PHARMACOLOGICAL STUDIES ON MELIA AZADIRACHTA

[N. O. MELIACEAE]

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Part II.—Estrogenic and Antipyretic activity of Neem oil and its Fractions.

INTRODUCTION

The therapeutic uses claimed for neem in the indigenous system of medicine cover a wide field. It has been considered to be useful as an emmennogogue and a febrifuge[1]. It is well known that many plant products do possess estrogenic activity[2] and antipyretic action[3]. It may be recalled that the bitter glucoside, salicin obtained from the willow-bark possesses antipyretic action and formed the basis for the isolation of salicylic acid, which was later isolated from the oil of gaultheria also[4]. Since the testing of crude extracts only may lead to erroneous conclusions due to the absence of sufficient concentration of the active ingredients in the material, in evaluating the estrogenic and antipyretic activity not only the neem oil but also the bitter principles, nimbidin and nimbidol have been examined and the results are communicated in this paper.

MATERIALS AND METHODS

The method of preparation of the bitter principles–nimbidin and nimbidol —has been described earlier[5].

Estrogenic activity. Modified Allen and Doisy's vaginal smear method[6] was adopted to evaluate the estrogenic activity of the oil in rats. Female adult rats were ovariectomized and vaginal smears taken daily for a period of 2 weeks, following ovariectomy and animals that did not show negative smears for cornified cells were discarded. A group of 5 animals was used to estimate the activity of each fraction. Each animal in Group 1 was given estradiol dipropionate 0.5 γ in olive oil. Each rat in groups 2 and 5 received 0.5 cc. of neem oil and fatty ballast respectively. Rats in groups 3 and 4 were given 5 mg. of each of nimbidin and nimbidol respectively in olive oil. Actually nimbidin and nimbidol were dissolved in a little alcohol and the alcoholic solution added to the olive oil and then the alcohol was removed by evaporation. All the drugs were injected subcutaneously only once. From next day

onwards vaginal smears were taken for all the animals and observed for the cellular changes in the vaginal epithelium. Complete absence of leucocytes and appearance of a large number of cornified cells were taken as the criteria for the estrogenic activity.

Antipyretic activity: The method adopted was essentially that of Smith and Hambourger[7] with some of the modifications of Maren[8]. In order to maintain the pyrexia for a longer duration of about 48 hours the concentration of the yeast had to be increased to 20%. It was considered better to take the rectal temperatures at intervals of 2—3 hours instead of hourly intervals and also for a longer period upto about 40 hours instead of stopping the experiment after about 20 hours. Adult male albino rats weighing from 100 to 200 gm. were divided into groups of 5 each. One group was kept as an unfevered control and was not given any pyrogenic injection or drug. Another group received only the pyrogen (dried yeast) but no drug and thus served as the fevered control group. Rest of the groups received the pyrogen and also the drug. Except one group which served as unfevered control group all the other groups were fevered by injecting subcutaneously at 5.0 P. M. with a 20% suspension of dried yeast in 2% acacia saline in tap water at a dosage of 1 ml/100 gm. body weight of the animal. The unfevered controls were injected with 1 cc/100 gm. body weight of 2% acacia saline only. Next day at 12.0 noon, i.e. after 19 hours, the unfevered controls and the fevered controls were given 2% acacia saline while the other groups were administered the respective drugs orally as suspensions in 2% acacia saline or as solutions in water. One of the groups was given a standard drug of known antipyretic activity, viz. acetanilide. Rectal temperatures were recorded with an ordinary clinical thermometer inserted two inches deep into the rectum for one minute at the time of yeast injection, after production of pyrexia and at suitable intervals after the administration of the drugs. Animals had free access to food and water at all times.

RESULTS

Estrogenic activity : Response of the vaginal epithelium of ovariectomized rats to neem oil and its fractions is shown in Table I. Estradiol dipropionate was the standard drug used. The results indicate that neither the oil nor the fractions possess any estrogenic activity in the doses tried.

TABLE I.

Response of the vaginal epithelium of ovariectomized rats to neem oil and its fracti

Drug administered	dose	No. of rats per group	Cellular reaction after.....				
			Type of cells.	0	24	70	78
Estradiol dipropionate	0.5 *v*	5	L	2+	2+	+	-
			E	vf	vf	+	2+
			C	-	-	f	f
Neem oil	0.5 cc	5	L	2+	2+	2+	2+
			E	vf	vf	vf	vf
			C	-	-	vf	vf
Nimbidin	5.0 mg.	5	L	2+	2+	2+	2+
			E	f	f	f	f
			C	vf	vf	vf	vf
Nimbidol	5.0 mg.	5	L	2+	2+	2+	2+
			E	vf	vf	vf	f
			C	vf	vf	vf	f
Fatty Ballast	0.5 cc	5	L	2+	2+	2+	2+
			E	vf	vf	vf	vf
			C	-	-	-	-

L = Leucocytes; E=nucleated epithelial cells, C=Cornified cells; - = absence; vf=v
about less than 10% of the total cells; f=few comprising about 10 - 25% of the total
+ = present to the extent of about 25 - 50% of the total cells.

