In Vivo Anti-Fertility and Anti-Implantation Activity of Anacardic Acid Methyl Ester in Rats

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Summary

The practice of traditional medicine for fertility control in india is based on the use of plant as wall as chemical medicine for thousands of years. The aim of the present study is to evaluate the effect of anacardic acid methyl ester for fertility control study. This has been studied in rats to explore its anti-fertility activity and anti-implantation in vivo activity of the anacardic acid methyl ester in presence of ethanol were found to be 18%. The record of body weight maintained for 19 days starting from day 1 of pregnancy. This indicates that all rats of the control groups showed considerable weight gain over the period of 19 days. The weight gained by the rats and number of implants by treatment of anacardic acid methyl ester in presence of ethanol significantly. The results of this study suggest that the anacardic acid methyl ester in presence of ethanol possess hormonal properties that can modulate the reproductive function of the rats.

Keywords: anacardic acid methyl ester; ethanol*;* Anti-fertility; Anti-implantation;

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Introduction

The quest for the oral contraceptive agent that can control human fertility is as old as recorded history. Although a wide variety of synthetic contraceptive agents[20,22] are available, these cannot be used continuously among those anacardic acid methyl ester in presence of ethanol with claimed anti-fertility properties in medicine. In addition, an *in* vivo test showed that *induced* uterine contraction in rats (Calixto et al., 1991). The objective of the present work was to investigate the antifertility and antimplantation effect of the hormonal properties that can modulate the reproductive function of the rats.[6,9]

Materials and Methods

Chemicals
Sulfated zirconia (SZ), anacardic acid, Anacardic acid methyl ester.

Synthesis of anacardic acid methyl ester (AAME):

Sulfated zirconia (SZ), a solid acid with acid strength comparable to sulfuric acid, has been used for anacardic acid esterification. It is one of the most promising solid acid catalysts for esterification. Under the given right reaction conditions, SZ may be a suitable catalyst for the synthesis of anacardic acid methyl ester (AAME) up to 100% concentrations of anacardic acid by catalytic esterification . Good (AAME) conversion (80%) was obtained after 2 h of reaction using SZ (10 wt% based on anacardic acid content; 12 : 1 methanol : anacardic acid molar ratio) at 120°C. Higher reaction temperatures resulted in better yield. However, the elevated increase in methanol vapor pressure with reaction temperature becomes a deterrent for the use of temperatures above 150°C. Under such reaction conditions the esterification was found to be faster.

Experimental Animals

Animals were housed for present studies were newly borne female rats with 10 to 14 weeks of age. Which were ready for the first mating were used for study. The newly borne rats were separated from their mothers at their weaning age, and females were separated from males at the age of four weeks in order to prevent uncontrolled mating. Male rats were used for the test after checking their proven fertility[3] through a preliminary mating system with selected rats[8]. They all fed on commercial Amrut pellet diet and watered *ad libitum*

In vivo anti-fertility and anti-implantation test
The method described by Wiliamson et al (1996) 15 was employed for the experiment Virgin female rats of age between 10 and 11 weeks weighing between 175 and 200g were divided into control and experimental groups of 5 rats/group. One group was used for the anacardic acid methyl ester in presence of oil and the other for the anacardic acid methyl ester in presence of ethanol. The control groups were named as Co for the anacardic acid methyl ester in presence of oil and Ce for the anacardic acid methyl ester in presence of ethanol groups respectively. Each rat was kept singly in a cage to acclimatize without dosing for eight days. The experimental groups were treated with the above prepared sample orally at a dose of 300mg/kg-body weight/rat, while the control groups treated the anacardic acid methyl ester in presence of ethanol (70%) v/v in 0.5 ml volume. The anacardic acid methyl ester in presence of oil, while the anacardic acid methyl ester in presence of ethanol in 70 % v/v ethanol and oil at a ratio of 1:1.5. Males of proven breeding ability were introduced into each cage on the ninth day of treatment. Then vaginal smear and/or vaginal plug were checked each morning to examine the existence of sperm in order to confirm mating. The first morning mating observed was considered day-1 of pregnancy according to the methods employed by Desta (1994) [23] and Uguru et al. (1995)[13] The treatment of animals continued until day-19 of pregnancy, and the weights of the animals were taken daily. The control and

experimental rats were sacrificed on day-20 to determine the number of implants. The weights of the animals and numbers of implants were compared with those of controls. The experiment was carried out twice for each sample anacardic acid methyl ester in presence of ethanol. The anti-implantation[11] and anti-fertility[5] activities of each sample were calculated using the following formula (Williamson et al., 1996).

$$\text{Anti-implantation Activity} = \frac{\text{No of implants in control} - \text{№ of implants in test group}}{\text{No of implants in control group}} \times 100$$

$$\text{Anti-implantation Activity} = \frac{\text{No of animals showing no implantation}}{\text{Total No of animals}} \times 100$$

Statistical analysis

For the in vivo anti-implantation test, the mean ± SEM weight gain as well as the mean ± SEM number of implants in each test group was compared with the respective control groups.

