EFFECT OF THE ROOTS OF ALGERIAN BUNIUM INCRASSATUM ON BIOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL PARAMETERS OF MATURE FEMALE RATS

Nawel Attoui1*, Farid Berroukeche2,3, Kamilia Guedri4 and Fethi Touli5

1Department of biology, Faculty science of nature and life, Laboratory of Vegetal Resources Valorization and Food Security in Semi-Arid Areas South West of Algeria. Tahri Mohamed University of Bechar, Algeria
2Laboratory of Physiology, Pathophysiology, and Biochemistry of Nutrition, Department of Biology, Faculty of Nature and Life Sciences, Earth and Universe, University of Tlemcen 13000, Algeria
3Faculty of Medicine, the University of TAHRI Mohammed., Bechar, B.P 417 Kenadsa road, Bechar 08000, Algeria
4Laboratory of Toxicology and Ecosystems Pathologies, Faculty of Exact Sciences and Nature and Life Sciences, Larbi Tebessi University, Tebessa, Algeria
5Laboratory of chemistry and environmental sciences, Faculty of nature and life sciences, University of TAHRI Mohammed, Bechar, Algeria

*E-mail: atoui.naouel@univ-bechar.dz

(Date of Receiving-24-09-2020; Date of Acceptance-19-12-2020)

ABSTRACT

The present study’s objective was to evaluate the impact of Bunium incrassatum roots’ powder (Talghouda) as a dietary supplement on the evolution of biological, biochemical, and histological parameters in female Wistar rats. The animals were divided into two groups and given an orally standard diet supplemented with 15% of Bunium incrassatum roots powder, daily for 15 days. The obtained results showed that the roots of Bunium incrassatum induced an increase in the body and organs weight of Diet rats compared to the control, as well as effects on the biochemical parameters, characterized mainly by a significant increase in the glycemia (+44.44%), triglyceride (+56.81%), cholesterol (+11.90%), LDL (+4.08%) and HDL (+11.53%)and a decrease in creatinine (-3.65%), TGO (-6.92%) and TGP (-58.73%). In the histological study of organs, the thyroid of diet rats revealed large thyroid follicles, the colloid of the follicles was more or less developed compared to the control rats. Through the obtained results, it can be concluded that a standard diet supplemented with 15% of Bunium incrassatum roots powder may have a positive effect on biological, biochemical, and histological parameters.

Keywords: Bunium incrassatum, Diet, Biochemical, Histological, Rat

INTRODUCTION

Countless aromatic and medicinal plants with fascinating biological properties find applications in various fields such as pharmaceutical and medicine. In several developing countries, a large part of the population trusts traditional doctors and their medicinal plant collections to treat them (Benayad, 2008). In Algeria, traditional medicine, thus practiced, finds a favorable reception from local people who are, alas, sometimes prey to ignorant quackery that can be dangerous for their health (Wichtl et al., 1999). Bunium incrassatum (Boiss.) Batt & Trab, a medicinal plant belonging to the Apiaceae family, is widely distributed in the northern parts of Algeria and is vernacularly known as “Talghouda” (Quezel and Santa 1963). Bunium incrassatum is an economically significant medicinal plant, its roots (tubers) are quite nutritious, usually cooke das potatoes, and often eaten fresh. In the indigenous system of medicines, dried and powdered tubers are regarded as astringent, anti-diarrheal, and applicable against inflammatory hemorrhoids. Besides, this plant is used for bronchitis and cough treatment.

Previous studies on the phytochemical constituents of the genus Bunium revealed the presence of coumarins (Appendino et al., 1994), sesquiterpenes (Appendino et al, 1991), and especially essential oils (monoterpenoids) as frequent metabolites (Salehi, 2008). Other studies on the roots of Bunim incrassatun from Algeria showed the presence of fatty acids and coumarins (scopoletin and scoparone) in methanolic extract (Bousetla, 2015) and terpenes, sesquiterpens as major constituents in essential oil (Hayet et al., 2017). Furthermore, it is well documented that the essential oil and extracts from some Bunium species possess antihistaminic, antibacterial, and antifungal effects (Boskabady and Moghadas, 2004) besides antioxidant activity (Shahsavari, 2008). This study investigated the in vivo effects of Bunim incrassatun roots powder as a diet on some histological (liver, ovary, and thyroid), hematological, biochemical, and biological parameters in female Wistar rats.

