidant properties of HCP are dependent on the flavonoid contents of the HCP.

Key words: Hypericum perforatum L- Uranyl Acetate – Antioxidative - Flavonoid.
INTRODUCTION

Uranium is radioactive metallic element of high specific gravity. It is chemically classified as a heavy metal and weakly radioactive. Uranium is found as an oxide, uraninite or mixed oxide, pitchblende or complex salt. Occurs in nature as in rocks, soil, rivers, oceans, plants and animals. Consequently, all humans are exposed to naturally occurring uranium through ingestion, inhalation and skin contact (Craft, 2004). Internalized in the body, the soluble components migrate throughout the body and uranium concentrates in the bone, kidney, and liver. When the uranium enters the body, it binds with bicarbonate and proteins. This binding action helps prevent soluble uranium from interacting with most body tissues (Gilman, et al., 2009). Uranium from the environment enters the human body by ingestion with food and drink and by inhalation of respirable airborne uranium-containing dust particles or aerosols (Domingo, et al., 2004)

*Hypericum perforatum* L. is widely distributed through temperate areas of the world, such as North Africa, western Asia and Europe. It contains flavonoids, tannins and essential oil Pavlovic et al., (2006). *Hypericum perforatum* (HCP) contains many compounds, such as coumarins, unsaturated fatty acids, polyacetylenic derivatives, aphrodisiac, aperient, and sesquiterpenes. Therefore, it has various pharmacological activities, such as cholangogue, stomachic, diuretic, and tonic effects (Baba, et al., 2005). *Hypericum perforatum* (HCP) is highly considered as antioxidant, seeds and herbs. Results revealed that there is a positive correlation between the total antioxidant and their phenolic and flavonoid contents (Righetti, et al., 2008). Flavonoids are potent antioxidants, free radical scavengers, and metal chelators and inhibit lipid peroxidation. Phenolic compounds are considered as a major group of antioxidant molecules that contribute to the antioxidant activities of herbs and oil of their ability to scavenge free radicals. *Hypericum perforatum* (HCP) have possess therapeutic effects in different areas and it could be considered as beneficial source of materials for human health. Mazandarani et al., (2012). Plant essential oils and its extracts have been used for many thousands years, particularly in pharmaceuticals, food preservation, and natural therapies (Imelouane, et al., 2010 and Junko and Toshio, 2011). Moreover, Essential oils also are potential sources of novel antimicrobial compounds especially against bacterial pathogens.

The volatile compounds in tubers of HCP have been investigated previously. Hence, an investigation of the volatile compounds present in tubers
and leaves species can give a better understanding of the compounds present. Therefore, this paper study the amelioration of uranyl acetate induced toxicity on male rats by using *Hypericum perforatum* herb as well as the chemical composition of essential oils of the tubers and leaves species of HCP growing in Ukraine (MacLeod and Gonzalez, 1982, Bruggink, et al., 2005 and Javier, et al., 2011).

**MATERIALS AND METHODS**

- **Materials:**

*Hypericum perforatum* L. powder and oil were obtained from Agricultural Seeds, Medicinal Plants and Herbs Company, Cairo, Egypt. Uranyl acetate was obtained from SIGMA Company for Pharmaceutical Industries Cairo, Egypt. 30 male albino rats of Sprague Dawley strain weighing (150±10g) were purchased from Agricultural Research Center, Giza, Egypt. The rats were provided *ad libitum* with tap water and were fed with standard diet.

- **Methods:**

**Preliminary phytochemical screening of crude extracts of volatile oils and aqueous extracts of HCP:** Tannins and resins were detected in the plant sample according to the method of (El-Badrawy, 1996). Saponins substances were detected in different crude extracts under investigation according to the method of (Trease, 1961). Terpenes substances were detected in different crude extracts under investigation according to the method of (Finar, 1968). Flavonoids substances were detected in extracts of different samples using the method of (Geissman, 1962). Carbohydrates and glycosides were treated by Molish test according to the method of (Blabaa, et al., 1976).

**Experimental design:**

The experiment was carried out on 30 male Albino rats in two steps, in the first (one week), the rats were divided into two main groups. Control group (6 rats) fed on the basal diet. Control positive group (24 rats) fed on the basal diet and uranyl acetate 75 mg/kg/ rats/day by oral intubations for 4 weeks (Sarah Ghazi, 2014) rats were then divided into 4 groups (each 6 rats) as follows:

**Group (2):** Control positive group fed on the basal diet with oral uranyl acetate intubations for 4 weeks.

**Group (3):** Fed on the basal diet containing 5 mg/kg/diet HCP powder with oral uranyl acetate intubations for 4 weeks.
Group (4): Fed on the basal diet containing 5 ml/kg/ rats HCP oil with oral uranyl acetate intubations for 4 weeks.

