OPTIMISING THE DOSE OF EMBELIN, A POTENTIAL MALE ANTIFERTILITY AGENT, USING DIFFERENT ROUTES OF ADMINISTRATION

BY

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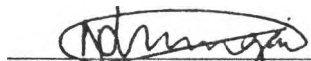
1996

DECLARATION

I, Nelly Nyambura Mungai hereby declare that this is my original work and has not been presented for award of a degree in any other University.



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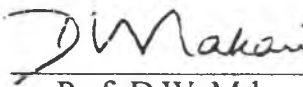

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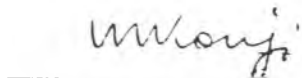

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DEDICATION

To my children

Wangari

Wanjiku

and

Mwangi

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ABBREVIATIONS

Name:

CP	-	Control phase
im	-	Intramuscular
p	-	Probability
PTP	-	Post treatment phase
TP	-	Treatment phase
vs	-	Versus
SEM	-	Standards error of measurement
PTL	-	Plasma testosterone levels
WHO	-	World Health Organization
G	-	Gauge

Unit

g	-	gramme
kg	-	kilogramme
mg	-	milligramme

µg	-	microgramme
min	-	minute
ml	-	millilitre
M	-	molar
nM	-	nanometer
°C	-	degree centigrade
iu	-	international unit
N	-	normal
µmol	-	micromole
nMol	-	nanomole
L	-	litre
%	-	percent

SUMMARY

Reproduction is a physiological process which involves the act of mating between two members of one species and of opposite sex with consequent production of offspring. At human level this physiological process is controlled by many factors such as behaviour, societal set up, economy and politics. One of the main ways of regulating sexual reproduction and therefore population growth in most of the groups has been through the use of contraceptives. However, most of the contraceptive methods available have been focused on the female.

In the recent past a lot of effort has been directed towards developing a contraceptive with suitable and acceptable male fertility regulating properties. One of the promising approaches to obtaining this male fertility-regulating agent has been in use of the higher plant extracts. Indeed some plants of the Myrsinaceae family have been known to have compounds (mainly benzoquinones) with male fertility-regulating properties. One such compound isolated from these plants is embelin. Results obtained with embelin so far indicate that it alters the histology of the testis and reduces the weight of the accessory sex glands, sperm count and their motility as it interferes with fertility in male rabbits by lowering plasma testosterone levels.

This project was aimed at investigating the minimum effective and optimum dose of embelin required to regulate fertility in male rabbit using three routes of administration namely subcutaneous, intramuscular and oral. In the latter case embelin was administered as a suspension as well as in tablet form. Plasma testosterone concentration was used as an indicator for fertility potential. The dose of embelin used was varied from 0.5 - 70 mg per kg of body weight. Plasma testosterone concentration was measured by radioimmuno assay (RIA) technique.

Results indicate that when embelin was administered intramuscularly a minimum dose of 5.0mg/kg of body weight was observed to lower plasma testosterone levels from a mean pre-treatment level of 8.32 $\pm$ 0.4 nmol/L to mean treatment phase level of 4.8 $\pm$ 1.2 nmol/L. This represented a 54 per cent decline in plasma testosterone levels. For the oral route a slightly higher than that used for the intramuscular route but minimum effective dose of 10.0 mg/kg of body weight was required to cause a significant ($P<0.001$) lowering of plasma testosterone from mean pre-treatment level of 12.2. $\pm$ 0.70 nmol/L to 6.0 $\pm$ 2.0 nmol/L during treatment. This was a 63 percent decline in the hormone levels. But when embelin was administered orally as a tablet of 50.0 mg base per kg of body weight there was slightly less lowering of plasma testerone than when it was administered as a suspension. The testosterone levels declined from mean pre-treatment levels of 5.5 $\pm$ 0.65 nmol/L to mean treatment levels of 4.7 $\pm$ 1.5 nmol/L this being a 40 percent decline

On administering embelin subcutaneously a minimum effective dose of 20.0 mg per kg of body weight was required to cause a significant ($P< 0.01$) lowering of plasma levels from mean pre-treatment levels of 22.6 $\pm$ 1.30 nmol/L to mean treatment levels of 19.8 $\pm$ 0.3 nmol/L representing a 12.4% decline.

The decline in testosterone levels when embelin was administered either intramuscualarly or as a suspension orally was almost instantaneous. But when administered as a tablet orally or is an injection subcutaneously the decline in the hormone levels was gradual. For the intramuscular route the effect of embelin in lowering testosterone levels was directly related to the dose of embelin administered implying that this effect of embelin was dose related.

Higher doses of embelin than those of intramuscular route were required for optimum effect when administered via oral route. This was perhaps due to the fact that some embelin was metabolised in the gastrointestinal tract before it was absorbed to get to the target organ where it was to exert its effect. When administered as a tablet form the effect was gradual and not very pronounced, this would imply that embelin was not released fast enough and in high enough concentrations to reach the site of action and have instantaneous and pronounced effect.

The gradual lowering of testosterone levels observed when embelin was administered subsutaneously was perhaps due to the fact that embelin got accumulated in fatty tissues, where some of it may have been metabolised and thus reducing its concentration that would get into the target organ This implies that the concentration reaching the target organ was not in high enough concentration to cause pronounced effect.

It is important to note that in all cases on withdrawing embelin the testosterone concentration returned to normal levels after treatment implying that its effect was temporary and hence reversible, this being a crucial property for any antifertility agent.

The observations made in the study indicate that embelin lowers plasma testosterone levels when administered at the right dose via the three major routes of drug administration This lowering of testosterone levels is most likely the cause of antifertility effect observed when embelin is administered to the rabbit From the study it would appear that the best route of administering embelin would be via the oral route Although embelin was required at higher doses when administered orally, the effect was almost instantaneous just like the intra-muscular route, despite the fact that no pain was inflicted on the animal when administering the drug Eventually the route would be economical as no medical staff or equipment would be required to administer embelin

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

Reproduction can be broadly defined as a physiological process which to most individuals implies the act of mating between two members of one species and opposite sex with consequent production of offspring. The term can also be extended to include replacement of damaged or dead cells in many body areas. At human level this physiological process is controlled by many factors such as behaviour, society and culture. As a result the reproduction pattern has untold effects on the economy and politics of a nation. All these factors therefore makes regulation of reproduction to be complex both at the personal and societal levels (Kessler and Standley, 1980).

1.1.2 Importance of controlled reproduction

Controlled reproduction in humans can also be referred to as contraception which is essentially the technology of eliminating the capacity to reproduce without eliminating affiliative sexuality. There are reasons why reproduction should be controlled. General uncontrolled production of individual cells is known to cause a variety of cancerous conditions while uncontrolled reproduction of members of a species can lead to crowding, stress, shortage of food and unhealthy competition for living space. At human level all this may lead to war and pestilence. Indeed family planning has so far been shown to lower infant, child and maternal mortality and morbidity and also to reduce the number of illegal abortions and their health hazards. Yet still much more can be achieved in family planning.

1.2 Family planning for males

For a long time family planning services have been geared towards the female while leaving out the male. Indeed there has been widespread use of the female

contraceptives which have to a great extent improved the social standards and behaviour of human kind. There is actually no sound argument why the development of a male contraceptive has been lagging behind that of the female. Of late however, strong biological evidence has been reached to justify why the burden of responsibility for conception control should be the males' priority (Greep, 1976). For instance, it is the male that triggers off the procreative process as the sperm must be ferried between the sexes. If the sperms are blocked from getting to the egg or are destroyed before reaching the egg, then conception cannot occur. Secondly, the males have a much longer fertile life than their female counterparts. Also the male is the sexual aggressor, while the role of a female in terms of sexual behaviour is that of a willing in many cultures and submissive partner to sexual intercourse. The males, therefore make up a target population for the implementation of conception control, and this is of great importance to the future welfare of the human kind (Greep, 1976).

From the foregoing, the search for contraceptives need not therefore be directed towards the female use only but the males too should share the contraceptive burden with their female counterparts. This way both male and female would have the health advantage of which partner to take the contraceptive, should one be found for the males (Lewis and Elvin - Lewis, 1977).

It is from the preceding that the World Health Organization (WHO) set on a task force to investigate the regulation of male fertility. The major objective of this task force was to produce safe, effective and reversible contraceptive methods for males which can be afforded by developing countries (Waites, 1989, WHO, 1990).

Fertility regulation in males can be divided into three major areas (de Kretser, 1974),

a) interruption of sperm formation either by direct action on the testis or by interfering with hormonal composition essential for spermatogenesis,

b) interference with epididymal maturation of sperm and

c) disruption of sperm transport.

As of today only two methods are available for widespread control of male fertility, that is, the condoms and vasectomy both of which interrupt sperm transport.

Of the various hormonal methods evaluated, the only effective compounds offering any hope for future use is testosterone alone or in combination with its derivatives (Pangkahila, 1991). However, due to the fact that it has to be given parenterally coupled with its inability to completely suppress spermatogenesis in all men, and the long duration between suppression and recovery of spermatogenesis after administration, it represent the self-explanatory drawback of the use of these compounds as male antifertility agents (de Kretser, 1980).

1.3 Higher plants: Source of male antifertility agents

Thus from the foregoing many attempts have been made in an effort to obtain a male-fertility regulating agent but without much success. But one avenue of research that seems to offer some hope is that of studying compounds extracted from higher plants (Farnsworth and Bingel, 1976). Indeed, the studies of safe, orally effective fertility-regulating agents from higher plants for use in humans is

not a foreign idea, and for a long time virtually every native culture has used plants in one form or another in an effort to control its population. A systematic attempt to evaluate these plants ethnomedically have shown that some are quite effective while some may be toxic (Farnsworth *et al,* 1980; Bingel and Farnsworth, 1980). Most of the compounds extracted from these plants so far were observed to exert their antifertility effect mainly on females but some had similar effect on males too (Farnsworth *et al,* 1980; Farnsworth and Bingel 1976).

The various compounds extracted from plants could regulate fertility because of one or more of the following effects;

i) estrogenic effect
ii) uterine stimulatory effect and
iii) post-coital contraceptive effect.

i) Estrogenic possessing compounds; example are isoflavones, coumestains and stilbenes but because of their generalised weak estrogenic effect they were found to be of little practical importance to human fertility regulation (Bingel and Farnsworth, 1980; Farnsworth and Bingel, 1976).

ii) Uterine stimulatory possessing compounds; an example of these group of compounds are the alkaloids. Unfortunately most of these compounds have additional pharmacological effects that exempt them from use as human fertility regulating agents (Farnsworth *et al,* 1980).

iii) Compounds with post-coital contraceptive effect; this group include compounds such as montanol, zoapatanol and pseudoholic acid (Briggs and Briggs, 1976). The compounds were isolated from *Mantanoa tomentosa* of the family Compositae. The plant has been used in Mexico as an

abortifacient and also to induce menstrual flow (Jiu, 1966; Levine *et al*, 1979) There are other plant-derived substances of known structures claimed also to have anti-implantation and/or abortifacient activity on the basis of animal results. Such compounds include, embelin (Kholkute *et al*, 1978), p-coumaric acid (Pakrashi and Shahe, 1978; Pakrashi and Pakrasi, 1979) and gossypol (Anonymous, 1978).

Compounds with post-coital contraceptive effect are more promising as potential contraceptives since they induce antifertility effect via peripheral organs such as the uterus and fallopian tubes without altering reproductive hormonal profiles (structures for some of these compounds are shown in Fig 1).

Thus sufficient evidence has accumulated to justify the continuation of research in an attempt to further develop these substances as male fertility regulating agents. Indeed, it is out of such research that a compound like gossypol has reached advanced stages of development as a male fertility regulating agent in the Peoples Republic of China (Anonymous, 1978; Bingel and Farnsworth, 1980; Dai and Dong, 1978).

Gossypol from the cotton plant, *Gossypium* spp of the family Malvaceae, is a dimeric sesquiterpene aldehyde which was discovered accidentally to be present in the cottonseed, stem and root. Gossypol has already been used as an oral contraceptive for males in People's Republic of China after having been shown earlier on to be effective both in animal experiments and also in clinical trials (Qian *et al*, 1980) The compound has been used as a tablet either as gossypol, gossypol acetic acid or as gossypol formic acid. The onset of infertility was observed to be dose related and the effect persisted for 3-5 weeks following withdrawal of treatment. Fertility gradually returned on withdrawing the treatment. For its mechanism of action, gossypol was shown to be spermicidal in humans (Waller *et al*, 1980) and also in mice (Coulson *et al*, 1980) It is thought

to interfere with testosterone biosynthesis (Lin *et al*, 1987) and not with libido

Unfortunately, gossypol has lately been observed to have several side effects, such as; slow onset of the antifertility effect and the danger of sterility after long term administration (Zhong *et al*, 1990), hypokalemic effect (Qian, 1979) inhibition of glutathione-s-transferase an enzyme that is involved in the detoxification of certain organic compounds including potential carcinogens in both rodents and humans. At high doses equivalent to those necessary for contraception gossypol was shown to cause discolouration of hair, diarrhoea, malnutrition, circulatory disorders and even heart failure (Maugh, 1981).