MATERIALS AND METHODS

Animals

Female Wistar rats used in this experiment, weighing 80g±4g, were obtained from Pasteur Institute (Algiers, Algeria). Rats were kept in individual polypropylene cages under standard conditions (i.e.,
temperature 22°C ± 2°C, relative humidity 60%) and a 12h light/dark cycle and were fed ad libium with standard laboratory chow diet and regular tap water.

**Plant Materials**

*Buñiun incrassatum* roots (tubers) were collected from the mountains of Sebdou, in the vicinity of Tlemcen (West of Algeria), and were identified by an expert in botany. The harvested materials were dried in the shade for 15 days, and the free-standing tubers were cleaned and spread on a dish to complete the drying. Once dried, the tubers were crushed for later use as an experimental diet.

**Diet preparation**

Female Wistar rats, at about 4 weeks of age, were divided into 2 batches of 6 rats each, the first batch received a standard diet (ONAB), and the second one received a diet enriched in Talghouda, this diet is composed of (85% ONAB standard diet and 15% Talghouda roots powder. A sachet of vanilla was added to the diet (1 sachet per 100g of diet) to mask the plant’s taste. In parallel, during 15 days of experimentation, the weight of the rats was regularly taken.

**Blood and organ sampling**

At the end of the experiment (after 15 days), the rats of each group were anesthetized after 12 hours of fasting with 10% chloral at a dose of 0.3 ml/10 g of rats.

After xyphopubic laparotomy from the abdominal vein, the blood was collected in heparinized tubes for biochemical analyses (urea, creatinine, blood sugar, triglycerides, total cholesterol, LDLc, HDLc, TGO, and TGP). While the organs: liver, kidneys, thyroid, and ovaries were carefully removed, rinsed with 9% NaCl, weighed, and soaked in 10% formalin for histological study.

**Histopathological examination**

Samples of liver, kidneys, thyroid, and ovaries from rats of different groups were collected directly after sacrifice. They were fixed in 10% neutral buffer formalin, embedded in paraffin, sectioned at 5μm thickness, and stained with hematoxylin and eosin. The stained sections were examined under a light microscope (Zeiss) equipped with a digital camera (Leica ICC50 W).

**Statistical Analysis**

Data were analyzed using MINITAB software (Minitab® 13.31). All values were represented as mean±S.E.M. Statistical significance was evaluated by one-way analysis of variance (ANOVA) test. Significance was measured using Fisher’s least significant for the exact P values and significant, differences are noted in the results. The difference between the groups was considered significant when p<0.05.

**RESULTS AND DISCUSSION**

**Evolution of Body and gain weight:** The results shown in (Figure 01) revealed the evolution of rat’s body weight following the daily administration, for 15 days, of B. incrassatum diet to mature female rats, where a significant increase in the body weight, as compared to control rats, was noted. Maximum evolution of body weight was observed on the sixth day of experimentation in the diet rats. However, it decreased notably until the tenth day of oral intake of Talghouda and stabilized after that, until the end of the experiment compared to control animals (Figure 02). In parallel, concerning the gain weight, the oral supplementation of B. incrassatum revealed a significant increase in weight of 405.5% compared to the controls.

**Organs weight evolution:** The obtained results (Table 1) showed that oral administration of B. incrassatum diet for 15 days Induced a highly significant increase in ovary relative weight (p<0.01) and a significant reduction in liver relative weight (p<0.05), compared to the control groups. No significant changes were noted in kidney and thyroid relative weights between the two groups of rats in parallel.

**Effect of the B. incrassatum diet on biochemical parameters of blood:** Variation of some plasma parameters of the hepatic functions (Transaminases GOT and GPT), renal (Urea and Creatinine), as well as the lipidic balance (Cholesterol and triglyceride, LDLc and HDLc) and carbohydrate (Glucose) in Wistar rats receiving an oral diet of 15% talghouda compared to controls were recorded in the following figures (Figures 3,4 and 5).

