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INVESTIGATION OF THE TOXICITY POTENTIAL OF *TRIGONELLA FOENUM GRAECUM* (LINN)

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ABSTRACT

Extract prepared from the seeds of *Trigonella foenum graecum* Linn, was administered twice a week for four weeks, at dosages of 1.0, 1.5 and 2.0 gkg^{-1} body weight, to male albino Wistar rats. At the end of fourth week, the animals were sacrificed by transection of the jugular vein and testes, kidneys and liver of each rat were processed for histopathological assessment. The extract of *Trigonella foenum graecum* at all dose levels, had necrotic effect on the liver and the kidney tissues. Spermatogenesis was observed in the testes at dosage levels of 1.5 gkg^{-1} and 2.0 gkg^{-1} of the extract.

INTRODUCTION

Trigonella foenum graecum (Linn), family leguminoceae, "Fenugreek" is mostly a low plant with pinnately three-foliate usually denticulate leaves with stipule adnate to petiole. The flowers are yellow, white or blue. The fruit is a beaked long or short indehiscent pod and many seeded. *Trigonella foenum graecum*, has a strongly scented odour of cumarin. The plant is mostly found in southern Europe, Asia, North Africa and India (Bailey, 1949). It is also found in Nigeria, mostly Northeastern Nigeria. Its indigenous names in Northeastern Nigeria (Borno State) include "Hilbe" (Hausa) and "Wulwa" (Kanuri).

There are about 70 species of the genus Trigonella. Among these only two species are of economic importance. These are *Trigonella foenum graecum* and *Trigonella coerula*. Many of these have been used for their medicinal and other properties. In India the leaves are used as contraceptive while the seeds are used as antidiabetic and also consumed as spice (Bailey, 1949). In North Africa, the seeds are used to fatten young girls in preparation for marriage (Cameroon and Carmichael, 1941).

The aqueous preparation of the seeds are also used by the traditional medicine practitioners to treat diabetes mellitus. The anti-hyperglycaemic effect (Ribes *et al.*, 1984, Amin *et al.*, 1988, and Ajabonor *et al.*, 1988), spermicidal effect (Dhawan *et al.*, 1977) and anti-ulcer activity (Al-Meshel *et al.*, 1985), has been noted in rats and dogs. The aqueous extract of the seed is also claimed to have an aphrodisiac activity (Al-Meshel *et al.*, 1995). Previous investigations have so far dwelt on providing scientific support for the many uses claimed by medical practitioners of the seeds of *Trigonella foenum graecum* and the exclusion of its possible adverse effects. The aim of this study is to investigate the effects of repeated administration of the aqueous seed extract of *Trigonella foenum graecum* on selected organs of the rat.

MATERIALS AND METHODS

Animals

Male albino rats of the Wister strain weighing 180 - 200g, which have been inbred for 4 years in the animal house of the Department of Pharmacology, University of Maiduguri, Maiduguri, were used. They were housed under standard lighting regimen and had free access to standard diet (Growers mash, ECWA feeds, Maiduguri, Borno State) and water.

Preparation of Extract

Dried seeds of *Trigonella foenum graecum* were purchased from the local market (Maiduguri). The seed were cleaned by winnowing to remove any chaff and stones. A 200g portion of the seeds was mixed with one litre of distilled water in a two litre beaker and boiled for 45min. It was allowed to cool to 40°C and then strained through cheese cloth. The liquid part was then filtered using Whatman No. 1 qualitative filter paper. The filtrate was then evaporated until the volume was reduced to 400 mL (1 mL of the extract representing 0.5g of the dry seeds).

Treatment Protocol

Fourty male Wister rats were randomly distributed into four groups of ten. Group one served as the control and received distilled water. Groups 2, 3 and 4 received the aqueous extract of *Trigonella foenum graecum* at doses of 1.0, 1.5 and 2.0 gkg^{-1}, respectively. Animals in the control group received a

quantity of distilled water equivalent to those in 2.0 gkg^{-1} group.

The extract was administered orally by means of a rat feeding needle of guage 20 and ball diameter 2.25mm (Harvard apparatus, U.S.A.). Animals received their doses twice a week for four weeks. Throughout the study period, the animals were observed daily for signs of toxicity or pharmacological reactions such as loss of appetite, loss of weight and depression. At the end of fourth week, the animals were sacrificed through ether anaesthesia and transection of the jugular vein. The testes, kidneys and liver of each rat were removed and observed grossly. These organs were then fixed in 10 per cent formal saline for histological studies. Paraffin sections were cut at 8 μm and stained with Haematoxylin and Eosin.