Group (5): Fed on the basal diet containing mixture of 5 ml/kg/ diet HCP powder and 5 ml/kg/rats HCP oil with oral uranyl acetate intubations for 4 weeks.

Body weights of the rats were measured three times a week during the four weeks. The percentage of daily changes in body weights was calculated.

Biochemical analysis:

Total cholesterol, HDL-cholesterol and triglyceride content were determined by enzymatic colorimetric method according to (Allian, et al., 1974, Richmond, 1973 and Fossati and Principle 1982) respectively. LDL-cholesterol and VLDL-cholesterol were calculated by the Friedewald Formula according to (Friedewald, 1972). Plasma alanine and aspartate aminotransferase enzymes activities (ALT and AST) and Bilirubin were determined according to the method of (Reitman and Frankel, 1957). Urea in serum was determined according to (Patton and Crouch, 1997). Serum uric acid and creatinine was estimated an enzymatic method according to (Trinder, 1969 and Henry, 1974). Malondialdehyde (MDA), Superoxide dismutase (SOD) and glutathione (GSH) were analyzed according to the methods described by Aebi, (1984) ;Nishikimi, et al., (1972) and Beutler, et al., (1963), respectively.

Statistical analysis:

All obtained data were statistically analyzed by SPSS computer software. An analysis of variance ANOVA was used and follow up LSD (SPSS) Computer program variation (Sümübülüoglu, et al., 1998).

RESULTS AND DISCUSSION

Preliminary phytochemical screening of hypericum perforatum (HCP) and aqueous extract

The phytochemical screening of tannins, saponins, resins, terpenes, flavonoids and carbohydrates of HCP oil and aqueous extract were detected and recorded in Table (1). It was noticed that tannins, saponins, resins, terpenes, flavonoids and carbohydrates were found in the HCP oil and powder.

Öztürk, et al., (2009) recommended that, hypericum perforatum, can serve as natural sources to develop the free radical scavengers and might prevent radical attack and therefore has antioxidative effect. Benedi, et al., (2004); Silva, et al., (2017) and Conforti, et al., (2019) found that, there is no previous studies reporting the chemical contents and antioxidant activity.
Table (1): Preliminary phytochemical screening of *Hypericum perforatum* (HCP) and aqueous extract

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Terpenes</th>
<th>Flavonoids</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Resins</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCP extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HCP oil</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Present - Absent

Effect of HCP powder and oil on body weight gain, feed intake and feed efficiency ratio of rats administrated uranyl acetate.

Data in Table (2) showed significant decreased in body weight gain and feed intake in control positive group compared with control negative group, while was an increase of feed efficiency ratio (FEP). Among all groups, the highest weight gain (BWG %) was noticed in the treated group with HCP oil, powder and mixture groups compared with control (+ve ) group. These results agreed with Moghaddam, *et al.*, (2016) who reported that there was an increase in body weight gain (BWG) for all treated group rats fed on of HCP oil, powder and mixture tubers comparing with the (+ve) group. The lowest value of the percentage body weight gain % was (8.04 ± 3.31%) was found in the control positive, while the highest percentage was (25.69 ± 2.18) recorded for the control negative. The treated rat groups showed that significant increase in body weight gain and feed intake compared with the control (+) groups, while was significant decrease in feed efficiency ratio. The treated rat groups, HCP oil, powder and mixture groups showed that non-significant in body weight gain and feed efficiency ratio (FEP) when compared to normal negative control group (-ve). Felmlee, (2009) and Moghaddam, *et al.* (2016) who suggested that, the weights of the animals increased significantly.