From the foregoing it would imply that gossypol is far from being an ideal male antifertility agent and there is need therefore for more intensified search for a male fertility regulating agent. When searching for a fertility regulating agent for males, the aim should be to obtain a compound that will limit the ability of the sperms to fertilise but that which will not affect libido. Thus any agent or device that would adversely influence libido in the male would be unacceptable (Greep, 1976).

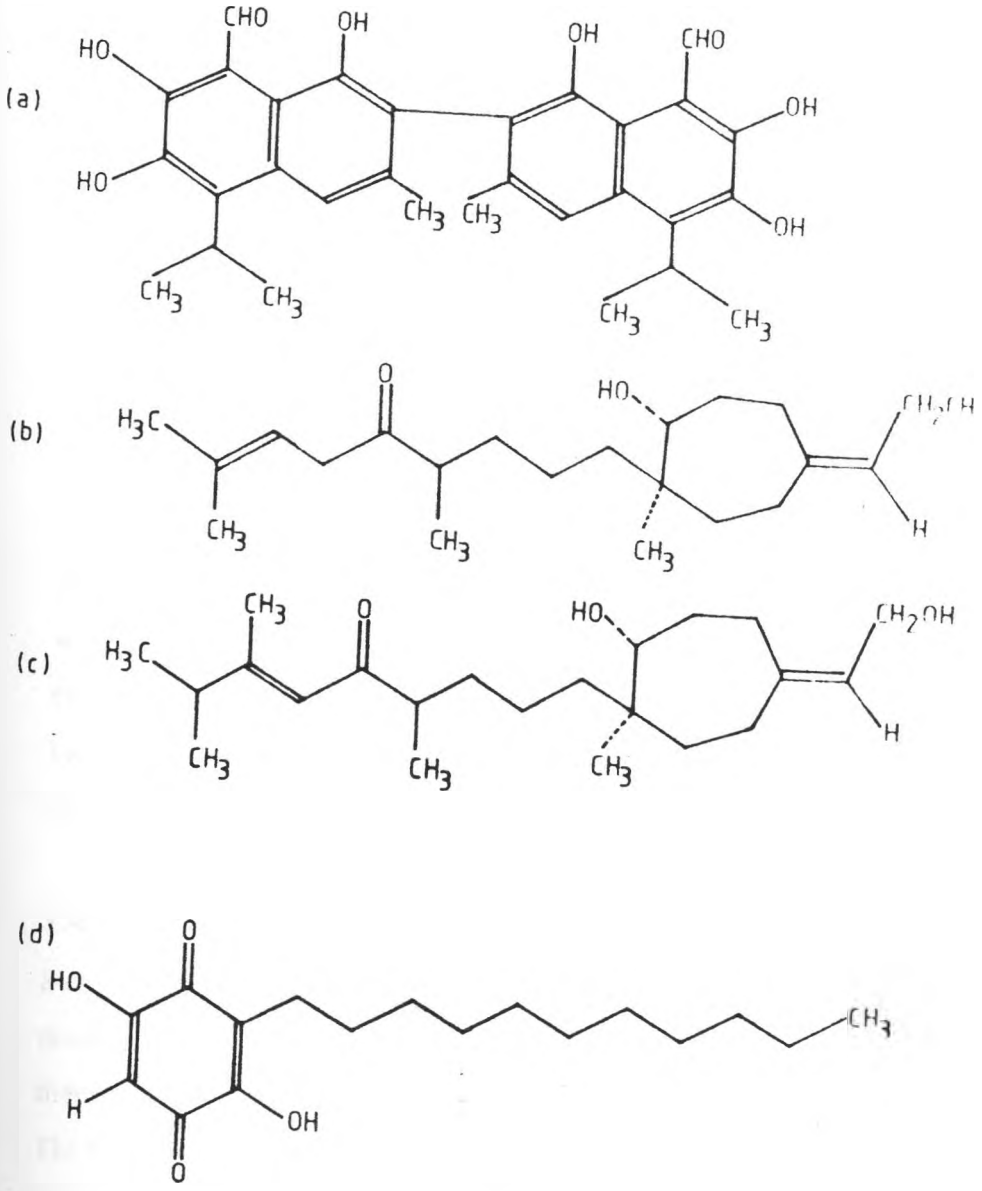


Fig. 1. Structures of some of the plant compounds with male antifertility effect

a) Gossypol

b) Zoapatanol

c) Montanol

d) Embelin

1.4. Embelin: A potential male antifertility agent

1.4.1 Background information

Embelin has so far been shown to be one such compound from higher plants which offers some hope as a possible future male antifertility agent. Encouraging results have been obtained with this compound in animal models like the rabbits and dogs (Aggrawal *et al*, 1986). Further studies have been carried out to determine the possible mode and site of action of embelin as a male contraceptive. Results obtained so far indicate that embelin exerts its antifertility effect by inhibiting testosterone biosynthesis thereby disrupting spermatogenesis (Githui, 1990). The specific site on the testosterone biosynthetic pathway which has been proposed as possible target for embelin inhibition namely; 17, α- hydroxylase which converts 17, α- hydroxyprogesterone to androstenedione and acetic acid (Githui, 1990) (see scheme I for possible inhibitory site of embelin on testosterone biosynthesis pathway). Further work was carried out which indicates the specific enzyme site inhibited by this compound to be 17, α-hydroxylase (Makawiti, 1992).

1.4.2. Embelin: Classification and distribution in nature

Structurally embelin is a benzoquinone, a group of compounds that are widely distributed in nature and have a wide range of biological activities associated with them. In higher plants the benzoquinones are found in the family Myrsinaceae. The Myrsinaceae family belongs to the Primulus order and about a thousand plants classified into thirty-three genera have been described (Englers, 1964). Of those plants, eleven species have been grouped into three genera namely, *Ardisia, Myrsine and Maesa* (Watt and Meyer-Brandwijk, 1962). The three genera grow in southern part of Japan and are also found in India where they are referred to as *Embelia ribe* Burm F. (Ogawa and Natori, 1968). In India the plant is regarded as one of the most important source of crude drugs among the indigenous medicines and its berries are locally known as *'vidanga'* (Hussein and Srivasteva, 1979). Plants in the family Myrsinaceae from Japan have been studied and show

the presence of embelin and/or rapanone, maesaquinone, acetyl quinone and other related quinones (Ogawa and Natori, 1968).

In Kenya the Myrsinaceae family is represented by four species in four different genera, that is, *Maesa lanceolata* Forsk, *Myrsine africana L. Rapanea malanphoes L.* Mez and *Embelia Schimperi* Valtke (Midiwo *et al*, 1988). These plants grow generally as shrubs in moist and shady places at altitudes of about 1500 meters. The species *Myrsine africana* and *Maesa lanceolata* are mainly found on the lower ranges of the Aberdares and Mount Kenya while *Rapanea melanphloes* is mainly found near Nunguni hills in Machakos district. These plants have been shown to have the following benzoquinones, embelin, maesaquinone and maesanin (see Fig. 2 for structures) which are widely distributed in the plant seeds, bark and leaves. Seeds have been shown to give the highest yield of 1-10% (Midiwo *et al*, 1988)

All benzoquinones have the quinone moiety and an alkyl side chain. The structure of some common naturally occurring benzoquinones are shown in Figure 2

1.4.3. Pharmacological properties of benzoquinones

Benzoquinones are a highly reactive group of compounds with a lot of biological activity attributed to them. They are known to exhibit anthelmintic property. This was first observed in India when powdered berries of *Embelia ribes* Burm were administered orally as indigenous medicine (Nadkarni, 1954, Guru and Mishra, 1966; Pranjpe and Gokhale, 1932) This property of benzoquinones was also reported in Kenya where the berries are used in traditional medicine as anthelmintic (Kokwaro, 1976).

Benzoquinones have been shown to have insecticidal properties, thus embelin when mixed with wheat samples at very low concentration of about 0.187%

exhibited high efficacy by causing both adult and larva mortality (Chander and Ahmed, 1983). It was further observed that even after eight months of storage, embelin did not have any toxic effects on germination of the wheat grains (Chander and Ahmed, 1985; 1987). These observations confirmed earlier work by Deshmukh and Borle (1975) who showed that the petroleum ether extracts of the *Embelia ribes* Burm berries had insecticidal properties against *Dactyrotus certhani*. The same were also observed to protect green grams from *Callosobruchus chinensis* L. infestation by causing mortality of the insects (Chander and Ahmed, 1982).

The benzoquinones, embelin, maesaquinone and rapanone or their derivatives have been demonstrated to be antibacterial, antifungal or antiamoebic (Shah *et al*, 1984; Rao *et al*, 1985).

Benzoquinones are also known for their antifertility effects. Embelin for example has been reported to have fertility regulating activity in both males and females. This was first noted in India whereby aqueous and alcoholic extracts of *Embelia ribes* Burm berries when fed orally to the rats in high doses were reported to prevent implantation but were also noted not to exhibit any toxic effects (Arora *et al*, 1971; Bhargava and Dixit, 1985). The male antifertility property of embelin as mentioned earlier has also been shown in rats (Githui, 1990; Gupta *et al*, 1989; Seth *et al*,1983) and in rabbits (Makawiti *et al*, 1992). It was also noted that feeding Bounet monkeys with powdered berries from *Embelia ripes* caused a reduction in semen volume and sperm mobility (Jayaraman, 1977). Embelin was also shown to have antifertility effect by altering testicular histology, glycogen levels, sperm counts and weights of accessory glands in male rats (Aggrawal *et al*, 1986). Reversible contraceptive like activity due to embelin was reported in male dogs whereby azoospermia was observed (Dixit and Bhargava, 1983).

1.4.4. Physical properties

Embelin is a 2,5-Dihydro-3-undecyl-1,4- benzoquinone. It has a melting point of 140-143 °C and gives a single homologous spot on TLC with an RF value of 0.56 in chloroform : methanol (1:1) solvent (Kaul *et al*, 1929). When ran on oxalic acid activated silica gel using n-hexane : ethyl acetate : acetic acid in ratio of 17:2:1 as the mobile phase an R_F value of 0.4 is obtained (Midiwo *et al*, 1988)