Results

In vivo anti-implantation/anti-fertility activities

Table 1 summarizes the number of rats with implantation and the total number of implants in each group. It shows the percentage anti-implantation and anti-fertility activities exhibited by each sample in comparison to the corresponding controls. It was noted that some of the rats in test groups did not show implantation. As shown in Table 1, the highest rate of anti-implantation activity of AAMEO was 81.3% while the anti-implantation and anti-fertility activities of AAMEE sample were 37% and 20% respectively.

Table 1: The percentage anti-implantation and anti-fertility activities of each sample given to the corresponding "n'' number of rats at a dose level of 300mg / kg / rat as calculated after laparotomy made on day 20 of pregnancy.

Sample Groups	n	No of rats Showing implantation	Total No of Implants	Activity (%) Anti-implantation	Anti-fertility
C^o	10	10	96	0	0
AAMEE	10	08	48	50	20
AAMEO	10	04	18	81.3	60
C^e	10	10	96	0	0
AAMEE	10	08	58	37	20

o = controls for the oil group e = controls for the ethanol group

The body weight recorded for 19 days starting from day 1 of pregnancy showed that all rats of the control groups have considerable weight gains. The body weight gained by rat in most test groups showed a significant difference ($P < 0.05$) with their corresponding controls (Table 2). The weight gained by the rat groups LRW ($t = - 4.624$, $P = 0.002$) was significantly less than the controls. However, when compared with the controls, LLW and LLE treated rats there was no significant difference in their weight gains ($t = - 2.238$, $P = 0.056$ and $t = - 0.968$, $P = 0.361$ respectively).

Table 2: Average rat at a dose level of 300-mg/kg g rols. weight gained by rats treated with each given orally for 19 days as compared to control

Sample Groups	n	Weight gain in g (Mean ± SE)	P- value	95% CI of the mean Lower	Upper
AAMEE	10	60.9 ± 8.8	0.056	-40.21	0.61
AAMEO	10	38.4 ± 9.1	0.002	-63.48	-21.24
C^o	10	80.7 ± 1.2			
AAMEE	10	68.3 ± 10.5	0.361	-36.73	15.01
C^e	10	79.2 ± 4.1			

o = control for oil group e = control for ethanol group

Table 3: Average number of implants counted in those rats showing implantation upon laparotomy made on day 20 of pregnancy both in the control and treated groups.

Group	n	№ of rats showing implantation	№ of implants (Mean ± SE)	P- value	95% CI of the mean Lower	Upper
AAMEE	10	08	4.8 ± 1.2	0.007	-7.89	-1.71
AAMEO	10	04	1.8 ± 1.2	0.000	-10.81	-4.79
C^o	10	10	9.6 ± 0.5			
AAMEE	10	08	5.8 ± 1.5	0.070	-6.73	0.33
C^e	10	10	9.0 ± 0.3			

o = control for oil group e = control for ethanol group

The number of implants in the LRW treated rats was significantly less (t = - 5.982 and P 0.000) than the average number of implants in the control group. The Rats treated with LLW (t = -3.578, P = 0.007), showed significant differences with their respective control group. No significant difference was, however, observed between the average number of implants in rats treated with LLE (t = - 2. 09, P = 0.070) and the control group.

Discussion

The results demonstrated that anacardic acid methyl ester in presence of ethanol *has* anti-fertility and anti-implantation activities. The anti-implantation activity observed by LRW might indicate the presence of one active ingredient in the anacardic acid methyl ester in presence of ethanol.

Their estrogenic nature (Waller 1987, Debella et al. 1999) although it is not conclusive, the absence of implantation sites in some group of rats in the present study might be due to the effect of the anacardic acid methyl ester in presence of ethanol. It is possible that anacardic acid methyl ester in presence of ethanol can produce anti-fertility effect through their ability to alter the estrus cycle of the animal (Makonnen et al., 1997). Therefore, the number of rats showing no implantation in the present study might be due to a prolonged diestrus phase, which gives no chance for fertilization.

The anacardic acid methyl ester in presence of ethanol exhibited significantly greater uterine contractions than the anacardic acid methyl ester in presence of oil. Those anacardic acid methyl ester in presence of ethanol that showed anti-implantation activities in vivo. However there was no sign of abortion such as vaginal bleeding or weight loss observed during the in vivo test. This might be because (on one hand) the anacardic acid methyl ester in presence of ethanol concentration used for in vivo test was too low to induce abortion or on the other hand the uterine contracting effect of the anacardic acid methyl ester in presence of ethanol. could be due to other pharmacological mechanisms that were not sufficient to cause abortion. The anacardic acid methyl ester in presence of ethanol mechanism(s), of anti-implantation or uterine contraction observed in this study requires further investigations. Moreover, fractionation of the anacardic acid methyl ester in presence of ethanol. is a worthwhile anacardic acid methyl ester isolate the active compounds for the observed activity.

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