THE EFFECT OF INDIAN LIQUORICE ON FERTILITY POTENTIALS OF MALE RATS

A.E. LIGHA and A. C. OYIBO

Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa States, Nigeria.

Abstract - The use of herbs as an adjutant form of medical treatment is gaining popularity all over the world. This study is aimed at determining the effects of Indian liquorice on the fertility potentials of male Wistar rats. Fifty-five rats divided into seven groups were used in this study. Doses of 200 and 400mg/kg of the extract were used. The parameters considered were the weight of the testis, malondialdehyde concentration, hormonal levels, histological characteristics and sperm analysis. We observed a significant change in the weight of the testis, inhibition of testicular tissue peroxidation, testosterone levels, sperm concentration and motility were changed in comparison to the negative control. Obvious cellular changes were also observed in the liquorice-treated groups. These changes were brought back to almost pre-treatment values in the reversal groups. The changes observed in groups co-treated with vitamin E were minimal and statistically insignificant. The results suggest that the seed extract of Indian liquorice has a negative effect on the fertility potentials of the male rats and shows oxidative properties.

Key words: Indian liquorice, infertility, antioxidant, lipid peroxidation

INTRODUCTION

Traditional plants are used as a source of treatment of diseases in different parts of the world. The use of plant extracts as an adjutant form of medical treatment is currently enjoying great popularity. The use of herbs to treat disease is almost universal among non-industrialized societies (Dasilva et al., 2002). A number of traditions came to dominate the practice of herbal medicine at the end of the twentieth century. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization (WHO) estimates that 80 percent of the world’s population presently uses herbal medicine for some aspect of primary health care (traditional medicine). Earlier in the 1990s, approximately one-third of people surveyed in the United States had used at least one unconventional therapy during the previous year (Eisenberg et al., 1993) Traditional healers are still consulted in Nigeria as a first choice, probably because traditional medicine blends readily into the socio-cultural life of the people. A report from Adomi (2006) shows that therapy is still sought in plants by the Togolese.

Herbal remedies are fast growing and might take over from over-the-counter (OTC) drugs. Some of these supplements include Aloe Vera products, Chinese balms and other herbal concoctions that are preferred by the Yoruba-speaking people of Nigeria as first choice.

The need to control fertility cannot be overemphasized. Apart from overpopulation, more than half a million women die annually due to compli-
cations related to pregnancy and childbirth, which amounts to one woman dying every minute of every day (WHO/UNICEF, 1996).

Numerous plants have been used historically to reduce fertility and modern scientific research has confirmed antifertility effects in at least some of the herbs tested (Oderinde et al, 2003; McNeil et al., 2003; Olabiyi et al., 2006; Koneri et al., 2006).

Indian liquorice is a wild plant, growing best in fairly dry regions of low elevation. It grows in tropical and subtropical areas such as Nigeria, India, Sri Lanka, the West Indies, and South China. In fact, it is now naturalized in all tropical countries (Dwivedi, 2004). Its botanical name is *Abrus precatorius* and it belongs to the Fabaceae family. Other common names include Jequirity and Crab’s eye. The seed contains abrine, abriline, glycyrrhizin, gallic acid, protein, trigonelline, calcium, lipolytic enzymes, pectin, lectin and precatorine, gums and lignan (Rajaram and Janardhanam, 1992; Ivan, 2003). It is also used for the treatment of conjunctivitis, epilepsy and externally it is applied to treat abscesses and stomatitis (Hhabra et al., 1990). It is also traditionally used against leucoderma, wounds, alopecia, asthma, tubercular glands, fever, ulcer and tumor (Khare, 2004; Vaidyarathnam and Varier 1995). The saponin component of liquorice root, liquiritoside, has shown *in-vitro* anti-inflammatory activity (Anam, 2001). The lectin component of liquorice has also shown properties of bactericidal and non-specific immune response *in-vitro*.

Although the toxicity profile of most medicinal plants has not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts (Oluyemi et al., 2007a). Synthetic contraceptives currently in use are effective but are associated with a high incidence of side effects such as amenorrhea, menorrhagia, polymenorrhea etc. There is a need to search for new antifertility agents with minimal side effects, availability, accessibility, affordability and reversibility.

