Extraction of Flavonoid compounds from Nigella Arvensis Linn seeds & to study their physiological effects on female reproductive system.

Mossa M. Marbut*, Najdat Ali S. Al-Kadhi*, Kahtan A. Al-Mzaein**.
*Dept. of Physiology, College of Medicine, Tikrit University.
**Dept of Physiology & Pharmacology, Veterinary Medicine, Baghdad University.

Abstract

Nigella arvensis is a grassy plant with green to blue flowers & small black seeds. It is known as seed of blessing belong to genus Nigella & family Ranculaceae. Black seeds have been used in public medicine as curative substances for treatment of many diseases. It has therapeutic uses like worm infestation treatment, antiallergic, antiviral & anti-inflammatory. Thus, the aim of this study is to extract, identify the flavonoid compounds from Nigella arvensis L. seeds (black seeds) & to study its physiological effective dose on female reproductive system of mature mice. The effect of different doses of extracted flavonoids on the ovarian and the uterine weight: For assessment of effective physiological dose, daily different doses of the crude flavonoids (above or below than recommended dose 27.5 mg/kg of B.W. were given orally by gavage needle to 60 immature female mice, from weaning days (post natal day 17).

Six groups of immature female mice including 10 mice/group were divided randomly into two subgroups for each. The first and the second subgroups were treated for one and two weeks respectively. The results of this study showed that each kilogram of Nigella arvensis dry seeds contained 1.211 gm of the flavonoid compounds. The high performance liquid chromatographic (HPLC) analysis indicated the existence of 12 flavonoids of which only seven were identified. The identified flavonoids included: Catechin, Mericin, Rutin, Hydroxy Quarcertin, Kaempherol, Quarcerin and Hesperdin at a level of 77.34, 52.3, 47.3, 396.0, 95.6, 267.5, 36.56 μg/ml of extract respectively. There is no significant differences in the ovarian and the uterine weights during the first week among the treated and the control groups were recorded. While, a significant increase was found during the second weeks in groups which received 40, 50 and 60 mg/kg B.W as compared to 20 and 30 mg/kg B.W. flavonoid treated and control groups. No significant differences among 40, 50 and 60 mg/kg B.W. flavonoid treated groups were observed. Hence, 50 mg of flavonoids/kg.B.W. was selected in this study as an effective physiological dose for the following experiments.

Introduction

Nigella arvensis is a grassy plant with green to blue flowers & small black seeds. It is known as seed of blessing belong to genus Nigella & family Ranculaceae (1). Black seeds contain several active compounds such as flavonoids, which can be further subdivided into flavones, flavanols, isoflavones & flavanes (2,3). Black seeds have been used in public medicine as curative substances for treatment of many diseases. It has therapeutic uses like worm infestation treatment, antiallergic, antiviral & anti-inflammatory (4,5,6). Flavonoids are among the most widely distributed natural products in plants with 2000 different compounds occurring in free forms or glycosides (7). Recently, a renewed interest in flavonoids has been fueled by their wide physiological & therapeutic effects as antioxidant & estrogenic effects (8,9,10,11).

Thus, the aim of this study is to extract, identify the flavonoid compounds from Nigella arvensis L. seeds (black seeds) & to study its physiological effective dose on female reproductive system of mature mice.

Materials & Methods

Black seed were purchased from local market & classified by Iraqi national herbarium as Nigella Arvensis Linn. Quantitive & Qualitative analysis of Nigella arvensis seed were done & extract by high performance liquid chromatography (HPLC). Experiment One:
The effect of different doses of extracted flavonoids on the ovarian and the uterine weight.- For assessment of effective physiological dose, daily different doses of the crude flavonoids (above or below than recommended dose 27.5 mg/kg of B.W. by Cruz et al., (8) were given orally by gavage needle to 60 immature female mice, from weaning days (post natal day 17). Six groups of immature female mice including 10 mice/group were divided randomly into two subgroups for each. The first and the second subgroups were treated for one and two weeks respectively.

1.Group A (n=10):- Each subgroup was dosed orally single daily dose of flavonoid 20 mg/kg B.W.