Table (2): Effect of HCP powder & oil on body weight g,%, feed intake and feed efficiency ratio in rats received uranyl acetate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>body weight gain (g)</th>
<th>BWG (%)</th>
<th>feed intake (FI) (g/day)</th>
<th>feed efficiency ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>38.78 ±1.4</td>
<td>25.69 ±2.18</td>
<td>18.98 ±0.64</td>
<td>0.0048 ±0.003</td>
</tr>
<tr>
<td>Control (+)</td>
<td>12.68 ±3.4</td>
<td>8.04 ±3.31</td>
<td>15.76 ±1.39</td>
<td>0.0124 ±0.003</td>
</tr>
<tr>
<td>HCP powder</td>
<td>36.8 ±2.5**</td>
<td>23.38 ±4.59***</td>
<td>19.90 ±0.45</td>
<td>0.0054 ±0.004</td>
</tr>
<tr>
<td>HCP oil</td>
<td>35.90 ±4.20**</td>
<td>22.21 ±9.65**</td>
<td>19.43 ±0.67</td>
<td>0.0054 ±0.004**</td>
</tr>
<tr>
<td>mixture</td>
<td>35.37 ±3.3**</td>
<td>22.81 ±2.69**</td>
<td>18.65 ±1.24</td>
<td>0.0052 ±0.003</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of 6 rats. Significant with control group *p< 0.05** P< 0.01
Effect of HCP powder and oil on serum lipids pattern of rats administrated uranyl acetate.

Data in Table (3) showed administration of uranyl acetate to normal rats resulted in a significant increase in serum total cholesterol, triglyceride and LDL, VLDL cholesterol levels in control positive group compared with control negative group. The present data showed that uranyl acetate groups, receiving HCP mixture powder and oil, HCP powder and HCP oil had significantly lowered levels of serum total cholesterol, triglyceride and LDL, VLDL cholesterol compared to uranyl acetate group (+ve). These results agree with Santos-Gallego and Badimón (2012) who reported the beneficial effects of HCP in lowering cholesterol in serum. While Moghaddam, et al., (2016) found no benefit of using on blood cholesterol and triglycerides. HCP application both in feed and water combined being most effective in lowering the total blood cholesterol than the other application methods. On the other hand, significant increase in HDL-cholesterol for rats receiving mixture with HCP powder & oil, HCP powder and HCP oil groups compared to uranyl acetate control (+ve).

Results in Table (3), also indicated that all the protective groups HCP powder & oil showed decrease in atherogenic indexes (TC / HDLc and LDLc / HDLc) compared to uranyl acetate control (+ve). The hypolipidaemic effect of HCP may be due to increasing fecal lipid excretion and decreasing lipid absorption, Cieslak, and Filipiak-Florkiewicz (2002). Santos-Gallego and Badimón, (2012) proved that, an inverse relationship between low concentration of HDL-C and an increased risk for cardiovascular diseases (CVD). Arts, et al., (2011) found that, there was a strong inverse association between the intake of catechin and death from coronary heart disease, this inverse association was most pronounced in human at low risk of coronary heart disease.

Table (3): Effect of HCP powder & oil on serum lipids pattern in rats received uranyl acetate

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
<th>TC/ HDLc</th>
<th>LDL/ HDLc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td></td>
<td>80.01 ±26.67</td>
<td>90.34 ±9.8</td>
<td>23.16 ±5.7</td>
<td>38.34 ±1.5</td>
<td>18.12 ±2.01</td>
<td>2.07 ±0.01</td>
<td>0.62 ±0.20</td>
</tr>
<tr>
<td>Control (+)</td>
<td></td>
<td>100.01 ±33.33</td>
<td>175.16 ±13.76</td>
<td>40.33 ±17.97</td>
<td>23.34 ±4.69</td>
<td>35.12 ±3.01</td>
<td>4.28 ±1.42</td>
<td>1.77 ±0.59</td>
</tr>
<tr>
<td>HCP powder</td>
<td></td>
<td>92.85 ±30.95*</td>
<td>115.34 ±8.2*</td>
<td>36.40 ±5.7</td>
<td>31.87 ±2.1**</td>
<td>23.87 ±1.93</td>
<td>2.91 ±0.02</td>
<td>1.16 ±0.02</td>
</tr>
<tr>
<td>HCP oil</td>
<td></td>
<td>90.01 ±30.00</td>
<td>120.17 ±11.2*</td>
<td>33.45 ±9.6</td>
<td>29.38 ±3.7*</td>
<td>24.26 ±2.02</td>
<td>3.06 ±1.02</td>
<td>1.12 ±0.04</td>
</tr>
<tr>
<td>Mixture</td>
<td></td>
<td>80.93 ±26.97**</td>
<td>94.43 ±7.1**</td>
<td>26.67 ±1.73</td>
<td>34.60 ±2.34**</td>
<td>18.86 ±1.73</td>
<td>2.33 ±0.78</td>
<td>0.78 ±0.01</td>
</tr>
</tbody>
</table>

Each value is the mean± SD of 6 rats. Significant with control group *p< 0.05 ** P< 0.01
TC: Total cholesterol  TG: Triglyceride  HDLc: Low density lipoprotein cholesterol
LDLc: High density lipoprotein cholesterol  VLDLc: Very low density lipoprotein cholesterol

Table (3): Effect of HCP powder & oil on serum lipids pattern in rats received uranyl acetate
Effect of HCP powder and oil on serum ALT, AST, bilirubin, urea, creatinine and uric acid of rats administrated uranyl acetate.