**MATERIALS AND METHODS**

**Animals and treatment**

Adult male Wistar rats, weighing 200±10g (70-90 days old), were maintained in 12 h light and 12 h dark conditions at a temperature of about 32 °C ± 1 °C in an animal house. The standard laboratory chew and tap water were available *ad libitum*.

The plant material - India liquorice seeds - was obtained from a local market in Lagos and authenticated in the Department of Botany, University of Lagos. The seeds were ground into powder and Soxhlet extracted with distilled water in the Department of Pharmacognosy, University of Lagos. The yield was concentrated into a solid paste *in vacuo* at 50°C using a rotary evaporator. It was then stored at 0°C until ready for use. 200 mg/kg of the extract was administered to the rats and this was chosen because higher and lower doses have been used by other researchers to achieve antifertility effects (Rao, 1987; Sinha and Mathur, 1990).

Thirty-five rats were divided into seven groups of five animals each and the groups were as follows: Control: Fed with pelleted animal feeds and water *ad libitum*; Group A: treated with 200 mg/kg/day of extract for 8 weeks; Group B: treated with 400 mg/kg/day of extract for 8 weeks; group C: treated with 200 mg/kg/day of extract and 400 mg/kg/day of vitamin E concurrently for a period of 8 weeks; group D: treated with 400 mg/kg/day of plant extract and 400 mg/kg/day of vitamin E concurrently for a period of 8 weeks; Group E: treated with 200 mg/kg/day of plant extract for 8 weeks then stopped treatment for 8 weeks (withdrawal); Group F: treated with 400 mg/kg/day of plant extract for 8 weeks then stopped treatment for 8 weeks (withdrawal).

**Retrieval of tissue**

At termination of the treatment, the rats were anaesthetized with ketamine 1 mg/kg (intramuscularly (i.m.)), the chest was opened and blood samples collected by heart puncture. Plasma was separated
and stored at 0°C. Testicular pieces were collected in Bouin’s fluid for histology and rapidly frozen for malondialdehyde (MDA) estimation.

Organ weight

Organ weight was taken on the day of sacrifice. The testicles were separated by dissection after trimming off the attached tissues and weighed using the volume displacement method.

Morphometrical study

After removal, a testis was immediately fixed in Bouin’s fluid and embedded in paraffin. Sections of 5 μm thickness were taken from the middle portion of each testis, stained with hematoxylin and eosin (H-E) and examined under a light microscope.

Semen analysis

The caudal part of the epididymis was excised and transferred into normal saline for about 3 min for the spermatozoa to swim out. Then a drop of saline was transferred to the Neubauer counting chamber (hemocytometer) for semen analysis under the light microscope. Sperm count was done strictly according to the recommended protocol of the WHO (1999) manual.

Sperm concentration measurements

Hemocytometer chambers were prepared for counting according to WHO criteria of semen analysis. The spermatozoa were viewed and counted under a light microscope. The hemocytometer is divided into nine fields, but the spermatozoa were counted and recorded for only five random fields and the value recorded in millions (10⁶) (Tomlinson et al., 2001).

Sperm motility

Sperm motility was assessed using the WHO (1999) classification system, with only the three grades (a, b and c) reported: rapid forward progression, medium forward progression and slow forward progression. Each sample was assessed twice. For consistency, all readings were carried out at 37°C (WHO, 1999).

Hormonal assay

Testosterone levels were measured using an enzyme based immunoassay (ELISA) kit. The assay was carried out in five steps as previously described by Raji et al. (2006).

Determination of malondialdehyde concentration

The malondialdehyde (MDA) level was determined in the supernatant of the testicular homogenates by the modified method of Buege and Aust (1978). The concentration was calculated using the molar absorptivity of malondialdehyde that is 1.56×10⁶ M. Percentage oxidation was calculated as follows: 1-mean value of treated group/mean value of control x 100.

Statistical Analyses

Data were expressed as the mean ± SD and the significance of the difference was analyzed by the Student’s t-test. The values were considered significant at p < 0.05.