1.4.5. Toxic effects of embelin

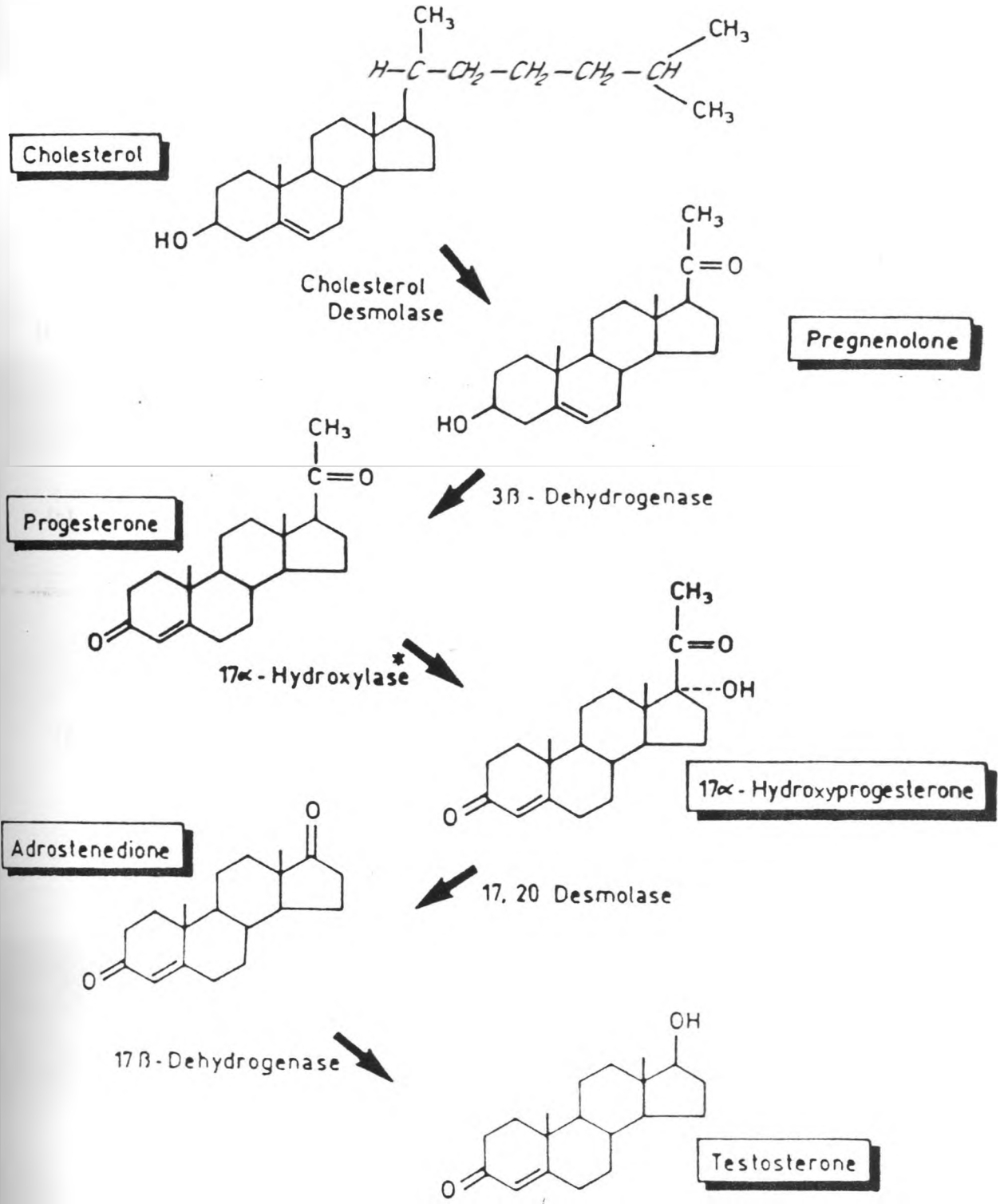
Due to the hope embelin seems to offer as a possible future male oral contraceptive, some further work has been carried out on toxicity of this compound. Available information indicate that embelin causes retinal pathology and visual defects. Thus embelin has been linked with optic atrophy among the Ethiopian population who usually take the compound for its anthelmintic properties (Low *et al*, 1985). This effect is supported by the observation that embelin causes retinal pathology and visual defects in chicks. Some studies on lipid metabolism using male albino rats show that there is impairment of lipid metabolism in testes, liver, brain and kidneys of these animals following embelin exposure. The defect in lipid metabolism led to accumulation of total lipids, cholesterol, triacylglycerols and free fatty acids in the testes, liver and kidneys while a decrease in lipid content was noted in the brain (Gupta and Kanwar, 1990, 1991). These changes were restored back to the normal levels once the drug was withdrawn. Benzoquinones have also been shown to interfere with oxidative phosphorylation in the mitochondria (Makawiti *et al*, 1990). Thus, maesaquinone and embelin were observed to uncouple mitochondria while maesanin inhibited electron transport and oxidative phosphorylation a property further confirmed in insects (Magiri, 1992).

It is important that further studies be carried out to establish any other possible toxic effects of embelin so that the benefits of embelin as an oral male contraceptive can be weighed versus the risks of its toxic effects and thus clear the way for its use.

Scheme I

The pathway for testosterone synthesis from cholesterol

* Indicate the enzyme that is likely to be inhibited by Embelin



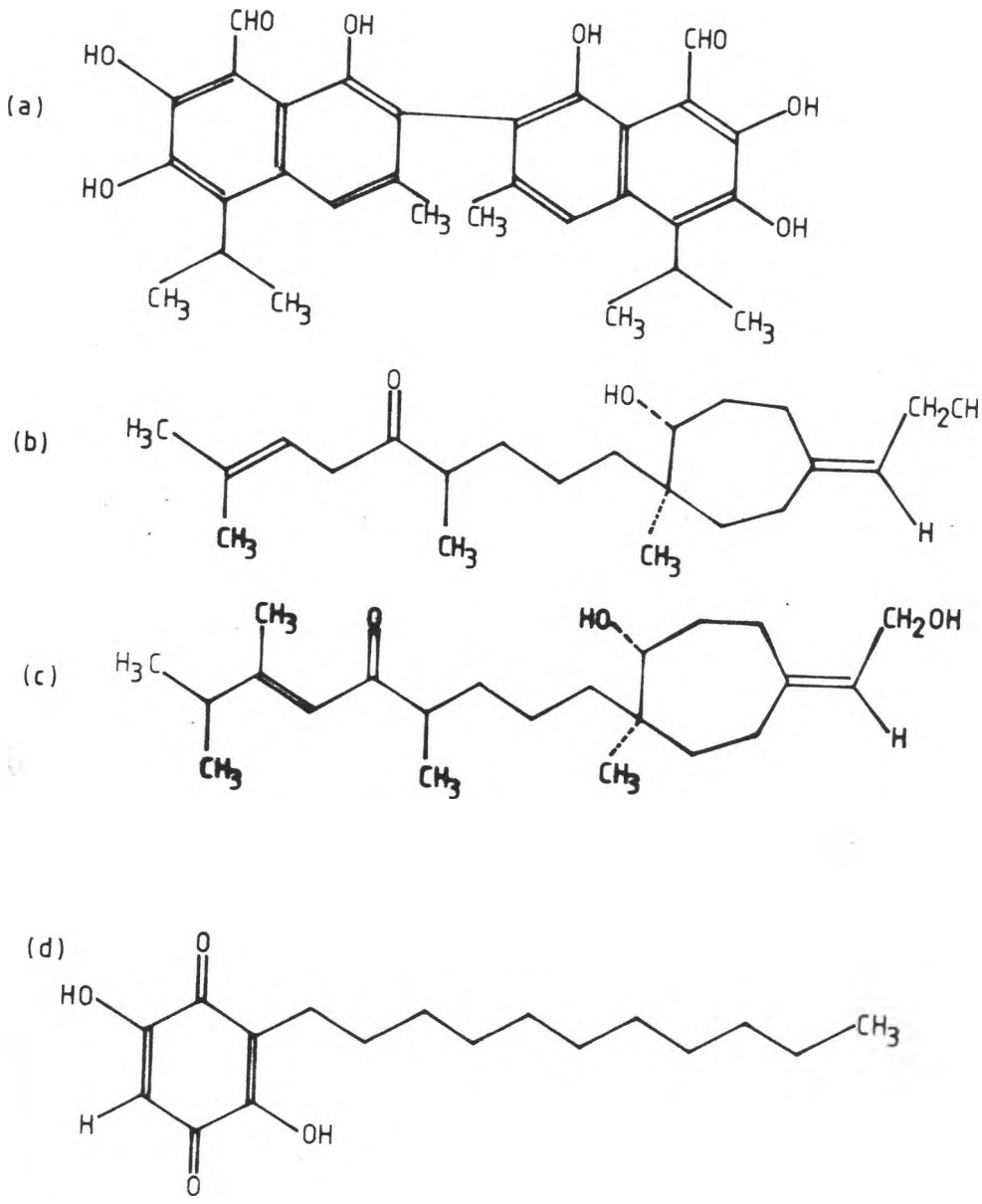


Fig. 2: Structures of some of the naturally occurring benzoquinones

a) Maesaquinone

b) Maesanin

1.5 RATIONALE

A lot of progress on family planning has been achieved in developed countries where by 1980's, over 95 percent of their total population were using one or more of the known methods of this practice (Riphagen and Lebert, 1989) But this has not been the case in developing countries, although a lot has been achieved at governmental level. In developing countries there is still a big difference between the population in need of and requiring such services and those actually practicing it. There are many reasons advanced for this anomaly although it is generally accepted that this difference could be compromised by better birth control technology. That is by the development of a wide range of effective, safe and acceptable contraceptives for both men and women for use in the widely differing cultural, religious and social settings that would meet their individual needs (Ishahakia and Bambra, 1992). This is where a promising male antifertility compound like embelin comes in.

Studies carried out on embelin so far by assessing the lowering of the plasma testosterone levels in male rabbits indicate that this compound may have some degree of antifertility potential Because of the hope embelin offers as a possible future male contraceptive, the current study intends to try and establish the suitable dose of embelin that would be most effective in lowering plasma testosterone levels and regulate fertility The study also hopes to determine the

most suitable route of administering this compound. This study would therefore be useful in laying out a basis for carrying out clinical trials of embelin.

The results obtained would be a milestone in achieving the WHO task force objective of regulation of male fertility by producing safe, effective and reversible contraceptive which can be affordable by developing countries (Diczfalusy, 1986; 1991).

1.6 AIMS AND OBJECTIVES

1.6.1 Aim:

The aim of this project is to investigate the most suitable dose of embelin for each of the three different routes of administration that would regulate fertility by lowering of plasma testosterone levels in male rabbits

1.6.2. Objectives:

1) To investigate the effect of embelin on plasma testosterone levels when administered via
    i) intramuscular route
    ii) oral route
    iii) subcutaneous route

2) To determine which of the routes above is most suitable for administering embelin so as to achieve maximum lowering of testosterone levels.

3) To identify the most suitable route of administering embelin based on results obtained under 1 and 2 above

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. Chemicals

All the chemicals used for extraction, suspension of embelin powder, tableting and testosterone assay namely chloroform, hexane, acetylacetate, acetic acid, oxalic acid, corn oil, diethyl ether, toluene, anhydrous sodium dihydrogen phosphate, anhydrous disodium hydrogen phosphate, sodium chloride, sodium azide and the scintran, [2,5-diphenyloxazole (PPO)] were purchased from Sigma chemical company, Poole, Dorset U.K. Other chemicals which were necessary for testosterone assay namely, anti-testosterone serum, testosterone standard, testosterone tracer, charcoal, gelatin and dextran were all provided by WHO Matched Reagents Programme (WHO, 1990). Heparin was obtained from Gedeon Richter Ltd., Budapest, Hungry.

2.2. Methods

2.2.1 Extraction of embelin crystals

Mature *Rapanea melanphloes* L seeds were sun dried for a period of two weeks Once dry, the seeds were ground into a fine powder using Lee house hold flour mill Embelin was then extracted from the crude powder following a modified method by Midiwo *et al.* (1988) as follows The powdered seeds (200g) were

soaked in 500 ml chloroform for a period of forty eight hours with occasional shaking, the mixture was then filtered using vacuum suction and the filtrate concentrated by evaporating off chloroform using the rotary-evaporator. To the concentrate a small volume of 10 ml hexane was added, and this was left overnight to allow crystallization to take place. This resulted in formation of yellowish orange crystals which were thereafter dried using vacuum suction. The extraction procedure is summarized in Scheme 2, page 31.

To confirm that the yellow crystals were embelin two tests namely, thin layer chromatography (TLC) and melting point (MP) were carried out.

2.2.2. Melting point determination

The melting point was determined using the Gallen Kamp melting point apparatus as follows: **Capillary tubes were sealed at one end by heating. To the open end the yellow-orange crystals were packed to the full. The tubes were then fixed in the apparatus which was fitted with an oil thermometer with a temperature range** of -10 °C to 360 °C. The crystals were electrically heated and the temperature at which they melted was noted. An average of six melting point determinations were done.

2.2.3. Thin layer chromatography

The thin layer chromatography (TLC) was performed using silica gel coated aluminium foil impregnated with 3% oxalic acid solution. The extracted crystals and a standard sample (previously purified by HPLC) of embelin crystals were dissolved in minimum amount of chloroform and then cochromatographed. The plate was eluted with a mixture of n-hexane : ethylacetate : acetic acid in the ratio of 17:2:1. After elution was complete and the plate dried, it was sprayed with ammonia solution for location of the spots. Purple spots were observed to be formed with both the sample and embelin standard.

2.2.4. Preparation of embelin for administration to the animals

The yellowish orange crystals which were confirmed to be embelin were made into a suspension, by suspending them in previously autoclaved and cooled corn oil. The concentration of the stock suspension was 100 mg/ml embelin in the corn oil. The suspension was then ready for administering to the animals.

2.2.5. Tableting of embelin for administration to the animals

On mixing the embelin crystals with magnesium carbonate, lactose and compressing with a tableting machine some dark brown tablets were formed. The tablets are shown in Plate 1 and were formulated to 50 mg base per tablet. These were used in the appropriate studies.

2.3 Animals

A population of fifty two (52) sexually mature white New Zealand male rabbits were used for the experiment. The animals weighed between 2 5 - 3 0 kg. They were bled in the University animal house and kept singly in cages of 45 cm by 45 cm by 30 cm. The cages were in a well-lit (12 hour light and 12 hour darkness) and ventilated room maintained at a temperature of 25 °C. Chow pellet, vegetables and tap water were provided *ad libitum*. The animals were kept in quarantine for two weeks for them to acclimatize to laboratory conditions.

