Testicular Cell Devastation in Wistar Rats on Administration of Aqueous Leaves Extract of *Symphytum officinale*

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**Abstract**

*Symphytum officinale* was historically used to treat a wide variety of ailments ranging from bronchial problems, broken bones, sprains, arthritis, gastric and varicose ulcers, severe burns, acne and other skin conditions. It has been documented that the presence of pyrrolizidine alkaloids in *Symphytum officinale* provoked toxic effect on the topical application or ingestion of the extract. We planned to study the effect of the aqueous extract of leaves of *Symphytum officinale* on male fertility in Wistar rats. The twenty four rats used for this study were randomized into groups A, B, C and D. Group A served as control and were orally administered 0.3 ml of distilled water; groups B, C and D received orally 0.2 ml, 0.4 ml and 0.6 ml of aqueous leaves extract of *Symphytum officinale* respectively for twenty eight days. The finding indicates that there was a significant (P<0.001) decreased in the body weight of group C and D compared to control group. The weight of the testes of groups C and D animals increased significantly (P<0.001) compared to control group A. The histological findings of the testes showed the presence of necrotic changes in the intestinal cells of the testes; they were massive multinucleated giant cells in groups C and D and loss of spermatides. The outcomes imply that aqueous extract of leaves of *Symphytum officinale* produced toxic effects in testicular morphology of the rats.

1 Introduction

In traditional medicine, plants have been used for hundreds of years to treat a wide variety of health-related disorders. The scientific research of plants used in traditional medicine with a multidisciplinary approach has increased worldwide, with more than 13,000 superior plants having been studied in the past five years, leading to sufficient scientific evidence on the pharmacological properties of these plants¹. In many developing countries, traditional medicines are widely utilized in the treatment of various ailments on an empirical basis. A variety of plants have been used for the treatment of ulcer, hypertension, diabetes and male reproductive function.

*Symphytum officinale* L. (Boraginaceae) or common comfrey is a perennial native of Europe and Asia and has been naturalized throughout North America. Comfrey grows well in rich, moist, low meadows, or along ponds and river banks, where it may reach a height of 1.2 m (usually 0.3-1.2 m). Both roots and leaves are reported to be used for medicinal purposes. It has been reported that *Symphytum officinale* extensively used in folk medicine for the treatment of various diseases such as joint complaints, muscle aches, arthritis, rheumatism, gout, gastrointestinal ulcers, burns healing and wounds healing. The roots of the *Symphytum officinale* are used in case of pulmonary complaints².

The roots and leaves of Symphytum species contain many active components including allantion (0.7 %), large quantities of mucilage, tannins, proteins, vitamins, mucopolysaccharides, steroidal sapponins, phenolic acids, symyptim, echimidine,
helipsupine, viridiflorine, echinatine, mainly rosmarinic acid and pyrrolizidine alkaloids. In dried roots of *Symphytum officinale*, the amount of total alkaloid content is 0.25 to 0.29 %, usually lower in leaves. Three major components; allantoin, rosmarinic acid and polysaccharides in *Symphytum* species are regarded as a reason of pharmacological effect and clinical efficacy of this plant. It is important to note that the pyrrolizidine alkaloid content of the leaf varies throughout the season.

The unsaturated pyrrolizidine alkaloid present in *Symphytum officinale* lead to toxic effect to body, and its use is controlled. Hepatotoxicity from *Symphytum officinale* is now rare, as it is widely accepted as being toxic when taken internally and oral formulations are restricted or banned in most countries. Hence information on the pyrrolizidine alkaloid content of the traditional preparations needs to be determined for individual products.

Therefore, we aimed to evaluated the effect of the aqueous extract of leaves of *Symphytum officinale* on male fertility in Wistar rats at different doses.

### 2 Materials and Methods

#### 2.1 Breeding of animals

Twenty four adult male wistar rats weighing 180-210 gm were procured from the animal house of Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State and bred in the animal house of Anatomy Department, Nnamdi Azikiwe University, Nnewi Campus. They were allowed for seven days acclimatization under normal temperature before tier weights were recorded. They were fed ad libitum with water and guinea feed pallets form Agro feed mill, Nigeria Ltd.

#### 2.2 Drug preparation

*Symphytum officinale* leaves were plucked from Okitipupa in Ondo State. It was identified at herbarium unit, Botany Department, Nnamdi Azikiwe University, Anambra State. It was sun-dried and then milled to a powder. The aqueous extract of leaves of *Symphytum officinale* was prepared by dissolving 300 mg/kg body weight in 10 ml of distilled water.

#### 2.3 Experimental protocol

Twenty (20) health adult male wistar rats were allocated into four groups (A, B, C and D) of six animals each. Group A serve as the experimental control and were orally administered 0.3 ml of distilled water; the experimental groups B, C and D were orally administered 0.2 ml, 0.4 ml and 0.6 ml of aqueous leaves extract of *Symphytum officinale* for twenty eight (28) days respectively. The control and experimental groups were anaesthetized using chloroform inhalation method and dissected. Testes tissues were harvested, weighed, and fixed in 10% formaldehyde for histological studies.

#### 2.4 Tissues processing

The testes tissues were transferred into an automatic processor where they went through a process of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in 10% formaldehyde. The testes tissues were washed over night in running tap water after four (4) hours in 10% formaldehyde. Dehydration of the fixed tissues was carried out in different percentages of alcohol 50%, 70% and 90% absolute. The tissues were then cleared in xylene and embedded in paraffin wax. Serial sections of 5micron thick are obtained using a rotary microtome. The tissue sections were deparaffined, hydrated and stained using the routine haematoxylin and eosin method. The stained sections were then examined under the light microscope.

