PROBABLE NEURO SEXUAL MODE OF ACTION OF CASIMIROA EDULIS SEED EXTRACT VERSES SILDENAFIL CITRATE (VIAGRA™) ON MATING BEHAVIOR IN NORMAL MALE RATS

SYED TABREZ ALI AND NABEEH I. RAKKAH
Department of Physiology, Faculty of Medicine
Umm-Al-Qura University, P.O. Box 7607, Makkah, Saudi Arabia

ABSTRACT
The present study deals with the aphrodisiac actions of the aqueous extract of the seeds of the hypotensive plant Casimiroa edulis on the sexual behavior of normal male rats. In this investigation 30 healthy male Wister strain white albino rats showing the brisk sexual activity age 15 weeks, weighing 400-450 grams were included. Female rats were artificially brought into estrous by hormonal treatment. Receptivity was checked by exposing them to the male rats and the most receptive females were selected for the stud. The mating responses including Mounting Frequency (MF), Intromission Frequency (IF), Mounting Latency (ML), Intromission Latency (IL), Ejaculatory Latency in first and second series (EL1 and EL2) and Post Ejaculatory Interval (PEI) were recorded after treating the animals with 250 mg/kg casimiroa edulis extract (test reference) and 5 mg/kg sildenafil citrate (standard reference) respectively orally per day for 7 days. Both the groups exhibited a significant increase in Mounting Frequency, Intromission Frequency, and first and second ejaculatory latencies, whereas Mounting and Intromission latencies and the Post Ejaculatory Interval showed a significant reduction than the controls. Although a similar pattern of mating behavior was observed among the test and the standard groups, however in all the cases as expected, sildenafil produced greater activity than the casimiroa edulis extract. These results suggest the possibility of a similar mode of action of casimiroa edulis and sildenafil citrate on mating behavior in these animals. Our work reported in this research thus provide preliminary evidence that the aqueous seed extract of casimiroa edulis possesses aphrodisiac activity and may be used as an alternative drug therapy to restore sexual functions probably via a neurogenic mode of action.

Keywords: Casimiroa edulis; sildenafil citrate; mating behavior; rat.

INTRODUCTION
In the present study we have tried to investigate the reproductive profiles of the aqueous extracts of the seeds of casimiroa-edulis Lia Llave et Lex, the "Zapote blano" (white Zapote) a tropical plant used by Mexican herb medicine since remote times (Power et al., 1911) as the "sleep-producer-fruit" when infusions of leaves or seeds were administered.

This pioneer phytochemical work was later followed by studies on casimiroa edulis (Kinkle and Romo, 1956; Merzels et al., 1957, Djerassi et al., 1958; Lozoya et al., 1978; Garcia et al., 1994), trying to corroborate the hypnotic properties attributed to this sweet fruit.

A number of chemical investigations of the seeds of casimiroa edulis have been reported causing a lowering of blood pressure in animals suggesting that pharmacological activity of casimiroa edulis seeds may be attributable to the presence of Nα, Nα dimethylhistamine (Major, 1968, Murphy, 1968).

In an initial report (Lozoya et al., 1977) have confirmed the vigorous hypotensive effect produced by the aqueous and alcoholic extract from casimiroa edulis seeds on cats, rabbits and dogs, together with the constrictor effect produced on the uterus by in vitro experiments on several animal species including human. More recently, the hypotensive principle has been isolated and its structure elucidated, corroborating the original and consistent use of this plant (Ortega, et al., 1978). The main use among the population is always related to cardiac or cardiovascular diseases (Magos et al., 1999).

From the literature cited above, it is now clear that the seeds and leaves of the plant casimiroa edulis have been used in Folk medicine as an hypotonic and sedative and more recently as an antihypertensive.

This compound illicit effect, including hypotension, indistinguishable from those of histamine (Lozoya et al., 1978; Garcia et al., 1994). The fall in blood pressure induced by histamine is characteristically transient (Gil et al., 1995; Godlewski et al., 1997), where as that caused by casimiroa edulis is remarkably long lasting (Lozoya et al., 1978; Garcia et al., 1994; Gil et al., 1995; Baisch et al., 2004; Maiti et al., 2007), and could thus be attributed instead of any of the various imidazoles derivatives found in...
the plant. The pharmacology of these constituents has not been determined in detail, but their histamine-like activity is not improbable, in view of their structural similarity with the biogenic amine.

Similar to the hypotensive nature of casimiroa edulis, is the compound sildenafil citrate (Viagra) a selective vasodilator that prolongs the action of cyclic guanosine monophosphate (cGMP), the primary mediator of vasodilation in the corpus cavernosum of penis by selectively inhibiting cGMP-specific phosphodiesterase type-5 (PDE5) facilitating smooth muscle relaxation and the flow of blood (Goldstein, 1998). These effects occur to some extent in other portions of the cardiovascular system as well (Weishaar, 1987).