For the treatment schedule the animals were divided into three groups according to the route of administration to be used.

Group I The animals in this group received embelin intramuscularly and they were further subdivided into four subgroup (a-d) each consisting of four animals. The embelin dosage was administered using a sterile 2 ml syringe and G19 needle and the dose was varied as follows:

Subgroup a 0 mg kg body weight this group received the corn oil only which was the vehicle used for suspending embelin and therefore served as the control

Subgroup b 0 5 mg kg body weight

Subgroup c 5 0 mg/kg body weight

Subgroup d 30 0 mg/kg body weight - this was the highest dose of embelin administered via this route

Group II The animals in this group were given embelin orally by use of a gastric lavage tube They were subdivided into six subgroups (a-f) each consisting of four animals The dose of embelin was again varied as follows

Subgroup a 0 mg/kg body weight these animals received only corn oil and thus served as the control.

Subgroup b 5 0 mg kg body weight

Subgroup c 10.0 mg/kg body weight

Subgroup d 50.0 mg/kg body weight

Subgroup e received a tablet orally which contained 50 mg embelin which was administered in form of powdered suspension of the corn oil.

Subgroup f 70 0 mg/kg body weight of embelin

Group III In this group the animals received treatment as a subcutaneous injection Again the animals were subdivided into three subgroups

(a-c) each consisting of four animals Embelin dose was varied as follows

Subgroup a 0 mg/kg body weight, that is, it received only corn oil and thus served as the control

Subgroup b 5 0 mg/kg body weight

Subgroup c 20 0 mg/kg body weight

For all the animals the treatment schedule was divided into three phases that is the pre-treatment or the control phase (CP) whereby the animals received no drug but only blood was collected The treatment phase (TP) in this phase the animals received the different doses of embelin while at the same time blood was collected There was then the post-treatment phase (PTP) or the recovery phase where only blood was collected but treatment was withdrawn

2.4 Collection of blood and storage

0 5 ml of blood was collected from the rabbit's marginal ear vein between 8 30 to 10 0 am under aseptic conditions using sterile 2 ml syringe and gauge 21 needle which were coated with heparin solution to prevent clotting of blood The blood samples were then centrifuged at 1500 g for 15 minutes at 4 °C Plasma was then separated and stored at -20 °C until assayed for testosterone

2.5 Testosterone assay

Testosterone was assayed using the radioimmuno assay method (RIA) which is an example of structurally directed assays that depends on interaction of a particular shape in the structure of the molecule being measured (i.e. the analyte) with another molecule that specifically recognizes that shape (the antibody). In the following assay the analyte was testosterone while the antibody was anti-testosterone serum and the tracer was tritium labelled testosterone. The assay was carried out as outlined in WHO matched reagents programme, (Sufi *et al*, 1990). The steps followed from sample preparation to the calculations are outlined below,

2.5.1 Extraction of testosterone from plasma samples

a) **The plasma samples were thawed and vortexed. Then 50 µl of plasma was** pipetted out and placed in a 5 ml glass tube.

b) **To the above glass tubes, 450 µl of the assay buffer was added.** (The assay buffer was composed of 2.53 g of NaH_2PO_4, 11.6 g Na_2HPO_4, 8.8 g NaCl, 1.0 g of previously warmed gelatin and 0.1 g of sodium azide in 1litre of distilled water; at pH 7.4)

c) 5 ml of diethyl ether was then added and the mixture vortexed for 45 seconds

d) The mixture was then allowed to stand for 5 - 10 minutes for the tube contents to separate After separation the lower aqueous layer was frozen using dry ice to allow the upper diethyl layer to be decanted easily into 7.5 ml vials

e) The solvent was then evaporated to dryness using a hot water bath The residue was dissolved in 2 ml of assay buffer by vortex mixing repeatedly at intervals of 10 minutes 500 µl each of the redissolved sample extract were used in duplicate for subsequent testosterone assay

2.5.2 Assay of testosterone

For testosterone assay the following tubes were prepared in duplicate:-

1) **Sample tubes which contained: 500 µl of the sample; 100 µl of antiserum; 100 µl of tritium labelled testosterone**

2) Standard tubes contained 500 µl of standard testosterone (instead of the sample) plus all the other contents

3) Total count tubes (TC) which contained only 800 µl of the buffer solution and 100 µl of the tritium labelled testosterone

4) Non-specific binding tube (NSB) which had 600 µl of the buffer and 100 µl of the tritium labelled testosterone

5) Buffer blanks tubes (Bo) contained the following, 500 µl of the buffer, 100 µl of antiserum and 100 µl of tritium labelled control

Quality control (QC) tubes were also included in each assay The tube contents were incubated for a period of 4hours after which the tubes were transferred to an ice-bath where 200 µl of ice-cooled dextran-coated charcoal (0.625 g of charcoal and 0.0625 g dextran in 100 µl of buffer) was added to each tube except in Total Count (TC) tubes. See Table 1 for a summary of contents of assay tubes. The contents were then vortexed and left to stand for 30 minutes after which they were centrifuged at 4 C for 15 minutes at 1500 g using a Beckman model TJ-6 centrifuge connected to a TJ-R refrigeration unit. The supernatant from each tube was decanted into scintillation vials containing 5 ml scintillation fluid (30 g of PPO in four litres of toluene) and counting was done in a Packard Tricarb scintillation counter

Table 1: Summary of contents of assay tubes

	Buffer	Standard or sample	Antiserum as working Dilution	3H Testo- working Dilution		Dextran Coated charcoal
Total Count (TC)	800 µl	-	-	100 µl	I N C U B A T E at 4 °C	-
Non-specific Binding (NSB)	600µl	-	-	100µl		200µl
Buffer Blanks (Bo)	500µl	-	100µl	100µl		200µl
Standard or sample	-	500µl	100µl	100µl		200µl

2.5.3 Calculation of results

The calculation of testosterone levels were done as follows:

For each set of samples (which were in duplicate) and also for the standards (which were in triplicate), the mean counts were determined. The percentage binding for the standard was calculated from the formula below:

$$\frac{\text{mean counts (standard - NSB)}}{\text{mean counts of the buffer blanks}} \times 100$$

From the calculations, the standard curve was plotted as percentage binding of the

standards against the various concentrations on a log-logit graph The concentration was expressed in fentomoles per litre (fmol/L) The percentage binding of the test samples were calculated in a similar manner to that of the standard and concentration of testosterone in fentomoles was read from the standard curve The dilutions undergone by the samples due to the assay procedure were calculated and testosterone concentration expressed as nanomoles per litre (nmol/l) The sensitivity of the assay at two standard deviations below the maximum specific binding was ± 0 51 nmol l Mean inter-assay coefficent of variation was 12 6% and the intra-assay coefficient of variation was 2 2% at 8 50 ± 0 18 nmol l The mean testosterone levels for each dose of embelin administered were then plotted against time of treatment plus or minus one standard error of measurement (SEM).

2.6. Statistical analysis

All the data was analyzed statistically using the student 't' test and the following formula was used.

for paired samples where $n = n_2 = n$

then

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1 - S_2}{n}}}$$

while for non-paired samples where $n_1 \neq$ to n_2, then t was calculated as follows

$$t = \frac{\bar{X} - \bar{X}_2}{\sqrt{\frac{(n_2 - n_1)S^2_1 + (n2 - n1)S^2_2(\frac{1}{n_1} + \frac{1}{n_2})}{(n_1 + n_2)^{-2}}}}$$

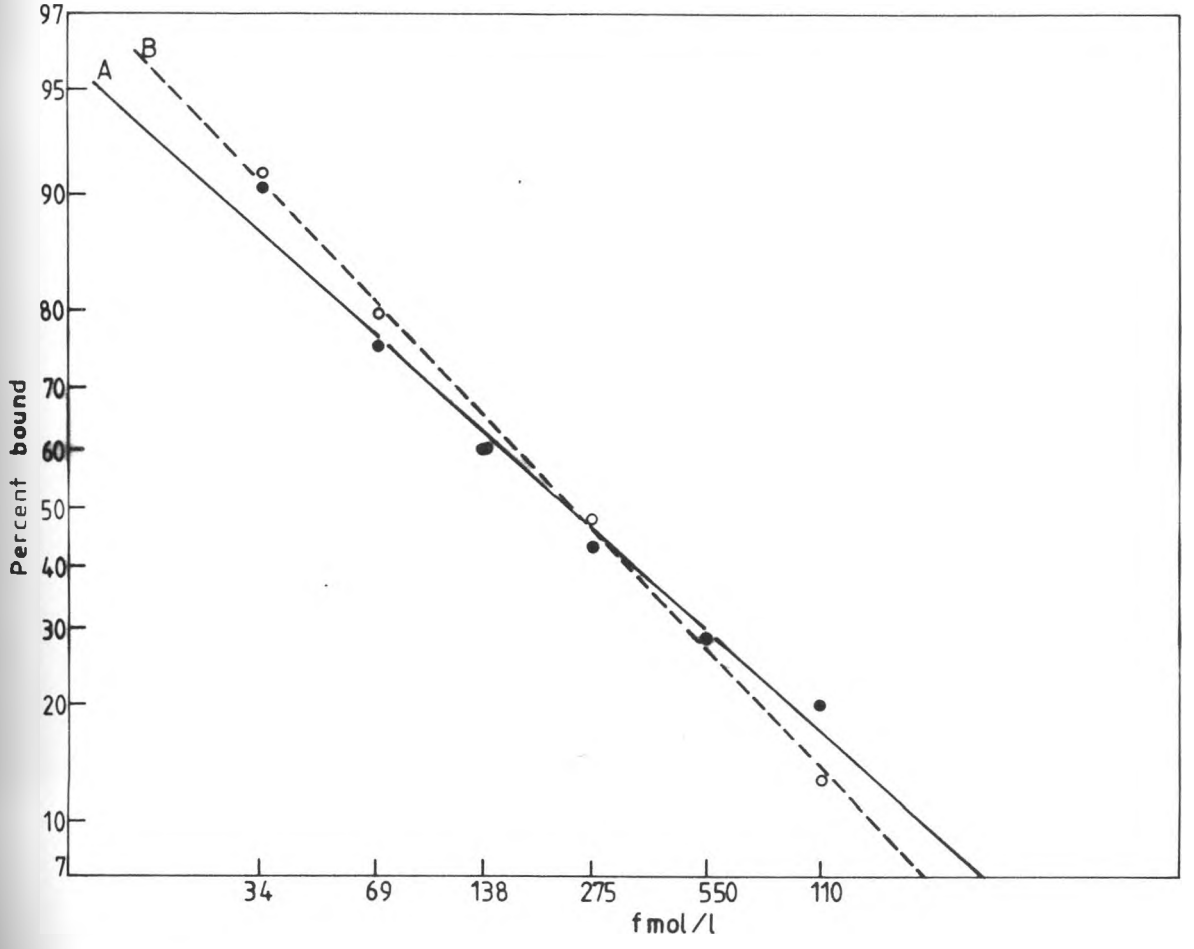
Where by

$\bar{X}$ = mean value of testosterone concentration in one phase

n = number of samples

S = standard deviation

The dose effect on the various routes used to administer embelin were compared during the three phases of treatment Differences with a probability *(p)* value of less than or equal to 0 05 was considered to be significant



The Standard Curve for testosterone plotted on log-logit graph

The concentration of the standards were expressed in fentomoles per litre (fmol/L) Two standard curves were plotted inorder to determine the best fitting curve which was found to be curve B

SCHEME 2

A flow diagram showing extraction of embelin starting from dry *Rapanea melanphloes* seeds