Data in Table (4) showed significant increase of ALT, AST, Bilirubin, Urea, Creatinine and Uric acid in control positive group compared with control negative group, while was significant decrease (P<0.01) in ALT level for rats receiving mixture of 21.64 ± 7.4U/ml when compared to rats of +ve control which administrated uranyl acetate it 30.82± 4.29 U/ml.

As clear from Table (4) significant decrease (P<0.01) and (p< 0.05) in AST were observed in the groups treated with mixture of 26.40± 3.93 mg/dl and HCP powder was 39.26± 7.2 mg/dl when compared with rats administrated uranyl acetate which was 45.4± 3.50 mg/dl (+ve control group). Therefore, it is possible to suggest that these extracts are safe and might have protection against hepato cellular damage as evidenced by normal serum levels of AST and ALT. A low significant decrease (P ≤ 0.01) and (p< 0.05) in serum urea and creatinine and uric acid levels in mixture group and HCP oil were detected in comparing with positive control. Renal toxicity is a major adverse effect of uranium, and the metal has toxic effects on the liver as well. These results are in agreement with those reported by Eid, (2009) who found a significant decrease in serum creatinine for groups fed on different levels (5, 10 and 15%) of HCP.

On the other hand, it has been found that decrease in bilirubin for group treated with mixture, HCP powder, HCP oil compared with control positive group Table (4). It was likely that the antioxidant activity of the extracts produced better response in such stressful conditions. These results are near from Domingo, et al., (2014) which gives an indication on the hepatotoxic effect of uranyl acetate of rats recieving intragastrically 75mg / kg / b.w uranyl nitrate. These results of treatments are in agreement with those reported by El-Hofi, (2005). The results in this study are in contrasted with the results of Smit, et al., (1987) who observed an increase in plasma levels of ALT and AST when rats were exposed to depleted uranium in their drinking water. These contradictory results are probably due to the quantity of uranium used and the duration of contamination.
Table (4): Effect of HCP powder & oil on serum ALT, AST, bilirubin, urea, creatinine, and uric acid in rats received uranyl acetate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>HCP powder</th>
<th>HCP oil</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/ml)</td>
<td>12.45 ± 5.85</td>
<td>30.82 ± 4.29</td>
<td>28.17 ± 7.8</td>
<td>29.59 ± 5.0</td>
<td>21.64 ± 7.4**</td>
</tr>
<tr>
<td>AST (mg/dl)</td>
<td>28.40 ± 4.8</td>
<td>45.4 ± 3.50</td>
<td>39.26 ± 7.2</td>
<td>38.19 ± 6.1</td>
<td>26.40 ± 3.93**</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.45 ± 0.03</td>
<td>1.02 ± 0.03</td>
<td>0.55 ± 0.09**</td>
<td>0.62 ± 0.05</td>
<td>0.44 ± 0.06**</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>12.25 ± 2.9</td>
<td>29.70 ± 3.2</td>
<td>25.17 ± 3.7</td>
<td>26.13 ± 3.6</td>
<td>23.72 ± 2.6**</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.80 ± 0.10</td>
<td>2.64 ± 0.15</td>
<td>2.31 ± 0.18</td>
<td>2.25 ± 0.17</td>
<td>1.07 ± 0.11**</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>2.83 ± 0.38</td>
<td>4.04 ± 0.58</td>
<td>3.23 ± 0.4</td>
<td>3.18 ± 0.5</td>
<td>2.54 ± 0.6**</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of 6 rats. Significant with control group *p< 0.05, **p< 0.01

ALT: Alanine aminotransferase enzymes
AST: Aspartate aminotransferase enzymes

Effect of HCP powder and oil on antioxidant and oxidative stress parameters of rats administrated uranyl acetate.

As evident from Table (5) showed significant decreased in (SOD) and (GSH) in control positive group compared with control negative group. While was an increase of malondialdehyde (MDA). There was significant decrease p< 0.05 in malondialdehyde (MDA) were observed in rats fed on mixture group of compared with positive control in which superoxide dismutase (SOD) revealed an increase significantly (P< 0.001) and (P< 0.01) in treated groups when compared with control + ve group administrated uranyl acetate.

