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Appendix I

MB-50

As Revised: March 1981

A method to test the effects of plant extracts on the fertility of male rats

1. Rationale

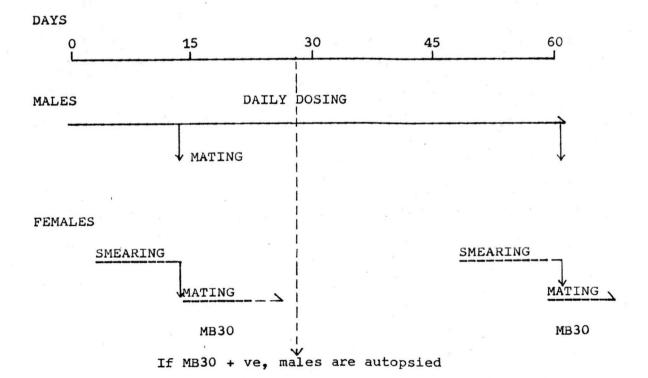
The activity of plant extracts on epididymal function (and, if required on testicular function and spermatogenesis) is examined by oral administration of the extract to male rats. The rats are then mated with females to assess their fertility.

2. Procedure

A. Synopsis

EPIDIDYMAL EFFECTS

TESTICULAR EFFECTS



- (1) Epididymal Effects (15 day dosing) Male rats are dosed with extract for fifteen days. On days 14 and 15 each male is housed with 2 females in proestrus selected in accordance with the MB30 protocol. The males are autopsied, their testes and epididymides weighed and epididymal sperm examined. The females are autopsied on day 16 of gestation. The number of pregnant animals, the number of implantation sites, the number of normal and abnormal fetuses and the number of corpora lutea of pregnancy will be recorded and reported exactly as for MB30.
- (2) Epididymal and Testicular Effects (60 day dosing). Male rats are dosed with extract for 60 days. If required, they may be mated at days 14 and 15 (as above) and the males autopsied on day 30 if the females are not pregnant. This would reduce the amount of extract required and advance the date of autopsy of the males consequent on a positive MB30. If the MB30 is negative, the males would continue to be doses to day 60, when they are mated (day 59 and 60) autopsied; their testes and epididymides weighed and epididymal sperm examined. The females are autopsied on day 16 in gestation. The number of pregnant animals, the number of implantation sites, the number of normal and abnormal fetuses and the number of corpora lutea of pregnancy will be recorded and reported exactly as for MB30.

B. Animals

Proven fertile Sprague Dawley male rats (10 weeks old, 250-350 g) and virgin females (eight weeks old, 180-200 g) should be housed, not more then four per cage according to cage size.

A large group of females (at least 80) should be smeared to establish their estrous cycles (as per MB30 protocol).

C. Dosing

Males are randomly assigned to experimental and control groups, each group contains six animals. The extract should be administered orally by flexible stomach tube or metal feeding needle immediately after weighing the animals. The animals should be dosed according to their daily weights. Extracts should be administered in the highest possible dose; the limiting factor is the viscosity of the extract solution in a volume not exceeding 1 ml. Dosage should be reported in g of extract per kilogram body weight. The dosage should be made up daily, or every other day, the remainder being kept in the refrigerator. Dosing should continue for 15-60 days according to requirements. The testis volume should be measured at the beginning of dosing and at autopsy.

NOTE: Where appropriate, aliquots of the extract may be frozen at -20°C and an aliquot thawed each day for dosing. If this procedure is adopted, aliquots should be flash frozen using liquid nitrogen or an ethanol-dry ice mixture.

D. Pairing

(1) Epididymal Effects (15 day dosing) On the evening of day 14 of dosing, the male should be placed with two females in proestrus. On the morning of day 15 he male should be removed and given a final dose. On the evening of day 15 of dosing the male is placed with two further females in proestrus. On the following morning, the male is autopsied. The females housed individually or in pairs are given no treatment and are autopsied on day 16th of gestation, according to the MB30 protocol.

(2) Epididymal and Testicular Effects (30-60 day dosing) If the MB30 at day 15 Wespositive, the males are autopsied at day 30. If not, dosing may be continued until day 60 when the procedure under (1) above is performed.

E. Autopsy

(1) Male - The volume of the testis is measured before dosing begins, i.e. on day 1. The volume is measured at autopsy (day 15,30 or 60). The testis and epididymis are removed and weighed separately. The epididymis is placed in saline, opened with fine cissors or needles and the motility of sperm assessed (simple motile vs. non motile)

(2) <u>Female</u> - On day 16 of gestation the animals should be autopsied. The uteri and ovaries should be removed, the number of corpora lutea of pregnancy in the ovary should be counted, the uterus should be opened longitudinally and the number of normal and abnormal (dead and/or degenerate) fetuses recorded. The placenta, fetus and membranes should be removed and the number of implantation sites recorded.

F. Report

(1) Male - Change in testis vol. Experimental Testis Wt. Experimental

Control

Control

Activity of sperm (?) Experimental

Control

(2) <u>Female</u> - The report and procedures outlined here assume a 90% pregnancy rate in the control animals. Levels 1-4 indicate the importance of results in order of priority. Date should be included for both control and treated groups in each case. As indicated in the flow sheet, MB30 + 40 is the line of first priority.

Result

Procedure

Level 1

Number of animals pregnant Number of animals treated 30% reduction; retest at higher dose.

40% reduction; result positive, proceed to MB40/41.

Level 2

Number of implantation sites perpregnant animals

40% reduction; retest at higher dose.

60% reduction; proceed to MB40/41.

Level 3

Number of normal fetuses per pregnant animal

60% abnormal; retest at higher dose.

80% abnormal; proceed to MB40/41.

NOTES - As for MB30.

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