In an unpublished preliminary study, we noted a marked relaxation of the rat corpus cavernosa necessary for penile erection and sexual stimulation at the standardized dose of 250 mg/kg of the seed extract of casimiroa edulis. Similar effects have been reported in the literature with sildenafil citrate as well (Moraled et al., 1998).

Keeping in view the similarity between the mode of action of both the compounds as a potent peripheral vasodilator and as a positive inotropic agent both in vivo and vitro, present study has been designed to determine the mechanisms involved on the sexual responses of the aqueous extract of the seeds, the part of the plant. The pharmacology of these constituents has not been determined in detail, but their histamine-like activity is not improbable, in view of their structural similarity with the biogenic amine.

**Materials and Methods**

**Preparation of the Casimiroa edulis extract**
The aqueous extract of casimiroa edulis seeds was prepared as described previously (Gil and Horacio, 1991; Baish et al., 2004). Briefly, ripe fruits of casimiroa edulis were obtained and after being deflated, the dry powdered kernels were extracted successfully by maceration at room temperature with hexane, dichloromethane-methanol, methanol and finally water. Organic solvents were eliminated in a rotatory evaporator; water was removed by lyophilization. For the pharmacological tests, the solid aqueous extract thus obtained (approximately yield: 5.5%) was dissolved in isotonic NaCl solution to a concentration of 100 mg/ml.

**Aphrodisiac studies**
Wister strain white albino male and female rats, age 15 weeks, weighing 400-450 grams and 250-300 grams respectively, were obtained from the institutional animal house facility and were hosted singly in separate standard cages at standard laboratory environment (22-24°C room temperature, 60-70% humidity, 12 hour light-dark cycle, free access to solid pellet diet).

Female rats were artificially brought into estrous by administrating oral suspension of ethyl estradiol (Schering-Germany) 48 hours before the mating by the established methods (Szechtm an et al., 1981). An additional 1 mg/ml subcutaneous injection of progesterone (Schering-Germany) was given to all female rats 6 hours before the mating. Receptivity was checked by exposing them to the male rats and the respective females were selected for the study. The recipient female rats were introduced into the male cages of all 3 groups and the mating responses including mounting frequency (MF), intromission latency (IL), ejaculatory latency in first and second series (EL1 and EL2) and post ejaculatory interval (PEI) were recorded by established methods (Dewsbury et al., 1970; Rutna et al., 2000). Statistical analysis for all the parameters was done using one-way analysis of variance (ANOVA) method.

**Results**
The data for the mounting frequency (MF) after the oral administration of 250 mg/kg. casimiroa edulis extract and 5 mg/kg. sildenafil citrate dose in 10 male rats and in 10 age-matched controls is shown in (fig. 1). The values of the mounting frequency obtained by the casimiroa edulis extract/sildenafil citrate treated rats compared with the values obtained by the control group showed a highly significant increase (p<0.0001). A significant increase...
was again noted in intromission frequency in both the casimiroa edulis extract (p<0.001) and sildenafil citrate (p<0.0001) groups compared with the control group (fig. 2). Similarly values for the ejaculatory latency tests both in first and second series further indicated a highly significant increase (p<0.0001) in the casimiroa edulis/sildenafil treated groups compared with the controls (figs. 3 and 4 respectively).

**Fig. 1**: Effect of the seed extract of casimiroa edulis and sildenafil citrate on mounting frequency in normal male rats. Values are Mean ± SE (n=10).

*Note: n = Total number of the rats examined. Casimiroa edulis/sildenafil citrate treated values are compared with age matched control rats.

* p<0.0001

**Fig. 2**: Effect of the seed extract of casimiroa edulis and sildenafil citrate on intromission frequency in normal male rats. Values are Mean ± SE (n=10).

*Note: n = Total number of the rats examined. Casimiroa edulis/sildenafil citrate treated values are compared with age matched control rats.

* p<0.0001, ** p<0.001

In contrast an inverse relationship was noted when the mounting latency, intromission latency, and post-ejaculatory interval were studied in the casimiroa edulis and sildenafil citrate treated rats (figs. 5, 6 and 7 respectively). In comparison with control group, the test group (casimiroa edulis) showed a significant decrease in mounting latency and intromission latency (p<0.001), whereas this decrease was found to be highly significant in the standard group (sildenafil citrate) (p<0.0001). In a similar manner, values of the post ejaculatory interval

**Fig. 3**: Effect of the seed extract of casimiroa edulis and sildenafil citrate on ejaculatory latency in first series in normal male rats. Values are Mean ± SE (n=10).

*Note: n = Total number of the rats examined. Casimiroa edulis/sildenafil citrate treated values are compared with age matched control rats.