Treating rats with HCP protective after induction of uranyl acetate showed significant increase in glutathione (GSH) (P<0.01), this was observed on rats fed on mixture group comparing with positive control, also a significant increase (P< 0.05) were observed in rats fed on mixture, HCP powder and oil.

From the present study, we can conclude that β-bisabolene and other volatile oils or supertences (70.7 % and 63.1 % respectively) were the major components in the essential oil of HCP leaves, would play an important role as an the antioxidant activity (Abd El-Aziz, et al., 2015).
Table (5) Effect of HCP powder and oil on antioxidant and oxidative stress parameters in rats received uranyl acetate

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>SOD (mg/L)</th>
<th>GSH (U/mL)</th>
<th>MDA (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td></td>
<td>0.54 ±0.12</td>
<td>9.27 ±0.6</td>
<td>11.26 ±1.4</td>
</tr>
<tr>
<td>Control (+)</td>
<td></td>
<td>0.26 ±0.09</td>
<td>4.22 ±1.54</td>
<td>19.2 ±0.8</td>
</tr>
<tr>
<td>HCP powder</td>
<td></td>
<td>0.67 ±0.05***</td>
<td>5.07 ±1.5</td>
<td>14.4 ±1.09</td>
</tr>
<tr>
<td>HCP oil</td>
<td></td>
<td>0.65 ±0.17***</td>
<td>5.16 ±0.69</td>
<td>15.6 ±0.9</td>
</tr>
<tr>
<td>Mixture</td>
<td></td>
<td>0.75 ±0.2***</td>
<td>7.7 ±0.88**</td>
<td>12.5 ±1.7*</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of 5 rats. Significant with control group *p< 0.05  ** P< 0.01 ***P< 0.001

CONCLUSION:
From the previous results this research can conclude that the administration by uranyl acetate has a negative effects, and the active components of HCP powder, oil and mixture reflects an important role in the inhibition of these effects on the different parameters that has been taken in consideration in this study. HCP may be contain more free radical scavenging molecules and it can be used as a potential source of natural antioxidants and nutrients. Its use as a natural antioxidant source appears to be an alternative to synthetic antioxidants. This study could be useful for further exploitation and application of the HCP powder, oil and mixture.

REFERENCES:


الملخص
تحسين السمية الناتجة عن خلات اليوسانتي على ذكور الفئران باستخدام عشبة حشيشة القلب

تعرضت هذه الدراسة لمعرفة التأثير الوقائي لعشبة حشيشة القلب على السمية الناتجة عن الإصابة بخلات اليوسانتي على ذكور الفئران، وتم تقسيم عدد 30 ذكر فئران أليبيو إلى مجموعتين أساسيتين: المجموعة الأولى وهي المجموعة الضابطة (N=15)، أما المجموعة الثانية (N=15) وهي مجموعة الکنترول الموجبة حققت لمدة 88 يوم بدماء خلات اليوسانتي بجرعة تساوي 0.5 ملجم / كجم / يوم عن طريق انبوب الفم وقسطل على 4 مجموعات كالآتي: مجموعة (2) موجبة الکنترول الموجبة (غير معالجة). مجموعة (3) مسحوق حشيشة القلب 5 ملجم / كجم / الوجبة. مجموعة (4) زيت حشيشة القلب 5 ملجم / كجم / الوجبة. مجموعة (5) المخلوط كلا من مسحوق وزيت حشيشة القلب 5 ملجم / كجم / الوجبة و5 ملجم/كجم وزن الفأر.

واظهرت النتائج أنه في المجموعات الوقائية حدث انخفاض كبير في مستوى الكولسترول الكلي في الدم والدهون الثلاثية، ALT، AST، LDL، LDL، البروتينات، والكرياتينين، وحمض اليوريك، و(GSH، SOD، و HDL) و(BWG) مقارنة مع مجموعة الکنترول الموجبة. وأظهرت النتائج أن كلا من مسحوق وزيت حشيشة القلب يقلل وحسن من معظم مؤشرات الكيموجيوفاي التي سببتها خلات اليوسانتي حيث عشبة حشيشة القلب وزنها من الأغذية الوقائية الغنية بالمركبات الفينولية.

الكلمات المفتاحية: حشيشة القلب - خلات اليوسانتي - مضادات الأكسدة - الفلافونويد