RESULTS

At doses of 1.0, 1.5 and 2.0 gkg^{-1}, no mortality was observed during the bi-weekly oral administration of the seed extract of *Trigonella foenum graecum*. Gross examination of the organs did not reveal any pathological changes due to treatment with the extract.

General histopathologic evaluation of organs, revealed a dose dependent effect of the extract on the structure of the organs, with the effect increasing with dose. Histopathologic changes in the kidney of animals exposed to 1.0 gkg^{-1} of the extract, showed tubular necrosis with reduced luminal size (8 - 25μm) and leucocytic infiltration into the interstitium compared to the control (Fig.1). Increasing the dose of the extract to 2.0 gkg^{-1} caused marked tubular necrosis with some cytoplasmic vacuolation and coalescence of tubular epithelium. There was increased luminal diameter (13-30μm, Fig. 2).

When compared with the control (Fig.3) the structure of the testes exposed to an extract dose of 1.0 gkg^{-1} revealed normal germ cells at various stages of gametogenesis. There appeared to be many spermatozoa which occupy the inner 1-2 layers of the seminiferous tubules. The luminal diameter was slightly reduced (15-30 μm wide). The luminal diameter of the seminiferous tubules increased slightly (18-40 μm across) when the dose was increased to 1.5 g kg^{-1}. At a dose of 2.0 gkg^{-1}, the diameter of the tubular lumen increased further (15-50 μm across) with a slight increase in tubular diameter (from between 112-135 μm at 1.5 g kg^{-1} to 125-140 μm). The germ cells showed more spermatocytes and spermatids, appearing in many layers with corresponding increase in the number of spermatozoa which occupy the inner 4-6 layers of seminiferous tubules.

Figure 5 shows (photomicrograph of the normal liver) normal extensive interlacing sinusoidal system radiating from the central vein. Hepatocytes are large with centrally placed nuclei. But the general architecture changed when exposed to 1.0 g kg^{-1} of fenugreek extract. The sinusoids were congested with some necrotic changes in the hepatocytes. When the dose was increased to 1.5 gkg^{-1}, the hepatocytes showed fragmentation of nuclear chromatin (karyrrhexis), and coalescence with congested sinusoids. At a dose of 2.0 gkg^{-1}, there was marked tissue necrosis, congested sinusoids and infiltration by inflammatory cells (Fig. 6).

DISCUSSION

Generally crude drugs of plant origin are often prescribed by traditional medical practitioners without regard to their adverse or toxic effects. It is, therefore, pertinent to study the possible adverse effects of such preparations as a guide to their standardization and safe usage. The presence or absence of adverse effects emanating from the repeated oral administration of aqueous preparations of *Trigonella foenum graecum* has not yet been reported.

The liver and the kidney seemed to be more susceptible to the effects of the crude aqueous extract of *Trigonella foenum graecum*. The effect of the extract on the liver even in lower concentrations was similar to the hepatotoxicity demonstrated by carbon tetrachloride (CCl_4) in experimentally induced damage (Bowman and Rand, 1988). Both have necrotic effect on liver tissues but unlike the CCl_4, fatty liver was not observed in the present study. The extract also produced renal tubular necrosis at all dose levels. Changes in the kidney tubules as well as infiltration of leucocytes into the interstitium and the reduced luminal space at a dose of 2.0 gkg^{-1} may indicate that, the extract of *Trigonella foenum graecum* can impair kidney function.

The increase in daimeter of seminiferous tubular lumen with a slight increase in tubular diameter, the presence of spermatocytes and spermatids, and many spermatozoa, at 2.0 gkg^{-1} dose level, may indicate that, fenugreek at this dose level can facilitate spermatogenesis. Indeed the infusions of the extract have been used as an aphrodisiac to stimulate genital functions of men, in cases of impotence (Al-Meshal *et al.*, 1985).