* p<0.0001

**Fig. 4**: Effect of the seed extract of casimiroa edulis and sildenafil citrate on ejaculatory latency in second series in normal male rats. Values are Mean ± SE (n=10).

*Note: n = Total number of the rats examined. Casimiroa edulis/sildenafil citrate treated values are compared with age matched control rats.

* p<0.0001
showed a highly significant decrease (p<0.0001) both in the test as well as in the standard group, when compared with the controls.

Fig. 5: Effect of the seed extract of casimiroa edulis and sildenafil citrate on mounting latency in normal male rats. Values are Mean ± SE (n=10).
Note: n = Total number of the rats examined. Casimiroa edulis/sildenafil citrate treated values are compared with age matched control rats.
*p<0.0001, **p<0.001

Although a similar pattern of mating behavior was observed between the test and the standard group of animals, however in all the cases as expected, sildenafil produced greater activity than the casimiroa edulis extract. These results suggest the possibility of a similar mode of action of casimiroa edulis and sildenafil citrate on mating behavior in these rats.

Fig. 6: Effect of the seed extract of casimiroa edulis and sildenafil citrate on intromission latency in normal male rats. Values: Mean ± SE (n=10).
Note: n = Total number of the rats examined. Casimiroa edulis/sildenafil citrate treated values are compared with age matched control rats.
*p<0.0001, **p<0.01

Our results showed that 250 mg/kg aqueous seed extract of casimiroa edulis produced significant enhancing sexual activity in male rats as noted by their mating behavior tests.

In all the experiments, a significant increase was observed in mounting and intromission frequency as well as ejaculatory latencies both in the first and second series in both the test and standard groups when compared with the controls of the same aged rats, probably resulting due to an increase in potency and libido, an effect exerted by the casimiroa edulis extract comparable with the standard drug (sildenafil citrate) as expected with much greater tendency.

On the other hand, a significant reduction was noted in the mounting and intromission latencies in both the test and
standard groups than the controls, again indicating a significant progression in the sexual behavior of these animals, an effect exerted by the test as well as the standard drug. Post ejaculatory interval observations revealed the similar results.

Since post ejaculatory interval generally indicates the rate of recovery from exhaustion after first series of mating, as well as an index of sexual drive, a significant decrease in this parameter in our test and standard experiments indicates lesser exhaustion in the first series of mating, a sign of increased frequency of sex drive in these animals. These results are in conformity with the previous findings (Ottani et al., 2002, Pattij et al., 2005).

Effect of the hydro alcoholic extract of casimiroa edulis on nervous system activity in rats and mice has been reported previously (Mora et al., 2005). Oral administration of the casimiroa edulis extract in mice showed prolongation of pentobarbital-induced hypnosis, increased exploration of elevated plus-maze (EMP) open arms and partially protected from the pentylenetetrazol-induced convulsions. These results provide evidence that hydro alcoholic extract of casimiroa edulis may contain sedative principles of anxiolytic and antidepressant properties affecting the nervous system activity. Further evidence for a neurogenic effect of casimiroa edulis comes from the anticonvulsive studies of the aqueous extract of the seeds and leaves of this compound in rats. Garzon et al. (1999) noted a maximum protection of 70% at the 2 and 4th hour with 100 mg/kg. casimiroa edulis seed extract in pentylenetetrazole induced seizure in rat. Similar observations have been made in a previous study by using the leave extract of this compound (Navarro et al., 1995).

Sildenafil enhanced neurogenesis in young rats has been reported by augmenting neurogenesis in aged rats after focal cerebral ischemia (Zhanq et al., 2006). In these experiment treatments with sildenafil citrate, improved functional recovery thus suggesting that inhibition of PDE5 activity by sildenafil augments neurogenesis in the sub ventricular zone of the aged ischemic rats although these rats had reduced number of neural progenitor and stem cells in sub ventricular zone. Casimiroa edulis has also been reported to have its hypotensive and vaso relaxing activity through the activation of adrenergic mechanism via mediating nitric oxide release (Baisch et al., 2004).

Significant improvement in sexual activity in our study may be interpreted as an effect of casimiroa edulis that stimulates the neural mechanism. The extract may induce changes at the neurotransmitter levels or at the neuronal levels in a similar manner like sildenafil citrate by enhancing the relaxant effects of nitric oxide in response to sexual stimulation. Casimiroa may promotes penile erection in these rats by blocking the activity of type 5-PDE, which causes cGMP to accumulate in the corpus cavernosum of the penis leading to an increased sexual drive. Similar suggestions have been made previously with other compounds (Suresh et al., 2000).

Our work reported in this research thus provide preliminary evidence that the aqueous seed extract of casimiroa edulis possesses aphrodisiac activity and may be used as an alternative drug therapy to restore sexual functions probably via a neurogenic mode of action. Further studies are however recommended to determine the exact dose and the mode of action of this compound to enhance the sexual performance in both vivo and vitro.

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