#### 2.5 Statistical analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet’s test. A P < 0.001 value was considered as statistically significant.

### 3 Results

#### 3.1 Morphometric analysis of body weight

Table 1 demonstrated on administration of aqueous leaves extract of *Symphytum officinale* to animals of group C and D lead to significant (P<0.001) decreased in the body weight compared to control group.

**Table 1:** Comparative study between the mean initial and final body weight change in all the groups (A, B, C and D) before and after administration of the leaves extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (gm)</th>
<th>Weight Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Group A (Control group)</td>
<td>184.60±4.60</td>
<td>208.30±7.80</td>
</tr>
<tr>
<td>Group B</td>
<td>188.10±1.70</td>
<td>203.10±4.10</td>
</tr>
<tr>
<td>Group C</td>
<td>193.70±3.30</td>
<td>176.40±2.50</td>
</tr>
<tr>
<td>Group D</td>
<td>199.40±2.40</td>
<td>167.10±2.30</td>
</tr>
<tr>
<td>F-Ratio</td>
<td>48.100</td>
<td>26.260</td>
</tr>
<tr>
<td>Probability of Significance</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P*<0.001 when compared with normal control group.

The decrease in weight of animals of group B was non-significant, and nearer to weight of control group.

#### 3.2 Morphometric analysis of testes weight
The administration of aqueous leaves extract of *Symphytum officinale* to animals of group C and D lead to significant (P<0.001) increased in the weight of the testes compared to control group (table 2). The non-significant increase in the weight of the testes of animals of group B compared to control, and nearer to weight of control group. This indicates lower dose of aqueous leaves extract of *Symphytum officinale* was safe for animals. While the higher doses of aqueous leaves extract of *Symphytum officinale* were toxic in nature.

**Table 2: Comparison of the mean relative testes weights of groups A, B, C and D**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testes weight (gm)</th>
</tr>
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<tbody>
<tr>
<td>Group A</td>
<td>2.10±0.04</td>
</tr>
<tr>
<td>Group B</td>
<td>2.15±0.14</td>
</tr>
<tr>
<td>Group C</td>
<td>2.46±0.30*</td>
</tr>
<tr>
<td>Group D</td>
<td>2.69±0.52*</td>
</tr>
<tr>
<td>F-Ratio</td>
<td>1.413</td>
</tr>
<tr>
<td>Probability of Significance</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.001* when compared with normal control group.

**3.3 Histological observations**

In testicle samples of control group, a normal histology was observed: seminiferous tubules with epithelium without peeling or artifacts (Fig 1A).

**Fig 1A:** Showing normal architectural structure of the testicular cells in group A

Group B received extract 0.2 ml showed normal arrangement of germinal cells, sertolic cells and leydig cells (Fig 1B). The group C and D treated with extract 0.4 ml and 0.6 ml, respectively leads destruction of some seminiferous tubules, characterized by epithelial detachment with cell fragmentation was observed (Fig 1C and 1D).

**Fig 1B:** Showing normal arrangement of germinal cells, sertolic cells and leydig cells in group B

**Fig 1C:** Showing necrotic changes in the interstitial cells and loss of spermatids in group C

**Fig 1D:** Showing necrotic changes in the interstitial cells and loss of spermatids in group D

Further apparent decrease in the diameter of the seminiferous tubules, accompanied with large empty spaces between them was observed. It appears necrotic changes in the interstitial cells and loss of spermatids of group C and D.

**4 Discussions**

The effects of aqueous leaves extract of *Symphytum officinale* on the testis were investigated to highlights the possible histological implications that could result following its consumption. The animals treated with higher dose of aqueous...
leaves extract of *Symphytum officinale* lead to increased significantly (P<0.001) compare to control group, while group B mean relative organ weight was statistically similar with the control group A. The histological findings indicates the presence of histological lesion in group C and D treated with high doses of aqueous leaf extract of *Symphytum officinale*. The result revealed disruption of the basement membrane of seminiferous tubules, near absences of sperm in the lumen and loss of internal cells of leydig cells.

The study, accounts for the first time that higher dose of aqueous leaves extract of *Symphytum officinale*, have toxic effects on testicle and also affect spermatogenic parameters of Wistar rats. These results compare with other studies that displayed these same injuries after administration of extracts of *Lagenaria breviflora*, *Carica papaya*, *A. precatorius* and *Martynia annua*. The mechanism of damage to the testicular epithelium has not been fully established, but several studies tested other plant extracts and described selective damage to spermatogonia, sertolicells and leydig cells. The findings exhibits that an increased dose of aqueous leaves extract of *Symphytum officinale* leads to damage in testicular epithelium, and it can produce adverse effect on sperm quality. It can be good choice for male fertility control; however, other physiological studies are required to support their use in humans.

5 Conclusions

From the study, we inferred that consumption of high dose of aqueous leaves extract of *Symphytum officinale* could cause damages to the testicular cells. In future protocol require to elucidate the mechanism of damage on testicular epithelium.

6 Conflict of interests

None declared.

7 Author’s contributions

EDU conceived the study; EDU and CIE did the literature review. IAC and NGU collected the data and carried out the statistical analyses. All authors read and approved the final manuscript.

8 References


UK J Pharm & Biosci, 2016; 4(4); 79.