2.Group B (n=10):- Each subgroup was dosed orally single daily dose of flavonoid 30 mg/kg B.W.

3.Group C (n=10):- Each subgroup received orally single daily dose of flavonoid 40 mg/kg B.W.

4. Group D (n=10):- Each subgroup received orally single daily dose of flavonoid 50 mg/kg B.W.

5. Group E (n=10):- Each subgroup received orally single daily dose of flavonoid 60 mg/kg B.W.

6. Group F (n=10):- Each subgroup received orally normal saline and served as control group. Ovarian and uterine weights of scarified animals were checked at the end of each interval periods for the assessment of effective physiological dose (9).

Experiment Two: Another experiment was designed to investigate the estrogenic effects of the flavonoids. Fifteen immature female mice 17 days old and weighing 10-12 gm were divided randomly into three equal groups and handled as follows:

1. Group A (n=5):- Mice received orally a single daily dose of flavonoids 50 mg/kg B.W. for two weeks.

2. Group B (n=5):- Mice received orally a single daily dose of estrogen 5 mg/kg B.W. (10) for two weeks.

3. Group C (n=5):- Mice received normal saline for two weeks to serve as control.

At the end of the experiment period, animals were weighed and killed by cervical dislocation. The uterus was cut just above its junction with the cervix and the junction of the uterine horns with the ovaries. The uterus was excised, trimmed free of fat and pierced to remove excess fluid. The uterus was then weighed to observe uterotrophic effect of flavonoids which may exhibit an estrogenic activity (11). Statistical analysis of data was performed on the basis of either one way or two way analysis of variance (ANOVA) using significant level of P<0.01 and P<0.05. Specific group differences were determined using Duncan test (12). All data are presented as a mean & standard error.

Results

The results of this study showed that each kilogram of Nigella arvensis dry seeds contained 1.211 gm of the flavonoid compounds. The high performance liquid chromatographic (HPLC) analysis indicated the existence of 12 flavonoids (Table 1) of which only seven were identified.

The identified flavonoids included: Catechin, Mericitin, Rutin, Hydroxy Quarcetin, Kaempherol, Quarcetin and Hesperdin at a level of 77.34, 52.3, 47.3, 396.0, 95.6, 267.5, 36.56 µg/ml of extract respectively. The percentage of identified parts was equal to 80.31%, while unidentified part represented 19.68%. Hydroxy Quarcetin and Hesperdin were represented the highest and the lowest concentrations of flavonoid compounds, respectively.

Tables 2 and 3 revealed that no significant differences in the ovarian and the uterine weights during the first week among the treated and the control groups were recorded. While, a significant increase was found during the second weeks in groups which received 40, 50 and 60 mg/kg B.W as compared to 20 and 30 mg/kg B.W. flavonoid treated and control groups. No significant differences among 40, 50 and 60 mg/kg B.W. flavonoid treated groups were observed. Hence, 50 mg of flavonoids/kg.B.W. was selected in this study as an effective physiological dose for the following experiments.

The results of experiment two showed that the group which received a single daily dose of 50 mg/kg.BW of flavonoids exhibited a significant increase (P<0.05) in the weight of the uterus (45.52 ± 1.08mg) as compared to the control (Table 4). Also, a significant differences in the weight of the uterus in flavonoids (45.52±1.08mg) and estrogen
Extraction of Flavonoid compounds from *Nigella Arvensis* Linn seeds & to study their………

(64.82±1.38mg) treated groups as compared to the control was recorded.