A highly significant increase in blood glucose in the diet rats (p<0.01), compared control animals (Figure 3.A). However, no changes were observed in urea’s renal plasma parameters (Figure 3.B) and creatinine (Figure 3.C).

**Histological changes**

**In liver:** Microscopic observation of the liver’s histological sections from control and diet groups showed a normal Centro-lobular vein without histopathological particularity, which presents red blood cells. The hepatic parenchyma closed mono to binucleated cells tracing small passages called trabeculae (Figures 06 and 07).

**In Kidneys:** Microscopic observation of histological sections of the cortical area of the kidney showed no structural difference in structure and density of Bowman capsules in control and diet animals. The epithelial cells of renal tubules are cubic and organized in a monolayer with no dilation of the tubular lumen in the two groups of rats (Figure 08 and 09).

**In Thyroid:** Histological sections of the thyroid revealed the presence of large thyroid follicles in diet rats compared to controls. The colloid of follicles in animals receiving diet was more or less developed compared to
Effect of the roots of Algerian *Bunium incrassatum* on biological, biochemical and histological parameters of mature female rats

### Table (01): Evolution of some organs relative weights (RW) in the Control and Diet rats

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control rats</th>
<th>Diet rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (g/100g)</td>
<td>5.20±0.55</td>
<td>4.72±0.28*</td>
</tr>
<tr>
<td>Kidney (g/100g)</td>
<td>0.98±0.01</td>
<td>1.05±0.08</td>
</tr>
<tr>
<td>Thyroid (g/100g)</td>
<td>0.22±0.01</td>
<td>0.26±0.22</td>
</tr>
<tr>
<td>Ovary (g/100g)</td>
<td>0.24±0.09</td>
<td>0.38±0.04**</td>
</tr>
</tbody>
</table>

**Discussion:** This work’s objective was to investigate the effects of a diet rich in *Bunium incrassatum* roots powder (15%) on mature female rats during 15 days of experimentation. Our results revealed that the increase in the final body weight of rats over their initial weight (13.8% at the end of experimentation) was not statistically significant compared to controls, as confirmed by Chentouh *et al.*, (2017) where mature female rabbits received different doses of organic extract of *B. incrassatum* roots by oral gavage (25, 50, 100 and 200 mg/kg/day) during 15 days. The increase in the final body weight of mature rabbits compared to their initial weight was not statistically significant, between 12.33 and 18.47% (Chentouh *et al.*, 2017). A similar result was reported by Salisu *et al.*, (2012), where the inclusion of 0, 15, 30, 45, and 60% of *Daucus carota* (Apiales) leaves’ flour in the diet of five groups of rabbits for 8 weeks demonstrated that rabbits with the highest weight and the gain ratio (p>0.05) was not significantly different from control rabbits. They also concluded that flour from dried carrot leaves can be incorporated up to 15% in the diet of growing rabbits without any adverse effects on their performance (Salisu *et al.*, 2012).

As shown in several previous studies (Couse *et al.*, 1999; Shivalingappa *et al.*, 2002), the administration of estrogenic or estrogen-like substances to impuberous or ovariectomized rats induced an increase in weight mass. Indeed, Pinilla *et al.*, (2001) proposed that this increase in the weight could be linked to a stimulation of animals’ appetite by the extract, which would result in an increase in their food consumption (Pinilla *et al.*, 2001).

In parallel, the present study results also showed an increase in the weight of the reproductive system compared to the control groups and a significant reduction in the relative liver weight in animals consuming 15% *B. incrassatum* diet compared to controls. In addition, no comparable changes in kidney and thyroid weights were observed between the two groups of rats. These results agree with those reported by Bayala *et al.*, (2006), where the treatment with the extract of *Medicago saliva* and *Salvia officinalis* increased body and reproductive organs weights due to the fact that both herbs are known to be excellent stimulants of appetite, promote weight gain and treat digestive disorders. The same study also showed that the aqueous extracts of *Habenaria floribunda* exhibited estrogen-like effects, with an increase in the fresh and dry weight of the uterus, a decrease in cholesterol levels in the uterus, and vaginal opening of ovariectomized spleens (Bayala *et al.*, 2006). As a result, the presence of β-sitosterol, oleic acid, and scopoletin in our studied plant (*B. incrassatum*), as reported by Bousetla *et al.*, (2015) can also explain this increase in the volume of these organs (Bousetla *et al.*, 2015).