The present study has shown that, the repeated oral administration of the aqueous seed extract of *Trigonella foenum gaecum* could have deleterious effect on the liver and the kidney but may stimulate spermatogenesis. Thus, providing an experimental support for the use of decoctions of *Trigonella foenum graecum* as an aphrodisiac in folk medicine. Although it may not be advisable to repeatedly administer such decoctions due to the possible adverse effects on the liver and the kidney. Further work is suggested on the

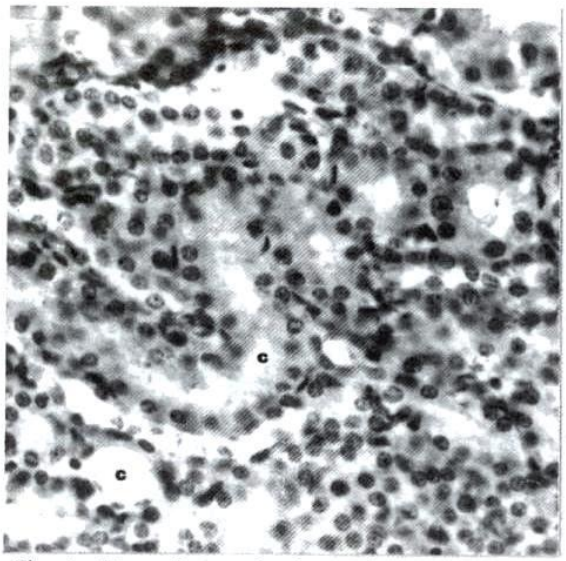

Fig. 1: Photomicrograph of a section of normal rate kidney (control) through the cortico-medullary junction showing patent tubular lumen (C) with intact luminal epithelium H & E stain. 400 x.

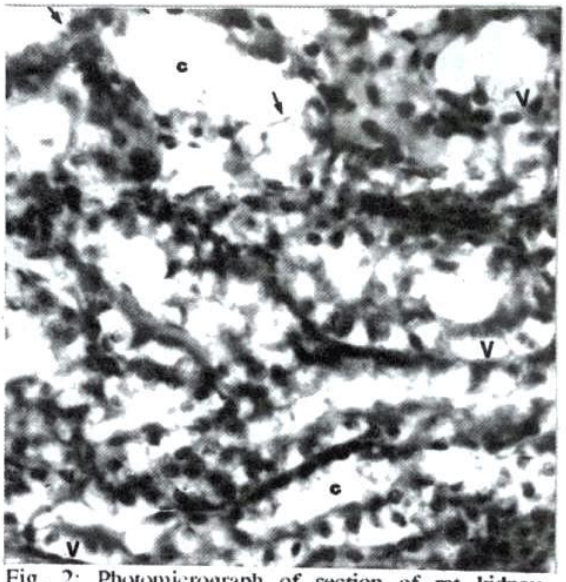

Fig. 2: Photomicrograph of section of rat kidney (treated with extract, 2.0 g/ml) through the corticomedullary junction showing tubular necrosis with vacuolation (v) of tubular epithelelium (short arrows) resulting in increased lumin diameter (C). H & E stain. 400 x.

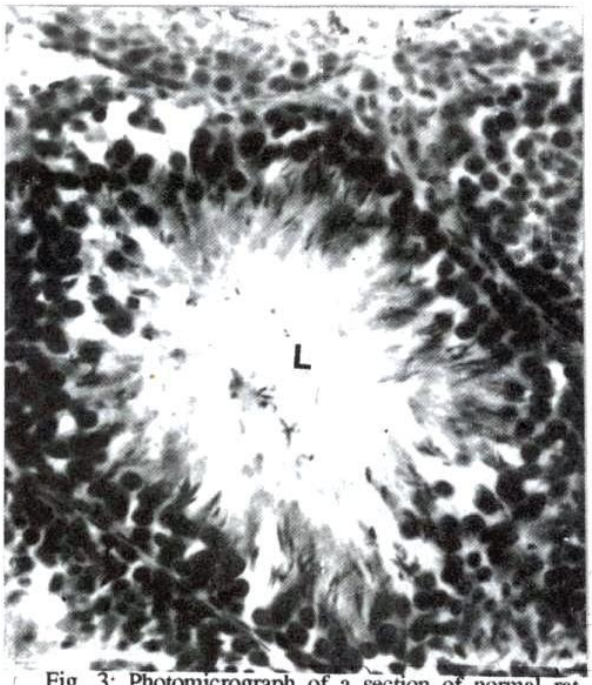

Fig. 3: Photomicrograph of a section of normal rat testis (control) showing seminiferous tubules with germinal epithelium. L is a tubular lumen. H & E stain. 400 x.