RESULTS

Organ weight

At sacrifice, liquorice-treated rats (200 or 400 mg/kg) showed a reduction in the testicular weights. However, the reductions were not statistically significant (p>0.05), as shown in Table 1. Rats co-treated with liquorice extract and vitamin E revealed an improvement in testicular weight.

Effect of liquorice on parameters of sperm analysis.

A significant reduction in both sperm concentration and motility was seen in the liquorice-treated groups. Co-administration of liquorice and vitamin E prevented a reduction in the sperm analysis parameters
to a reasonable degree. These parameters returned to almost normal pre-treatment values in the reversal groups, as seen in Table 2.

*Testosterone concentrations of control and treated rats*

Plasma testosterone concentrations were significantly decreased in the treated groups compared to the control (p < 0.05). The changes were more prominent in group B, which received 400 mg/kg liquorice, as shown in Table 3. The study also revealed a marked improvement of plasma testosterone levels after 8 weeks of cessation of treatment.

*Malondialdehyde concentration*

Malondialdehyde (MDA) concentrations were shown to be significantly increased in the liquorice-treated groups. Groups C and D that were co-treated with vitamin E revealed lower levels in comparison to those treated with liquorice. These effects were almost fully recovered after eight months of withdrawal of treatment, as illustrated in Table 4. In addition, the rate of oxidation is significantly higher in the treated groups (A and B) in comparison to both the vitamin E-treated and withdrawal groups, as shown in Fig. 1. The oxidation rate was found to be as high as 39% in the group treated with 400 mg/kg of extract and 15.87% in the 200 mg/kg extract treated. An oxidation rate of 5.37% was noticed in the reversal group treated with 200 mg/kg of the extract and 10.17% in reversal group treated with 400 mg/kg.

*Histopathological observations*

Testicular damage was evaluated and quantitatively compared by a pathologist blind to the nature of the experiments in the Department of Anatomical Pathology, University of Lagos Teaching Hospital, Lagos, Nigeria.

CONTROL: numerous seminiferous tubules containing germ cells of various stages of maturation; spermatogonia, spermatocytes, spermatids and spermatozoa, arranged with a normal polarity with the spermatogonia closer to the basement membrane and spermatozoa towards the lumen of the seminiferous tubules. The Sertoli cells (sustentacular cells) in between the spermatogonia were also observed. The basement membrane was not remarkable and the interstitial was loose and contained Leydig cells, as illustrated in Fig. 7. The germ cells were within normal limits of morphology.

The histological study in the liquorice-treated groups (A and B) showed a nuclear enlargement, chromatin clumping, nuclear vacuolation, cytoplasmic macrovesicles and isolated small collection of apoptotic cells. There was no significant inflammatory cell infiltration and the interstitium was not remarkable. In groups C and D, which were treated with vitamin E, the nuclear changes and swelling that were observed in group A were absent in the slides of these groups. So also, cell death was significantly not present and the interstitial spaces were seen to be widened, as shown in Figs. 4 and 5. There were fewer germ cells in some of the seminiferous tubules of group C compared to group D.

In groups F and G, the cellular changes seen in A and B, i.e. the swelling and nuclear changes as well as apoptosis, were barely seen, as shown in Figs. 6 and 7, indicating near restoration after 8 weeks of cessation of treatment.

**Table 1. Approximate organ weights using volume displacement technique.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>0.96 ±0.04</td>
</tr>
<tr>
<td>A</td>
<td>0.92±0.03</td>
</tr>
<tr>
<td>B</td>
<td>0.94±0.05</td>
</tr>
<tr>
<td>C</td>
<td>0.95±0.03</td>
</tr>
<tr>
<td>D</td>
<td>0.91±0.025</td>
</tr>
<tr>
<td>E</td>
<td>1.10±0.06 *</td>
</tr>
<tr>
<td>F</td>
<td>1.15±0.04 *</td>
</tr>
</tbody>
</table>