In addition, in our study, a standard diet supplemented with 15% *B. incrassatum* roots exhibited a hypoglycemic effect for rats on a medium-term diet lasting 15 days. However, no changes were observed in renal plasma parameters of urea and creatinine compared to control rats.

By analogy and not by homology, Azzi (2015),

---

**Table (01):** Evolution of some organs relative weights (RW) in the Control and Diet rats

**Figure 01.** Bodyweight evolution in control and Diet rats

**Figure 02.** Gain weight evolution in control and Diet rats

**4.4. In Ovaries:** Histological examination of the ovaries of control and diet rats revealed the presence of two generations of luteal bodies (CL) with no histomorphological differences between the two groups of rats. The old luteal bodies were composed of small pale eosinophilic luteal cells, while the luteal bodies of the current cycle were more basophilic and often had a fluid center. The antral follicles are less obvious since the ovary was composed mainly of luteal bodies. The evolution of the follicles was radial, which took place from the isthmus to the cortical area (Figure).
Figure 3. Variation of Glycemia, urea, and Creatinine in control and diet rats demonstrated that the ethanolic and chloroform extract of seeds of Citrullus colocynthis did not have a hypoglycemic effect in normal rats treated with intraperitoneal injection of 20 mg/kg of these extracts.

Likewise, Abd El-Baky et al., (2009), also noted that the extract of Citrullus colocynthis significantly lowered the levels of glucose, cholesterol, and triglyceride in the blood. The high plasma level observed in diet rats justified that B. incrassatum probably delayed insulin secretion.

According to Phillippe (1983), the plasma creatinine content told us about endogenous protein metabolism. In addition, this rate was proportional to the body’s muscle mass (Blacque-Belair, 1991).

During our experiment, three lipid parameters, triglyceridemia, total cholesterolemia, and HDLc were increased in diet rats. However, no significant changes were observed in the plasma LDLc level between control and diet groups.

With regard to cholesterol levels, the studies of Lundeen et al., (1997) and Samman et al., (1999) showed that the subcutaneous administration of estrogen to immature or ovariectomized rats reduced cholesterol levels in the systemic circulation, in the ovaries and uterine horns, which was the opposite of what has been observed.

In contrast, Mahmoodi et al., (2009) suggested that omega-3 fatty acid supplementation decreases plasma triglyceride levels and increases HDL cholesterol. We can explain these results by the presence of β sitosterol (Mahmoodi et al., 2009).

Lees et al., (1977) demonstrated that beta-sitosterol alone or in combination with phytosterols reduced plasma cholesterol levels. In parallel, Brufau et al., (2008) have shown that a daily intake of 2 to 3g of phytosterols reduced LDL-cholesterol concentration in the blood by about 10%. In the case of our study, the increase in HDL cholesterol is not always a sign of protection against lipid disruption. Still, it can also be a sign of compensation in response to an acute mechanism of dyslipidemia (Brufau et al., 2008). In addition, an experimental period of 15 days remains insufficient to confirm these deductions.

Stambouli and Sebbagh, (2018), added that B. incrassatum is used by herbalists to treat thyroid disorders. This may possibly explain the changes in plasma lipid parameters in female rats given 15% powder roots.

Indeed, the results reveal a significant reduction in TGP activity in the diet group compared to the control animals. On the other hand, no effect modification was observed in TGO activity between the two groups.

Huseini et al., (2009), showed that the treatment of type 2 diabetics with Citrullus colocynthis capsules (100 mg) 3 times/day and for 2 months did not ensure any significant changes in serum parameters (TGO, TGP, ALP, urea, and creatinine) (Huseini et al., 2009).

The effect of B. incrassatum bulbs on the histological structure of organs in female rats was also evaluated.

Indeed, the results did not reveal any histological change in the liver and kidneys. In contrast, histological sections of the thyroid showed the presence of large thyroid follicles in the diet rats compared to controls. The colloid of the follicles of the animals receiving B. incrassatum diet was more or less developed compared to the control rats.