**Table (1)** High performance liquid chromatographic results of *Nigella arvensis* flavonoids (seeds extract)

<table>
<thead>
<tr>
<th>Flavonoid Compounds</th>
<th>Sample R.T. (min.)</th>
<th>Standard R.T. (min.)</th>
<th>Concentration. (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>3.79</td>
<td>3.78</td>
<td>77.34</td>
</tr>
<tr>
<td>Mericitin</td>
<td>4.25</td>
<td>4.25</td>
<td>52.30</td>
</tr>
<tr>
<td>Rutin</td>
<td>4.45</td>
<td>4.41</td>
<td>47.30</td>
</tr>
<tr>
<td>Hydroxy Quarcetin</td>
<td>5.14</td>
<td>5.18</td>
<td>396.0</td>
</tr>
<tr>
<td>Kaempherol</td>
<td>5.77</td>
<td>5.77</td>
<td>95.60</td>
</tr>
<tr>
<td>Quarcetin</td>
<td>8.29</td>
<td>8.29</td>
<td>267.50</td>
</tr>
<tr>
<td>Hesperdin</td>
<td>9.49</td>
<td>9.49</td>
<td>36.56</td>
</tr>
</tbody>
</table>

R.T.=Retention time

**Table (2)** Effect of different doses of flavonoids on ovarian weight (mg/10gm B.W.) of immature mice.

<table>
<thead>
<tr>
<th>Dose of Flavonoid (mg/kg B.W.)</th>
<th>Weight of ovaries mg/10g B.W.</th>
<th>One week</th>
<th>Two weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>5.0±0.18</td>
<td>Bc</td>
<td>5.98±0.39</td>
</tr>
<tr>
<td>50</td>
<td>4.9±0.10</td>
<td>Bc</td>
<td>5.8±0.27</td>
</tr>
<tr>
<td>40</td>
<td>4.6±0.11</td>
<td>Bc</td>
<td>5.9±0.30</td>
</tr>
<tr>
<td>30</td>
<td>4.4±0.12</td>
<td>Bc</td>
<td>5.1±0.13</td>
</tr>
<tr>
<td>20</td>
<td>4.4±0.12</td>
<td>Bc</td>
<td>4.6±0.17</td>
</tr>
<tr>
<td>Normal saline</td>
<td>4.3±0.16</td>
<td>C</td>
<td>4.4±0.14</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SE Different letters denote significant differences between groups (P<0.05)

**Table (3)** Effect of different doses of flavonoids on uterine weight (mg/10gm B.W) of immature mice

<table>
<thead>
<tr>
<th>Dose of Flavonoid (mg/kg B.W.)</th>
<th>Weight of uterus mg/10g B.W.</th>
<th>One week</th>
<th>Two weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>33.8±0.46</td>
<td>Bc</td>
<td>46.6±1.69</td>
</tr>
<tr>
<td>50</td>
<td>33.6±0.50</td>
<td>Bc</td>
<td>45.4±0.40</td>
</tr>
<tr>
<td>40</td>
<td>33.2±0.58</td>
<td>Bc</td>
<td>44.6±0.99</td>
</tr>
<tr>
<td>30</td>
<td>30.0±0.70</td>
<td>Bc</td>
<td>33.0±1.49</td>
</tr>
<tr>
<td>20</td>
<td>29.8±0.66</td>
<td>C</td>
<td>30.6±0.44</td>
</tr>
<tr>
<td>Normal saline</td>
<td>29.0±0.70</td>
<td>C</td>
<td>30.0±0.98</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SE Different letters denote significant differences between groups (P<0.05)

**Table 4**: Uterotrophic effect of estrogen and flavonoids in immature Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>weight of uterus mg/10gm of B.Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid 50mg/kg B.W.</td>
<td>45.52±1.08</td>
</tr>
<tr>
<td>Estrogen 5mg/kg B.W.</td>
<td>64.82±1.38</td>
</tr>
<tr>
<td>Normal saline</td>
<td>29.63±1.86</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SE.
N=5/group.
Different letters denote significant differences between groups (P<0.05).
Discussion

The results of the present study showed that Nigella arvensis seeds extract contained 1.211 gm of flavonoid compounds/kilogram dry seeds (0.1211%), and the high performance liquid chromatography (HPLC) indicated the existence of 12 flavonoids, seven of them were identified representing only 80.31% of the total flavonoids. In addition to that, Hydroxy quercetin and Hesperdin were found to be the highest and the lowest flavonoids, respectively. However, the number of the flavonoids in Nigella arvensis seed extracts was relatively higher than that reported in other plants resources for instance, four flavonoid compounds, like kaempherol, luteolin, quercetin and myrictin in Iraqi and Yemeni zizyphus spina-christi (13), two flavonoids (Narirutin and Hesperdin) in orange Juice, Narirutin and Naringin in grape fruit (14) and quercetin as well as, Isoharmnetin in Artemisia absinthium leaves (15), but Nigella sativa seeds extract were found to be the richest flavonoid resources (22 flavonoid compounds) (16).