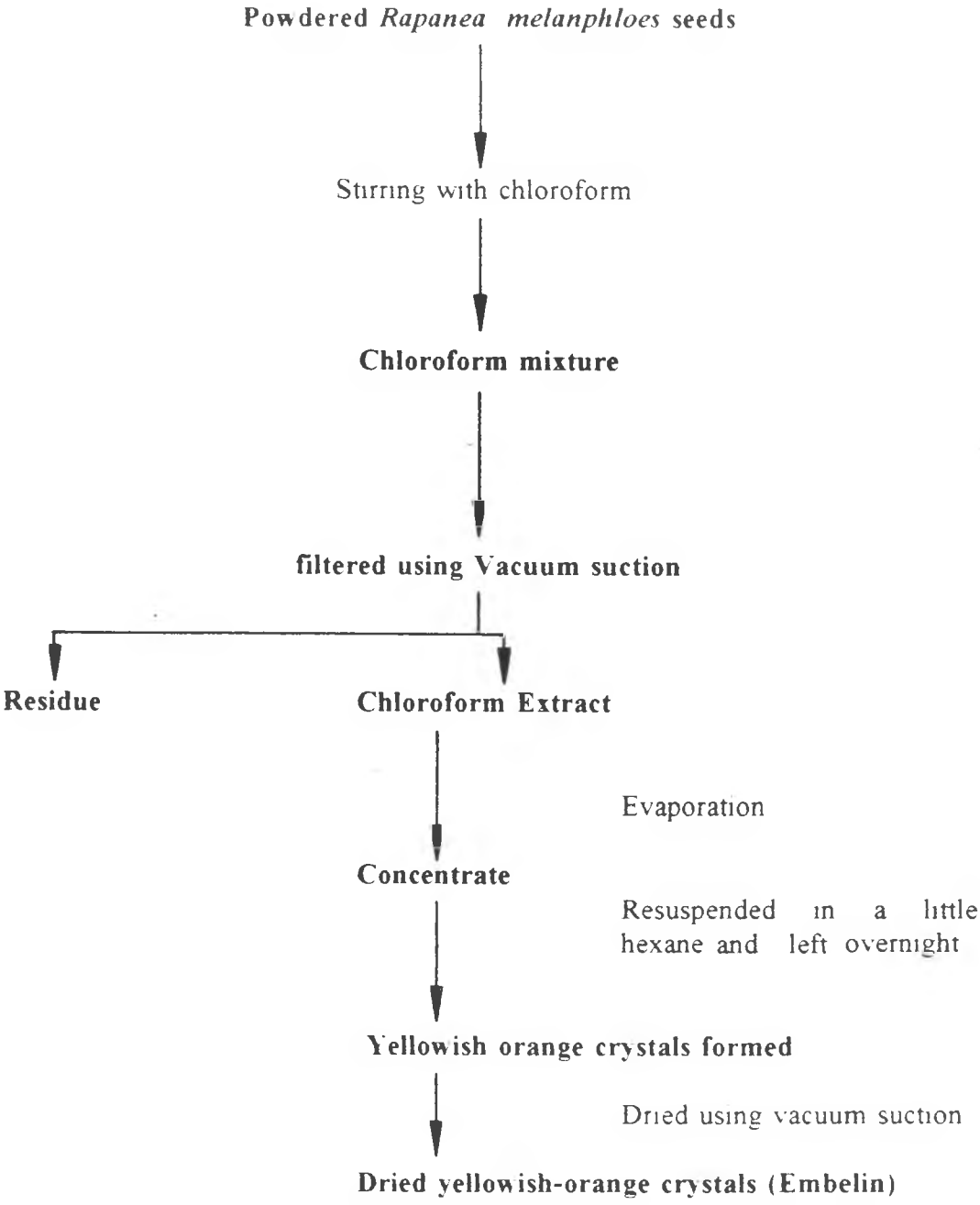


Plate one

The tablets formed from embelin crystals. The tablets were made by mixing magnesium carbonate and lactose and then compressing with a tableting machine.

CHAPTER THREE

RESULTS

3.1 Properties of embelin

The melting point of embelin as determined by Gallen Kamp method was found to be between 138-140 °C A thin layer chromotography of the extracted sample was carried out and its (R_x) value compared to that of embelin standard run on the same plate Results showed that the test sample and embelin moved the same distance on the TLC and the R_X values of the two were calculated and found to be the same (Plate Two).

In order to further investigate the property of the sample, the yellow orange spots on the TLC plate (one for the sample and the other for embelin) were sprayed with ammonia It was observed that both the sample and embelin standard spots turned purple The above observations on the melting points, R_x and the colour change on spraying with ammonia confirmed that the extracted compound was embelin This agrees with the characteristics of embelin extracted from the same plant seeds by Midiwo *et al* (1988, 1993)

3.2 Effect of embelin on plasma testosterone levels when administered intramuscularly

The effect of embelin injected as a corn oil suspension in varied doses of 0 5 mg, 5 0 mg and 30 mg per kg of body weight was investigated The actual changes in hormone levels at various stages of treatment are shown in Fig 3 and Table 2 while the changes from the normal (taken as 100%) during the same period of treatment are shown in Fig 4 The effects shown below were observed -

Effect of vehicle;

In this group of animals which received no embelin but only corn oil as the vehicle there was no significant change observed in the levels of testosterone (Fig 3 and Fig 4). A mean testosterone level of 6 2 ± 0 35 nmol/l was maintained during the three phases

Effect of 0.5 mg/kg body weight dose;

This dose of embelin was observed to have no effect on plasma testosterone in the whole course of treatment and therefore, the mean testosterone levels before, during and after treatment remained relatively constant at 11 7 ± 0 36 nmol/l (Fig 3, Fig 4, Table 2)

Effect of 5 mg/kg body weight dose.

When this dose was administered to the animals a significant drop($P<0.01$) in testosterone from mean control level of 8 32 ± 0 4 nmol l to mean treatment

levels of 3 8 ± 1 5 nmol l was observed This was an average decrease of 54% from the control (Fig 4) On withdrawing treatment the testosterone levels rose back to levels comparable to those before treatment (Fig 3 and Fig 4)

Effect of 30 mg/kg body weight dose:

When this dose of embelin was injected to the animals a similar but more pronounced effect to that obtained with 5 mg/kg body weight dose was observed The mean control testosterone levels dropped significantly($P<0.001$) from 8 7 ± 0 23 nmol/l to mean treatment levels of 3 2 ± 0.31 nmol/l. This represented a 63% decrease on the levels of the hormone. On withdrawing treatment the testosterone levels rose to levels comparable to those before treatment (8.8 ± 1.4 nmol/l).

The results are presented in Table 2, Fig 3 and Fig 4

Table 2

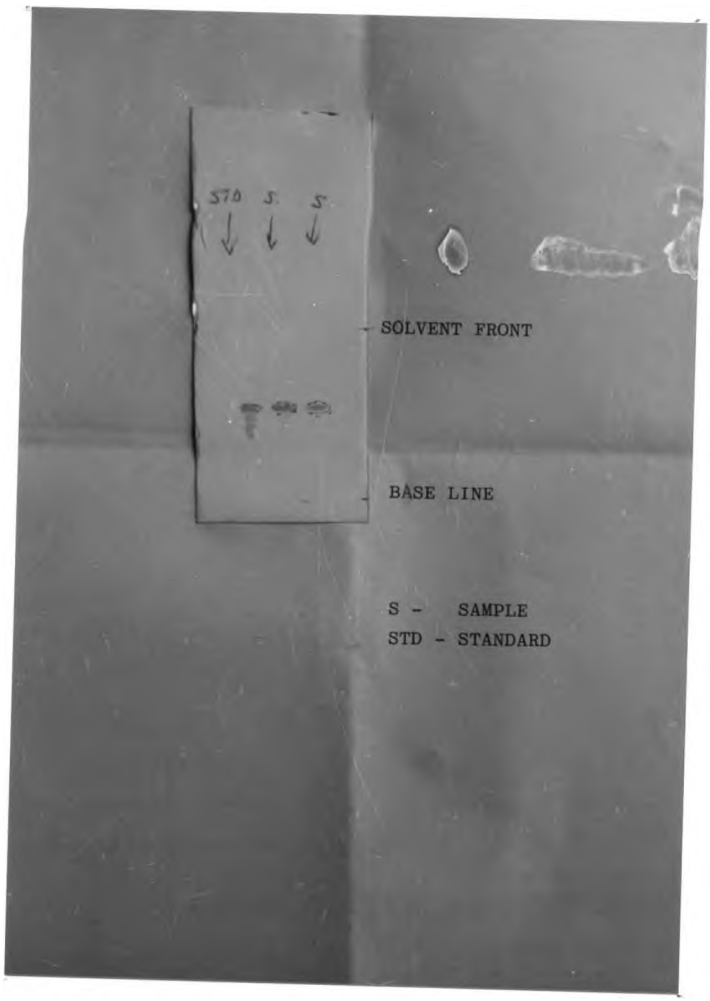
Changes in plasma testosterone levels in male rabbits following intramuscular administration of embelin

Plasma testosterone levels (nmol/l) mean ± SEM	Dose of Embelin in mg/kg body weight			
	0.0(Control)	0.5	5.0	30.0
Before Treatment (CP)	6.2 ± 0.35	11.7 ± 0.36	8.32 ± 0.4	8.7 ± 0.23
During Treatment (TP)	6.2 ± 0.35	11.5 ± 0.5	3.8 ± 0.15*	3.2 ± 0.31**
After Treatment (PTP)	6.2 ± 0.35	11.7 ± 0.36	10.0 ± 2.0	8.8 ± 1.4

The values show mean and SEM for 4 animals in each group. Significance of difference of measurements from the control phase by t-test. *$P<0.01$, **P 0.001.

Plate two

Thin layer chromatogram (TLC) of the yellow-orange crystal (embelin) run against the standard (STD).



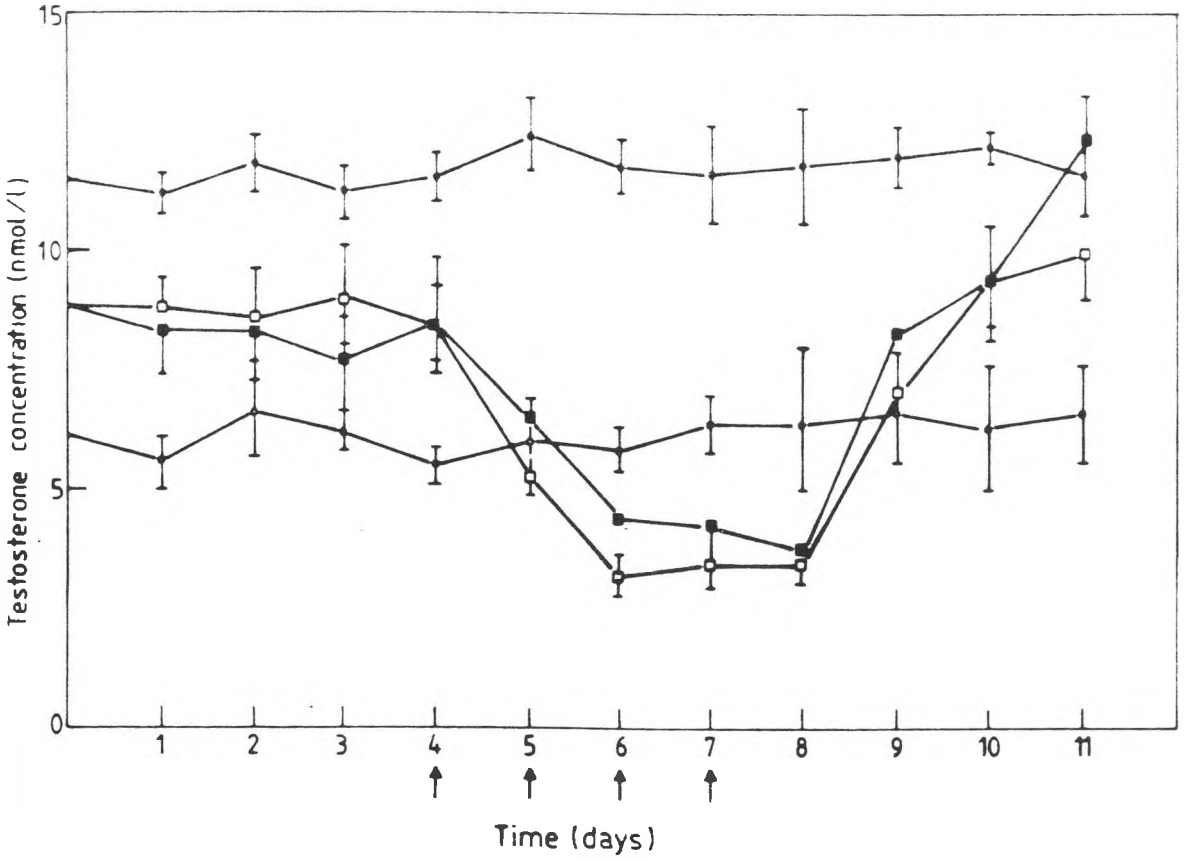


Fig. 3: Effect of embelin on plasma testosterone levels when administered intramuscularly.