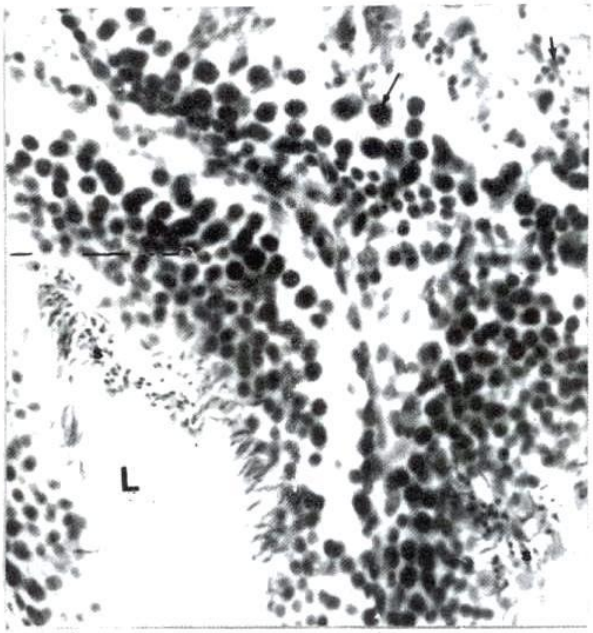

Fig. 4: Photomicrograph of section of rat testis (treated with 2.0 g/ml of extract) showing an increase in tubular diameter with corresponding increase in luminal size (L). The germ cells show differential increase in the number of spermatocytes (arrow), spermatids (small arrow) and spermatozoa (s). H & E stain, 400 x.

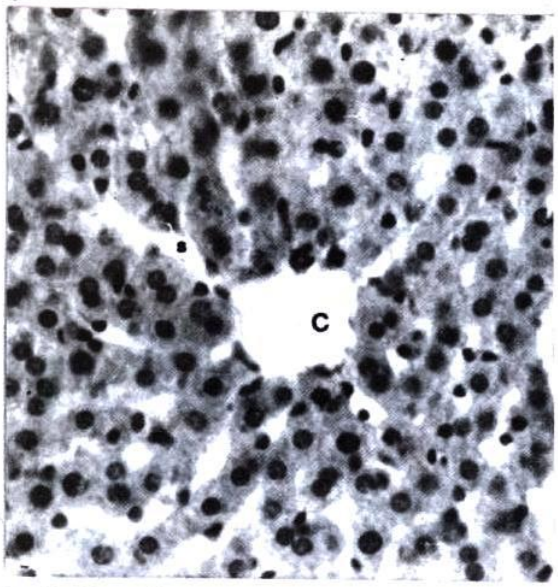

Fig. 5: Photomicrograph of a section of normal rat liver (control) showing an interlacing sinusoidal (s) system radiating from a central vein (C). H & E stain, 400 x.

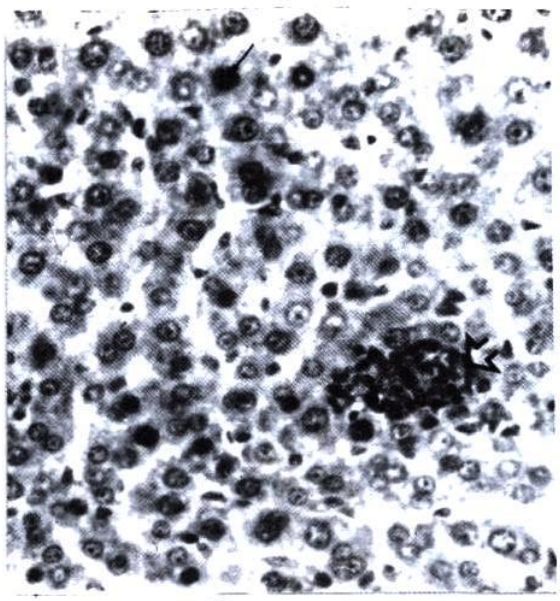

Fig. 6: Photomicrograph of a section of rat liver (treated with 2.0 g/ml of extract) showing marked tissue necrosis with pyknotic changes infiltration of a central vein (broken arrowhead). H & E stain. 400 x.

evaluation of the critical dose level where adverse effect of the extract on the liver and kidneys could be minimized, and yet maintaining its aphrodisiac and other effects.

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