Also, assessment of the effective physiological dose of the flavonoid which exhibited the maximum performance of female mice reproductive system was the aim of the current study. So, in this study ovaries and uterus weight in immature mice was used for the evaluation of flavonoid biological activity (9). And, the results in table 2 and 3 revealed a significant increases in the ovarian and uterine weights at the end of the second week in 40, 50 and 60 mg/kg B.W of flavonoids administrated groups as compared to the control and other treated groups.

However, the ovarian weight increment may be attributed to the stimulatory effects of the flavonoids on granulosa cells of the ovarian follicles inducing their numbers and growth development, as well as accelerating maturation processes at different follicular stages , in addition the blood supply and metabolic rate increasing (6,8,9,10).

The effect of phenolic and alkaloid extract from red onion Allium cepa L. on weight increments of ovaries and uterus of female mice was attributed to an induction of FSH secretion which is responsible for follicle growth and development, so the release of estrogen may effects renin angiotensin system and finally increases sodium and water retention and therefore uterine weight and wall thicknesses increases (17). The same observations was recorded by Al-Bekari et al., (18) concerning the weight of the ovaries and uterus of female mice treated orally by garlic extract. However, the estrogenic activity of the flavonoids in a number of hormonally responsive systems was recorded (19, 20).

The significant increment in the weight of the uterus may be attributed to the direct effect of flavonoids on cell division and proliferation induction of the uterine cells. Also, a spatial relationship between phenolic hydroxyl groups of estradiol and certain flavonoids was documented, so the close agreement between these configurations and between the associated chemical properties suggests that flavonoids binding to the cytosolic estrogen receptor can depress the same gene as true estrogens (9,17,21).

The results of the current study showed a significant increase in the uterine weight of immature mice, as compared to the control (table 4). This increment may be attributed to the affinity of flavonoids toward estrogen receptors, or it may participate in increasing the number and sensitivity of the estrogenic receptors (22,23).

In addition to that, the uterine weight increment in estrogen treated group can be explained that estrogen causes an increases in DNA and RNA –polymerases enzyme activity (22), which induces synthesis and secretion of the proteins (23), glycoproteins in the uterus (24), in addition to the phospholipids synthesis and glucose transportation through uterine cells, these leaded to increase uterine weight (25).

Murphy and Friensen (26), referred to the existence of insulin like growth factor-I (IGF-I) and their receptors in the uterus, which their locations was determined in myometrium layer (27), so ovariecetomized or immature rats treated with estrogen increases concentrations of IGF-I mRNA in uterus (26), therefore IGF-I play an important role in cell division through their functions as a modulator of growth hormone activates in muscular cells (28). These results are in...
agreement with results of other workers, (26, 29, 30).

However, in flavonoid treated group, the weights increment of the uterus was relatively lower than that recorded in the estrogen treated group. This is because of the low relative affinity of flavonoids and isoflavonoids to estrogen receptors (31,32), and is related to the structure of flavonoid ring system deviation or diposition of hydroxyl substituents from the optimal pattern present in 17-β estradiol (33).

In addition to that, extracted estrone from Zahdi date palm seeds significantly increased uterine weight (r=0.93) of immature female mice, through binding to the estrogen receptors and the increment was less than that noted with estradiol treated group (34). These results indicate that estrogen has the higher affinity than flavonoids towards estrogen receptors, and a more potent effect in inducing uterine growth.

References


34. AL-Haboby, B.T. Extraction of estradiol estrogenic compounds from date palm seeds and study their biological activity. Ms.C Thesis., Animal Resources Reproductive Physiology. College of Agri., Univ. of Baghdad.