○—○—○ Control

●—●—● 0.5 mg/kg of body weight

■—■—■ 5 mg/kg of body weight

□—□—□ 30 mg kg of body weight

The arrows indicate the days when animals received the varied doses of embelin

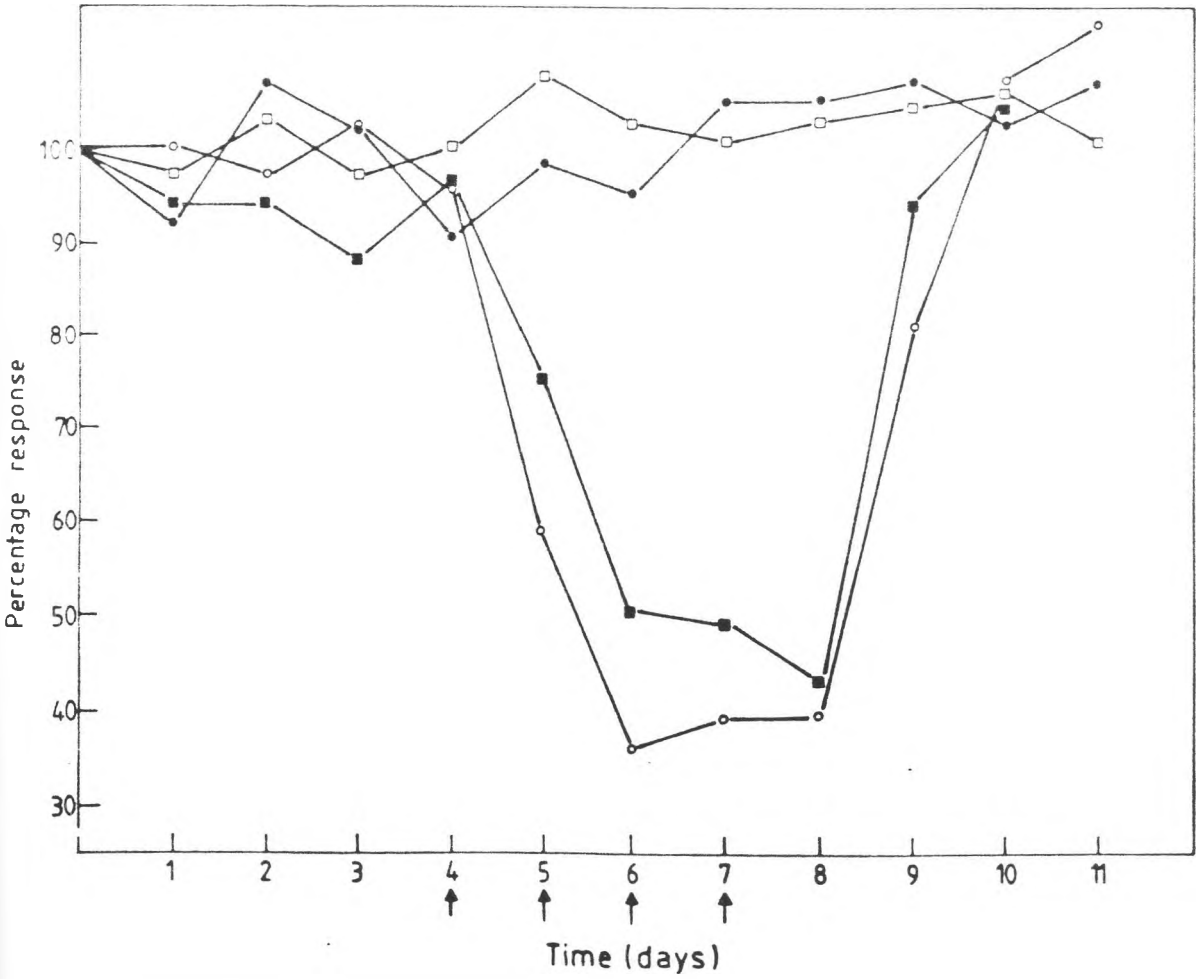


Fig. 4: Changes in Plasma testosterone levels given as percentages following intramuscular administration of embelin.

This was expressed as percent drop in testosterone levels from mean control levels to mean treatment levels.

●—●—● Control dose

□—□—□ 0.5 mg/kg body weight dose

■—■—■ 5 mg/kg body weight dose

○-○-○-○ 30 mg/kg body weight dose

The arrows indicate the days the animals received embelin

3.3 Effects of embelin on plasma testosterone levels when administered orally

The effect of embelin on plasma testosterone levels when administered to the animals in varied doses orally are shown in Fig 5a and 5b and Table 3 whereas variations from normal taken (100%) are shown in Table 4, Fig 6 and Fig 7. The doses administered were as follows 5 mg, 10 mg, 50 mg and 70 mg per kg of body weight as suspension while another 50 mg base was administered in the tablet form

Effect of the vehicle;

The group of animals which were treated with corn oil only also served as the control. It was observed that the mean testosterone concentrations before, during and after treatment remained constant. The values before, during and post-treatment phase were 14.0 ± 1.2 nmol/l, 14.0 ± 0.3 nmol/l and 14.2 ± 0.8 nmol/l respectively. This implied that there was no effect on plasma testosterone levels (PTL) after an oral administration of corn oil to the animals.

Effect of 5 mg/kg body weight oral dose;

When this dose of embelin was administered orally, plasma testosterone levels remained unchanged at the control, treatment and post treatment phases (Fig. 5a, Table 3 and Fig. 6)

Effect of 10 mg/kg body weight oral dose;

On raising the dose of embelin from 5 to 10mg/kg body weight plasma testosterone levels significantly decreased ($P<0.01$) from a pre-treatment mean control level of 12.2 ± 0.70 nmol/l to 4.55 ± 0.35 nmol/l during treatment. This represented a 63% decline in testosterone levels (Fig. 6). On withdrawing treatment the level rose to a mean post-treatment levels almost similar to those

before treatment (11 6 ± 0.72 nmol/l) The results are shown in Fig 5a and Table 3 This would imply that the effect of embelin on testosterone level was reversible

Effect of 50mg/kg body weight oral dose;

This dose of embelin gave a more pronounced but similar effect to that of 10 mg dose The mean plasma testosterone levels dropped significantly (P<0 001) from a mean level of 16 4 ± 0.83 nmol l before treatment to 4 8 ± 1 3nmol l during treatment This was a 71% decline in hormone levels (Table 4 and Fig 6) On withdrawing treatment the testosterone levels rose back to mean post-treatment levels of 15.7 ± 0.8 nmol/l (Fig 5a, Table 3).

Effect of 70 mg/kg body weight oral dose;

A 70 0 mg dose was the highest dose of embelin administered orally. This dosage caused a significant lowering (P<0.001)) of testosterone levels from 13.8 ± 0.8 nmol/l before treatment to 3.7 ± 1.6 nmol/l during treatment (Table 3). This was 73% decline of the hormone levels (Table 4, Fig. 6). On withdrawing treatment the plasma testosterone significantly (P<0.001) rose to levels beyond those observed before treatment phase of 16 1 ± 0 5 nmol/l (Fig 5a, Table 3)

Effect of tablet form of embelin;

The effect of embelin given as a tablet to the rabbits was investigated and the results are shown in Fig 5b and Table 3.

It was observed that embelin caused a significant lowering of plasma testosterone levels when administered in this form However the decrease (40%, Fig 7) was lower than that of the same dose of embelin when administered as a suspension (71%, Fig 6) Moreover the decline due to the tablet form of embelin was gradual (Fig 5b) whereas that of the suspension form was instantenous (Fig 5a)

Table 3: Changes in plasma testosterone levels following oral administeration of embelin

Plasma testosterone levels (nmol l) mean ± SEM	Dose of Embelin in mg/kg body weight					
	0(Control)	5	10	50	Tablet (50mg Base)	70
Before Treatment (CP)	14.0 ± 1.2	20.0 ± 1.65	12.2 ± 0.70	16.4 ± 0.83	5.5 ± 0.65	13.8 ± 0.8
During Treatment (TP)	14.0 ± 0.3	19.2 ± 0.3	4.55 ± 0.35**	4.8 ± 1.3**	3.3 ± 0.2*	3.7 ± 1.6**
After Treatment (PTP)	14.2 ± 0.8	20.6 ± 0.90	11.6 ± 0.72	15.7 ± 0.8	5.0 ± 0.57	16.1 ± 0.5

The values show mean and SEM for 4 animals in each group significance of difference of measurements from the control phase by t-test. *P<0.01. **P<0.001.

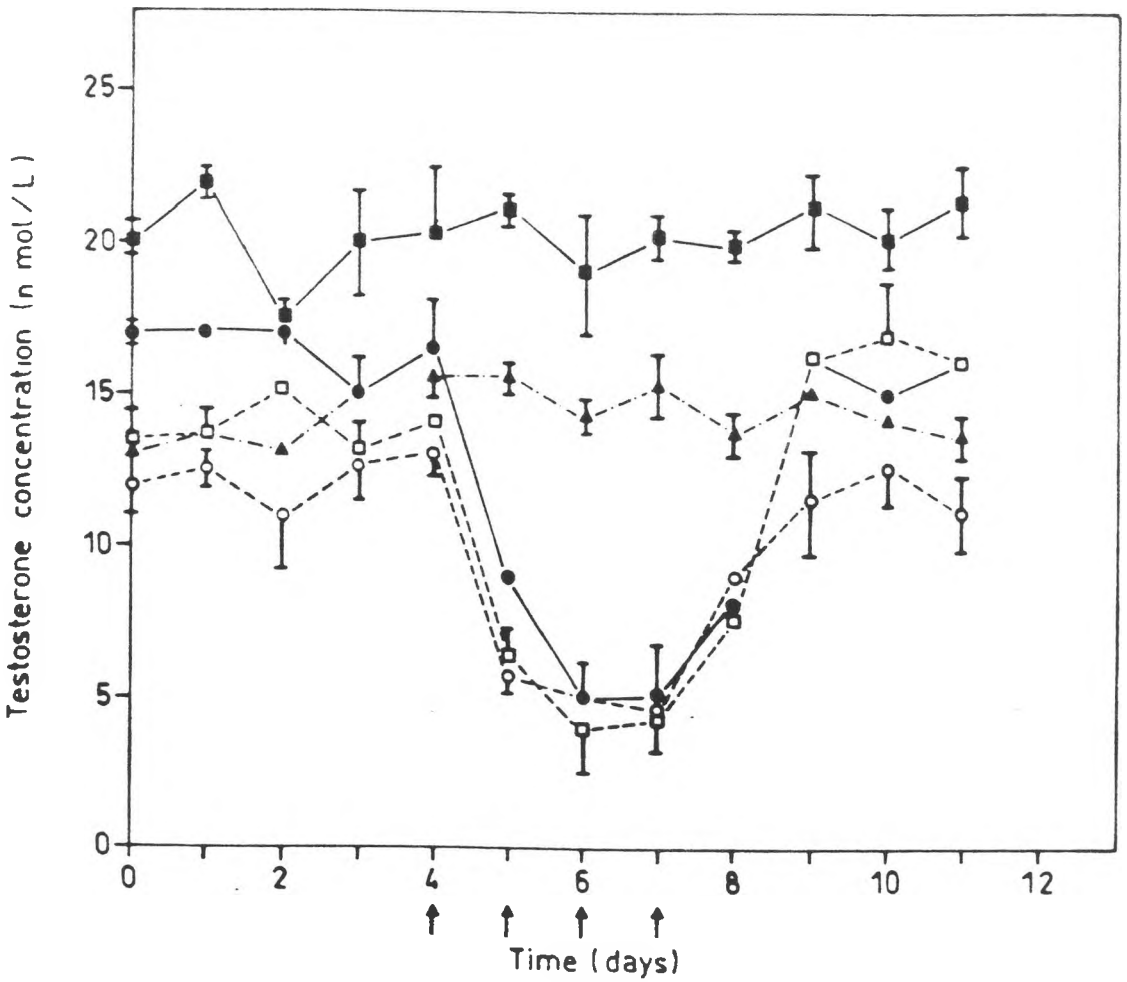


Fig. 5a: The effect of embelin on plasma testosterone levels when administered orally in form of a suspension.