* =significant difference (p<0.05)
Table 2. Semen analysis

<table>
<thead>
<tr>
<th>GROUP</th>
<th>AVE. SPERM CONCENTRATION (×10⁶)</th>
<th>SPERM MOTILITY</th>
<th>DESCRIPTION</th>
<th>VALUE OF SPERM x10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>157.34±0.13</td>
<td>16</td>
<td>70</td>
<td>EXCELLENT</td>
</tr>
<tr>
<td>A</td>
<td>134.08±0.21</td>
<td>23</td>
<td>19</td>
<td>FAIR</td>
</tr>
<tr>
<td>B</td>
<td>140.86±0.10</td>
<td>19</td>
<td>25</td>
<td>FAIR</td>
</tr>
<tr>
<td>C</td>
<td>159.22±0.09</td>
<td>18</td>
<td>53</td>
<td>GOOD</td>
</tr>
<tr>
<td>D</td>
<td>145.54±0.37</td>
<td>20</td>
<td>36</td>
<td>FAIR</td>
</tr>
<tr>
<td>E</td>
<td>162.32±0.17</td>
<td>18</td>
<td>67</td>
<td>GOOD</td>
</tr>
<tr>
<td>F</td>
<td>151.54±0.23</td>
<td>21</td>
<td>59</td>
<td>GOOD</td>
</tr>
</tbody>
</table>

Table 3. The effects of liquorice on serum testosterone and testicular tissue malonialdehyde concentrations

<table>
<thead>
<tr>
<th>TESTOSTERONE CONCENTRATION (nmol ml⁻¹)</th>
<th>MALONDEALDEHYDE CONCENTRATION (umol mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>4.90±0.08</td>
</tr>
<tr>
<td>A</td>
<td>2.05±0.06*</td>
</tr>
<tr>
<td>B</td>
<td>1.25±0.07*</td>
</tr>
<tr>
<td>C</td>
<td>2.40±0.02*</td>
</tr>
<tr>
<td>D</td>
<td>2.21±0.03*</td>
</tr>
<tr>
<td>E</td>
<td>3.07±0.01</td>
</tr>
<tr>
<td>F</td>
<td>3.86±0.02</td>
</tr>
</tbody>
</table>

Sample size (N)=5, SD= standard deviation, *=significant difference (p<0.05)

Fig. 2. A photomicrograph of a rat from control group (magnification x 100). The germ cells are within the normal limit of morphology with remarkable interstitium.

Fig. 3. A photomicrograph from group A; treated with 200 mg/kg of extract (magnification x 100) showing nuclear vacuolation, cytoplasmic macrovesicles and isolated small collection of apoptotic cells, without a remarkable interstitium.
DISCUSSION

Researchers have expressed their concerns about the rise in cases of male spermatozoa abnormalities (Kuku and Osegbe, 1989). Sperm factor constitutes about 40% of infertility and this is due to abnormalities of the male reproductive system (Ashiru et al., 1993). Infertility among couples in African societies, especially Nigeria, is causing increasing concern (Uriah et al., 2001). The infertility in Nigeria and Africa at large could be due to the fact that traditional healers are still consulted as the first choice because traditional medicine blends easily into the socio-cultural and economic life of the people. (Kela and Kufeji, 1995).

Fig. 4. A photomicrograph from rat Group B; treated with 400 mg/kg of extract (magnification x 100). A more severe form of Fig 3. with nuclear enlargement and chromatin clumping.

Fig. 5. Photomicrograph of rat from group D; treated with 400 mg/kg of extract and 400 mg/kg of vitamin E (magnification x 100). There are more germ cells in some of the seminiferous tubules of this group. The interstitium is narrowed and infiltrated with inflammatory cells.

Fig. 6. A photomicrograph from rat in group C; treated with 200mg/kg of extract and 400mg/kg vitamin E (magnification x 100). The nuclear changes and swelling that was observed in group A are remarkably reduced in this group. The interstitial spaces were seen to be widened.