The increase in ovarian weight can be explained by the presence of oleic acid and scopoletin in the extract of B.
Effect of the roots of Algerian Bunium incrassatum on biological, biochemical and histological parameters of mature female rats

**Figure 04.** Variation of some lipidic parameters in control and diet rats

During the neonatal period (PND 0 to 7), the ovary is a solid parenchymal mass without a medulla, cortex, or hilum. It consists of ovigerous nests, which are clusters of oogonia enveloped in undifferentiated mesenchyme. In the first few days, the ovigerous nests develop into follicles (i.e., a primary oocyte surrounded by a single layer of flattened pre-granulosa cells). Follicular development begins at the ovary center, where these primordial follicles become primary follicles (primary oocyte surrounded by a single layer of cuboid granulosa cells) and secondary follicles (primary oocyte surrounded by more than one layer of cuboid granulosa cells). During this neonatal period of development, the ovary is independent and insensitive to pituitary gonadotropins LH (luteinizing hormone) or FSH (follicle-stimulating hormone) (Picut et al., 2015).

Chen *et al.*, (2004) have shown that oleic acid supplementation induced the production of prostaglandin in the maternal endometrium. Choi *et al.*, (2015) has reported that perfusion with scopolet into a significant increase in cyclic adenosine monophosphate. It is well known that the gonadotropins FSH and LH act on their target cells via specific membrane receptors, respectively FSHR and LHR. Activation of these receptors by their ligands stimulates the production of AMPc (Changizi-Ashtiyan *et al.*, 2013).
Figure (06): Microscopic observation of a histological section of the liver of female control rats. VC, Centro-lobulaire vein, Arrow, hepatocytes. (H&E x400)

Figure (07): Microscopic observation of a histological section of the liver of female diet rats. VC, Centro-lobulaire vein; Arrow, hepatocytes. (H&E x400)

Figure (08): Microscopic observation of a histological section of female control rats’ renal cortical area. CB, Bowman’s capsule; TR, renal tubule. (H&E x400)

Figure (09): Microscopic observation of a histological section of dieting female rats’ renal cortical area. CB, Bowman’s capsule; TR, renal tubule. (H&E x400).

Figure (10): Microscopic observation of a histological section of the thyroid of female control rats. Collar, colloid of thyroid follicles; arrow, thyrocyte. (H&E x400).

Figure (11): Microscopic observation of a histological section of the thyroid of female rats. Collar, colloid of thyroid follicles; arrow, thyrocyte; (*), parafollicular cells. (H&E x400).
Follicular development is under the control of paracrine and autocrine factors. The infantile period (PND 8 to 20) is when the ovary’s granulosa cells begin to respond to FSH and LH (Parker and Picut 2016).

Histological examination of the control and diet rats ovaries revealed two generations of luteal bodies (LC) in the ovary with no histomorphological difference between the two groups of rats. The old luteal bodies are made up of small, pale eosinophilic luteal cells, while the current cycle luteal bodies are more basophilic have a fluid center. Antral follicles are less obvious since the ovary is made up mostly of luteal bodies. The evolution of follicles is radial from the isthmus to the cortical zone.

It is well known that the ovarian diameter depends on its follicle content which influences the size and diameter of the ovary by increasing the number of primaries, secondary, DeGraffe, and luteal body follicles. Another possible explanation for the increase in diameters could be returned to the role of follicular stimulating hormone (FSH) on the growth of the ovaries. The high concentration of this hormone stimulates the growth of ovarian follicles and directly influences the number of ovaries follicles that reach maturity (Hussain et al., 2018).

There is a significant increase in the number of primary follicles. This effect is due to the presence of sucrose, as confirmed by Chen et al., (2004). They showed that a sucrose concentration of 0.2 M in the cryoprotectant solution is more suitable for the cryopreservation of human oocytes (Chen et al., 2004). It is noted here that sucrose is the main compound of B incrassatum (Bousetla et al., 2015).
REFERENCES


The effect of supplementation with isoflavones on plasma lipids and oxidisability of low density lipoprotein in premenopausal women. Atherosclerosis, 147(2):277-283