▲---▲---▲	Control
■—■—■	5 m g/kg body weight dose
●—●—●	10 mg/kg body weight dose
○---○---○	50 mg/kg body weight dose
□---□---□	70 mg kg body weight dose

The arrows indicate the days the animals were treated with embelin

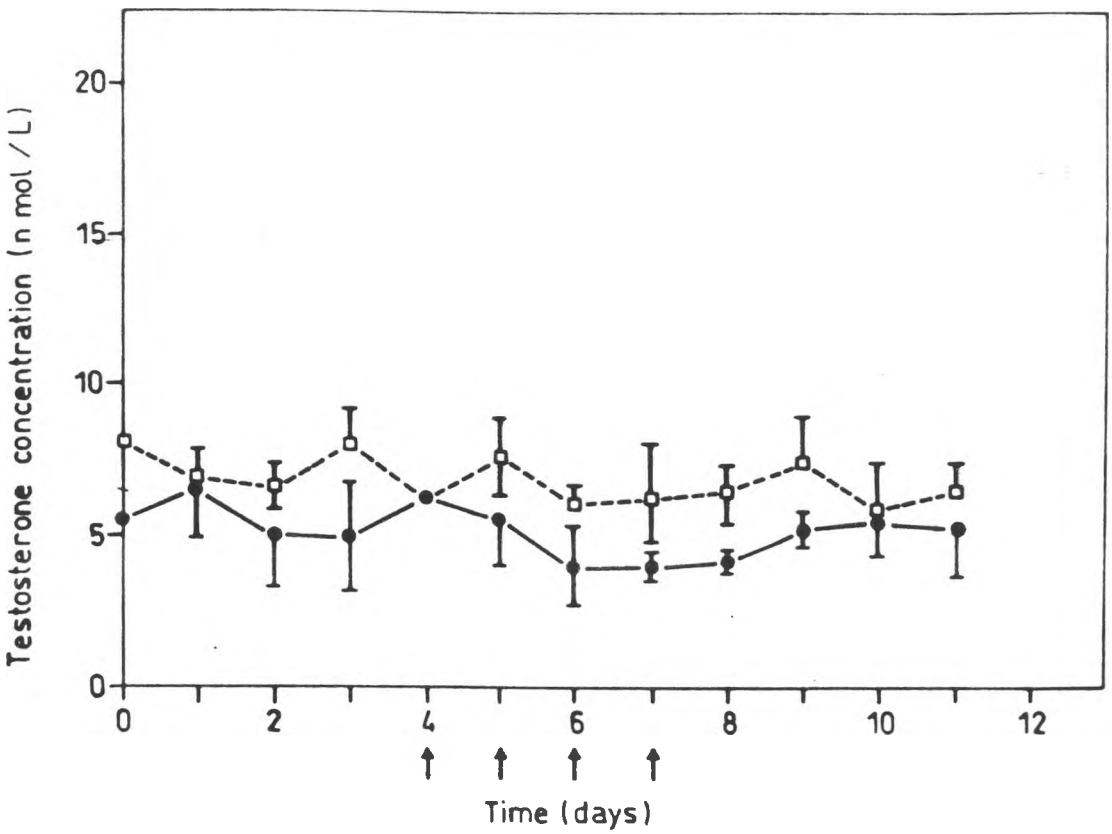


Fig. 5b: The effect of embelin when administered to the animals orally as a 50 mg base tablet.

□--□---□ Control

●—●—● Tablet with 50 mg/kg body weight base

The arrows indicate the days the animals were treated with embelin

Table 4

Changes on plasma testosterone levels given as percentages before and during oral administration of embelin

Dose in mg/kg Body weight	Percent decline in mean plasma testosterone levels
0.0 (CONTROL)	0.0
5.0	4%
10.0	63%
50.0	71%
70.0	73%
TABLET (50 mg base)	40%

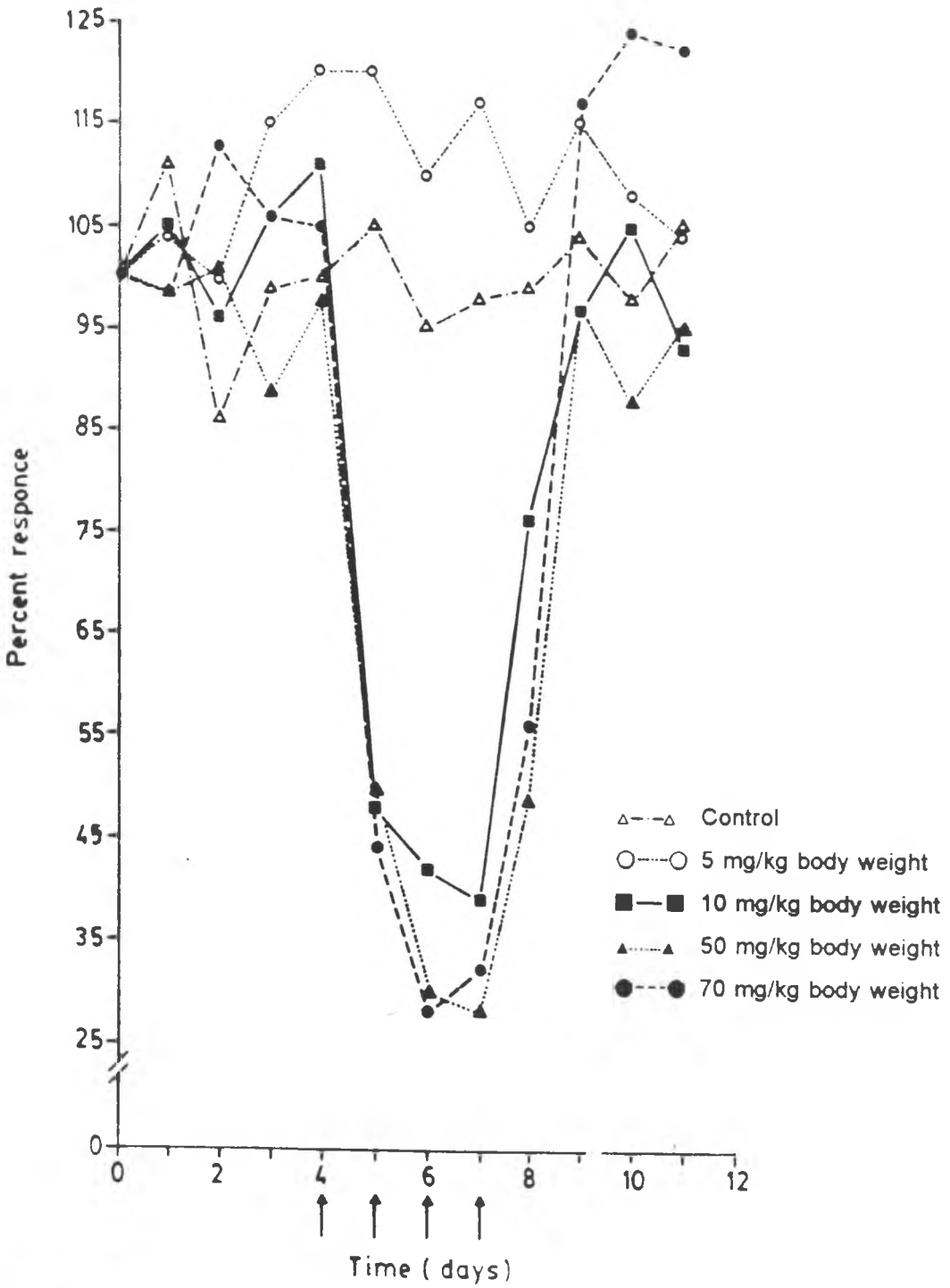


Fig. 6: Percent response of the animals to the oral doses of embelin when administered in form of suspension.

The arrows indicated the days the animals were treated with embelin

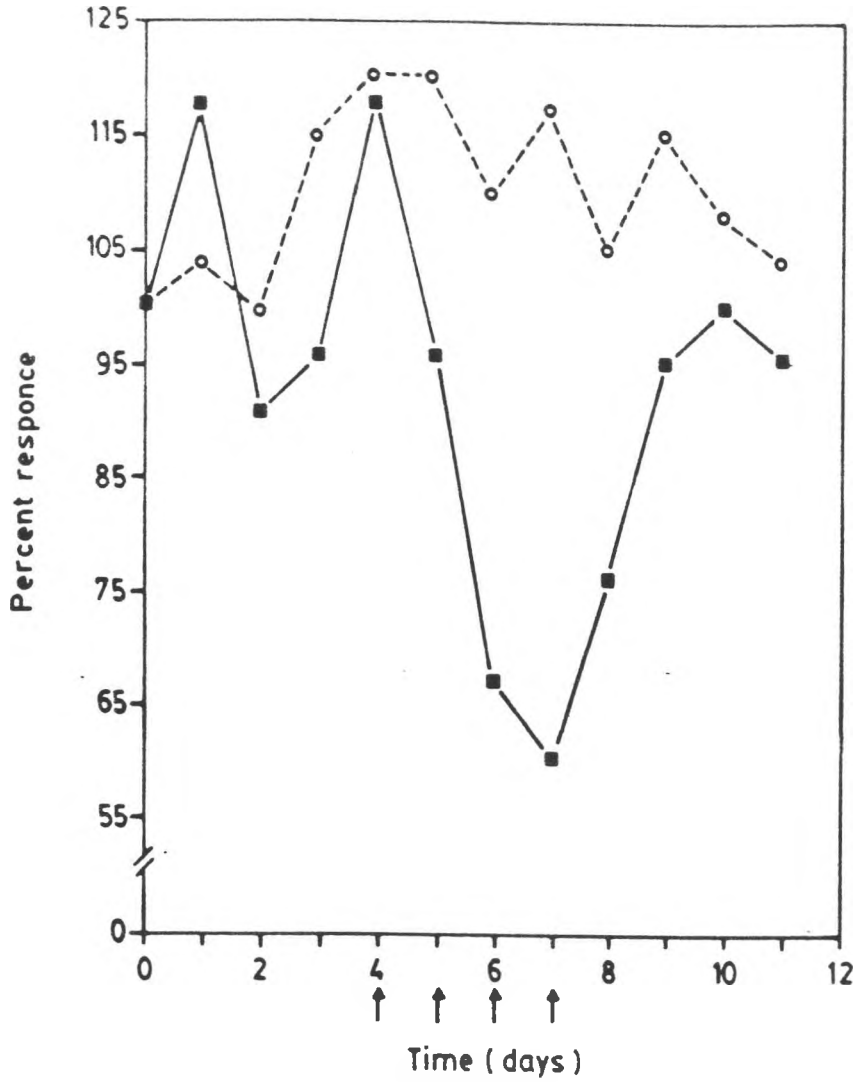


Fig. 7: Percent response of the rabbits to the tablet form of embelin (50 mg base). This was expressed as percent drop in testotestone levels from mean control levels to mean treatment levels.

○---○ Control

■—■ 50 mg base kg body weight (tablet)

Arrows indicate the days when embelin was administered to the animals

3.4 Effects of embelin on plasma testosterone levels when administered subcutaneously

The results on the effect of embelin on plasma testosterone levels when administered in varied doses from 5.0 to 20.0 mg per kg body weight subcutaneously are shown in Fig 8 and the mean plasma testosterone levels in the control, treatment and post-treatment phases for each dose of embelin administered are summarised in Table 5 The variations in testosterone levels from the control (100%) during the three phases are shown in Fig 9 The observations made were as follows -

Effect of corn oil;

In the control animals which had received only corn oil as the vehicle, the testosterone levels did not change but remained constant during the three phases of treatment (Table 5, Fig 8).

Effect of subcutaneous 5 mg/kg body weight dose;

Subcutaneous injection of 5.0 mg/kg body weight dose of embelin had no effect on plasma testosterone levels before, during and after treatment (Fig 8, Fig 9)

Effect of subcutaneous 20 mg/kg body weight dose:

Compared to the 5 mg/kg dose, when a 20.0 mg/kg body weight dose of embelin

was injected subcutaneously, there was no appreciable decrease in plasma testosterone, although there appeared to have been a very slight and gradual response (Fig 8) On withdrawing treatment, the testosterone levels remained significantly lower in the post treatment phase compared to the pretreatment phase (Fig 8, Table 5)

Table 5: Change in plasma testosterone levels following subcutaneous administration of embelin.

Plasma Testosterone levels (nmol/l) mean±SEM	Dose of Embelin in mg/kg body weight		
	0 0(Control)	5 0 mg	20 0mg
Before Treatment	22.2 ± 1.30	19.8 ± 1.9	26 6mg
During Treatment	22.2 ± 1.8	20 ± 0.3	18.9 ± 1.30*
After Treatment	20 6 ± 1.8	19.6 ± 3.0	20 6 ± 0.7

The values show mean and SEM for 4 animals in each group Significance of difference of measurements from the control phase by t-test *P<0.01.