Fig. 7. Photomicrograph of rat from group E; 8 wks withdrawal of 200 mg/kg of extract treatment (magnification x 100). Swelling and nuclear changes as well as apoptosis are barely seen. However, there is visible inflammatory cell infiltration.
Defective sperm function in cases of male infertility is mostly being caused by free radical activities (Sharma and Agarwal, 1996). There is growing evidence that spermatozoa are protected from the detrimental reactive oxygen species (ROS) effect by the powerful antioxidants in seminal plasma, since disturbances of sperm functions by ROS have been demonstrated in the absence of seminal plasma (Raji et al., 2007). In light of the above, it is not surprising that the testes of rats administered with vitamin E showed significant evidence of inhibition of lipid peroxidation. Vitamin E is a known powerful antioxidant with the ability to scavenge ROS. It also prevents the oxidation of essential cellular components because it is an excellent lipid-soluble, chain breaking antioxidant (Raji et al., 2007). The preventive function of vitamin E on testicular tissue oxidation and function cannot be overlooked since it has significantly prevented the cellular changes and cell death observed in group A animals in our research. The cellular testicular damage caused by Indian liquorice extract in our study supports that of Adedapo et al. (2007). An earlier study carried out by Sinha (1990) revealed a normal histological appearance that contradicts our histological findings in this study and this might be due to the higher dose used in this study.

The use of vitamin E in vitro has been also documented to improve sperm motility and viability (Verma and Kanwar, 1999), which is in agreement with our findings that show a protective effect in all the parameters used. Hughes et al. (1998) determined that in vitro supplementation of vitamins C, E, and urate separately have protective effects on sperm DNA integrity on irradiation.

The decrease in weight observed in the liquorice-treated rats, although not statistically significant, could be due to the reduced volume of the contents in the testes and lower numbers of testosterone-producing Leydig interstitial cells in the treated groups, clearly shown in the histological study. This result indicates that the extract suppresses Leydig cell steroidogenesis, which is in agreement with Amr Amin (2008).

The fertility parameters used in this study were almost restored in most rats by the end of 8 weeks after the cessation of the treatment, which correlates with work done by Sinha (1990). Hence, the effect of Indian liquorice on fertility and testicular functions in male rats is reversible. A similar result was observed in the estrous cycle of female rats by Okoko et al. (2008). In their study, estrous cycle analysis revealed that rats treated with 50 mg/kg body weight of methanolic extract of Abrus precatorius (Indian liquorice) seed for 32 days (8 cycles), produced an irregular pattern of cycling in 97.2% of the animals. This was characterized by a highly significant increase in the duration of the diestrous phase in the treated group when compared with the control. A highly significant decrease in the duration of the proestrus and estrous phases was also observed throughout the treatment period.

The effect of extract of liquorice on sperm function may therefore be caused by an increase in the lipid peroxidation as co-administration of vitamin E with liquorice leads to a significant decrease in the MDA levels, increased spermatogenesis and the near-normal level of the histological studies. The antifertility potentials of liquorice due to its oxidative properties might be due to the presence of some known...
constituents such as abrin (Bhaskar et al., 2008) and lectin (Myung-Sunny Kim et al., 2003)

CONCLUSION

The results of this study indicate that Indian liquorice extract administration had a harmful effect on the fertility potentials in male rats, probably due to an imbalance between the levels of ROS production and the natural antioxidant defense system. Various modalities can be used to protect spermatozoa from free radical-induced injury, such as diet that forms an important component of the antioxidant protection system; it supplies the major antioxidants such as vitamin C, vitamin E, and carotenoids, and for those patients who are suspected of having high levels of ROS, antioxidant supplements can also be considered. It appears that the primary site of action in this study may be the testes, however, its effect on the hypo-pituitary axis cannot be ruled out and further study is required to clarify this point.

REFERENCES


DaSilva, E.J., Baydon, E. and A. Badran (2002). Biotechnology and the developing world. Electronic Journal of Biotechnol-


Oderinde, O., Noroha, C., Oremosu, A., Kusemiju, T. and A.A. Okanlawon (2002). Abortifacient properties of aqueous extract of Carica papaya Linn seeds on Female Sprague-
THE EFFECT OF INDIAN LIQUORICE ON FERTILITY POTENTIALS OF MALE RATS