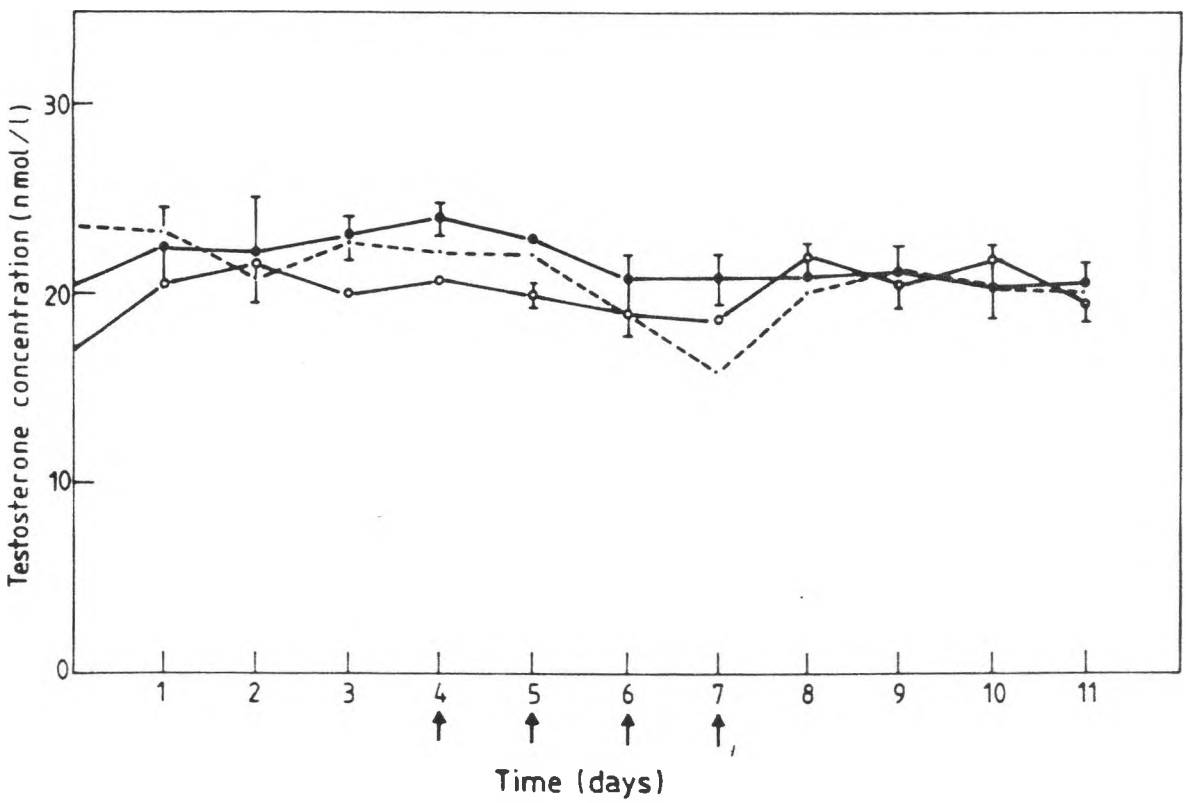


Fig. 8: Effect of embelin on plasma testosterone levels when administered subcutaneously

Results are expressed as variations in testosterone concentration versus time (days). Results are mean values ± SEM of four individual experiments.

●—● Control (vehicle only)

○—○ 5 mg/kg body weight dose

---------- 20 mg/kg body weight dose

Arrows indicate the days when the animals were treated with embelin

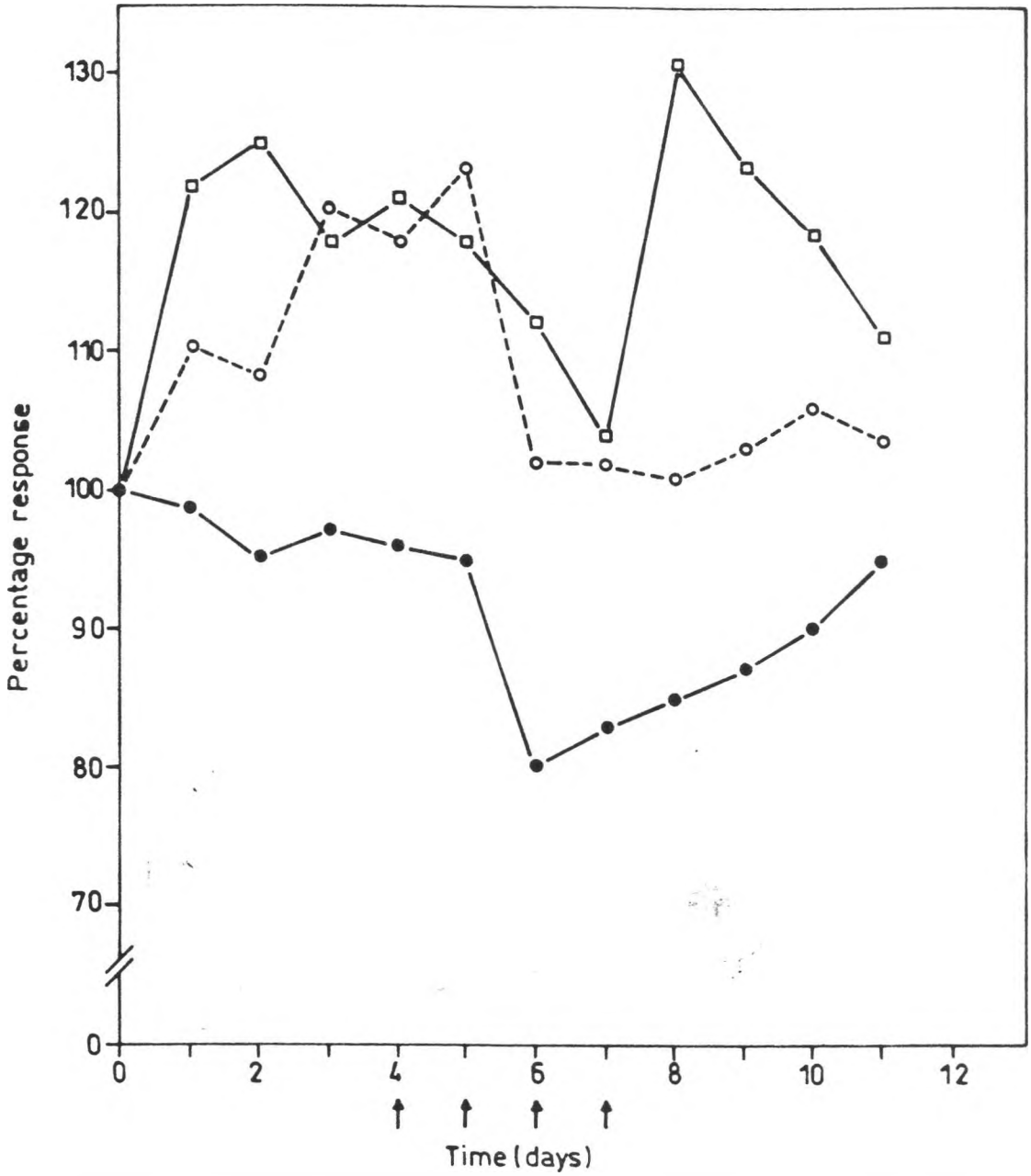


Fig. 9: Percent response of the animals to the different subcutaneous doses of embelin.

This was expressed as percent drop in testosterone levels from mean control levels to mean treatment levels

○----○ Control

□——□ 5 mg kg body weight

●——● 20 mg kg body weight

The arrows indicate the days the rabbits received embelin

CHAPTER FOUR

DISCUSSION

Studies carried out so far indicate that certain plant extracts have profound effect on male fertility. Thus gossypol which was accidently discovered (Abou-Donia, 1975), has been found to lower sperm mortility and therefore lowers the chances of conception (Zhong *et al,* 1990; Qian, 1980; Maugh, 1981). Embelin on the other hand has been reported to have fertility regulating activities on both male and females. In females embelin has been observed to cause an increase in ovarian weight (Prakash and Varshiney, 1980), inhibition of inplantation and resorption of uterus (Bhargava *et al,* 1984; Kholkute *et al,* 1978). Embelin has also been found to lower plasma testosterone levels in male rabbits (Githui, 1990, Gupta and Kanwar, 1991; Makawiti *et al,* 1992) and in male rats (Gupta *et al,* 1989) and in guinea pigs (Prakrash and Varshney, 1980) while powdered berries from *Embelia ribes* causes a reduction in semen volume and sperm motility in Bounet monkey (Jayaraman *et al,* 1971). Since this hormone is fundamental for spermatogenesis, maturation of sperm and for the maintenance of male sexual characteristic (Yoshiaki and Hiroshi, 1984) it is expected that it would interefere with fertility in the male and thus have its effect directly or indirectly on contraception. It has already been pointed out that any such compound would be a great step forward in contraception using the male as the target (Greep, 1976). Results obtained in this study show that administration of embelin intramuscularly, orally and subcutaneously at doses lower than 5 mg/kg body weight had no effect on plasma testosterone levels. This implied that these concentrations of embelin were not enough to cause the expected pharmacological response in the target organ. However, when the doses were increased the plasma testosterone levels decreased unevenly depending on the route of administration of embelin.

The observation that the decrease in plasma testosterone levels increased with the increase of the dose of embelin in all the three routes implied that the effect of the benzoquinone was dose dependent On withdrawing embelin after its administration through either the oral or intramuscular routes the plasma testosterone levels increased to concentrations equivalent to those before treatment, this implied that the effects of embelin were reversible and that it did not induce permanent damage to interstitial cells Similar observations on the lowering of plasma testosterone and recovery on withdrawing treatment were made by Githui (1990) when embelin was injected intramuscularly However, when administered subcutaneously, the lowering of plasma testosterone levels was not clearly noticeable and animals did not appear to recover to pretreatment levels within the experimental period compared to when the drug was administered intramuscularly and orally This may be explained by the possibility that embelin first accumulated in the adipose tissues from where it would be released slowly, the consequences of this would be that even after withdrawing the treatment, this release would still continue though at a lower rate and this may also possibly explain the observed failure to return to full recovery pretreatment levels Similar observations were made by Gupta and Kanwar (1991) when working with the same plant extract using the same route Alternatively, embelin could be eliminated faster when administered subcutaneously than when given in either of the other two routes (oral and intramuscular) This would lead to higher turnover and shorter half-life of the drug (due to metabolism) implying that a relatively higher concentration of embelin would be required to cause an appreciable lowering of plasma testosterone levels The findings were similar to those of Gupta and Kanwar (1991) who demonstrated an elevation of chloresterol in the testis of male rats when embelin was administered either subcutaneously or orally and a return to normal after withdrawing treatment These changes would be expected since synthesis of testosterone from cholesterol is inhibited by embelin at 17, α-hydroxylase step (Makawiti *et al.* 1992) This inhibition would result in the accumulation of cholesterol observed by Gupta and Kanwar (1991) and a

decrease in testosterone levels observed by Githui *et al*, (1991) and also in this study The inhibition of this enzyme is most likely reversible because on withdrawal of embelin, cholesterol and testosterone levels revert to normal (Gupta and Kanwar, 1990, Githui *et al*, 1991). It was noted that when embelin was administered intramuscularly and orally, the decline in plasma testosterone levels and the subsequent recovery were both instantaneous The testosterone levels were also noted to be slightly higher than those before treatment with embelin, this upsurge of the hormone was due to accumulated levels of leutenising hormone in the interstitial cells (Githui *et al*, 1991) This could perhaps be due to the rapid absorption of the benzoquinone especially through the gastrointestinal tract

It was also observed that besides the effect of lowering plasma testosterone levels embelin disrupted the maturation of spermatozoa (Githui, 1990). This implies that **the antifertility effect of embelin is not only through the lowering of testosterone levels but rather a combination of many effects the end result being the** antifertility effect

Conclusion and Recommendations

The study shows that

(i) the lowering of plasma testosterone levels by embelin when administered through the three routes are largely reversible and dose dependent

(ii) since testosterone is an obligatory requirement for male fertility, its lowering would therefore induce male infertility in the embelin treated animals By inference therefore, embelin appears to have a high potential as a male fertility regulating agent (contraceptive)

(iii) assessing embelin as a future male contraceptive it would appear that although slightly higher doses are required to exert antifertility effect when administered orally as opposed to intramuscular route, the former route appears to be the most reasonable for the administration as it would reduce the cost of trained health personnel and equipment and also as dosage convenience to the patients

(iv) in view of the observed lowering of plasma testosterone levels by embelin when administered subcutaneously, it can be inferred that this compound has the potential of being used as a subdermal contraceptive implant.

Further work on how best to formulate embelin preparation at concentrations that would have antifertility effects without interferring with libido (Githui, 1990), are interesting areas requiring future exploration in order to qualify embelin as a choice amongst the contraceptives available in the market today. This would go along way in trying to meet the ever increasing and varied individual requirements in contraception (Isahakia and Bambra, 1992; Waites, 1989). The study would also be a milestone in clearing embelin from the negative aspects of traditional medicine (Anokbonggo, 1992).

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