TABLE II

Effect of neem oil and its fractions on the pyrexia produced by yeast in rats

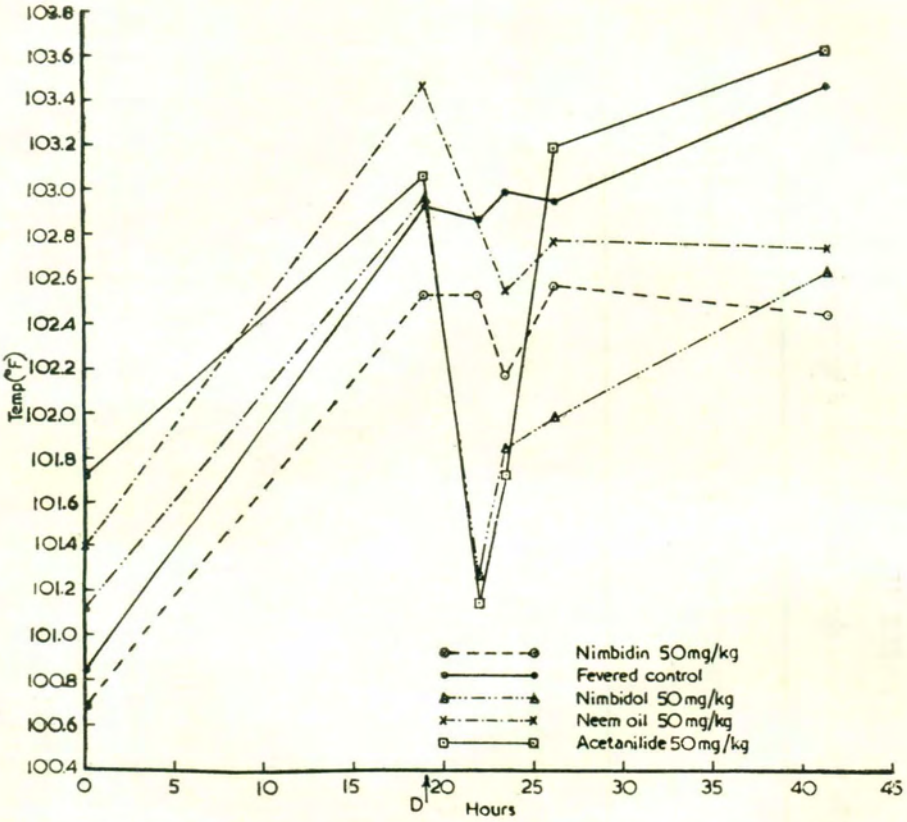
Drug administered	*Rectal temperatures in °F after.....hours				
	0	19	22	23½	2
Untreated fevered controls ..	100.8 (±0.28)	102.9 (± 0.38)	102.9 (± 0.19)	103 0 (± 0.37)	102.9 (±
Acetanilide 50 mg./kg. ..	101.7 (±0.20)	103.1 (± 0.15)	101.1 (± 0.63)**	101.7 (± 0.92)	103.2 (
Neem oil 50mg./kg..	101.4 (±0.34)	103.5 (± 0.22)	102.8 (± 0.30)	102.5 (± 0.28)	102.8 (
Nimbidin 50 mg./kg. ..	100.7 (±0.41)	102.5 (± 0.40)	102.5 (± 0.71)	102.2 (± 0.72)	102.6 (±
Nimbidol 50 mg./kg. ..	101.2 (±0.22)	103.0 (± 0.22)	101.3 (± 0.47)***	101.8 (± 0.44)	102.0 (

* Figures in brackets indicate standard errors

** Significant at 2 per cent level

*** Significant at 1 per cent level

Fig. 1.



Antipyretic activity: It was found that the temperature in normal rats which served as unfevered control group varied at different times of the day. Taking the variation in this group of rats into account, the necessary correction was applied to the other groups and the corrected temperature changes in each group are tabulated in Table II which summarises the results obtained in the experiment using 50 mg/kg of each test drug and the same dose of acetanilide and the results have been statistically evaluated. The data is graphically represented in Fig. 1. Drugs were given orally 19 hours after giving the yeast injection as indicated by the arrow at D in Fig. 1. The results show that in the fevered untreated group, with the dose of yeast injected, the maximum rise in temperature was seen between 17—19 hours. This temperature with slight fluctuations was maintained for nearly 7 hours when a secondary rise was observed. Acetanilide caused a sharp fall in the temperature which was of a short duration, with a similar steep rise to even above the previous level. Nimbidol showed a pattern similar to acetanilide but was more effective in controlling the temperature over a longer period. Neem oil exhibited

no antipyretic effect but seemed to suppress the secondary rise. Nimbidin acted in a manner similar to the neem oil in preventing the secondary rise while causing no reduction in temperature.

DISCUSSION

The statistical evaluation clearly indicates Nimbidol to be an effective antipyretic and more potent than acetanilide in controlling the temperature over a longer period of time. The other fraction, nimbidin and the neem oil exhibited no significant antipyretic action though both the substances prevented the secondary rise of temperature. Gujral *et al*[3] in their screening tests on the antipyretic activity of some indigenous drugs, have also found the decoction from the bark of *Melia azadirachta* to possess no antipyretic activity. The observations of Siddiqui and his co-workers indicate that the root bark[9] and the trunk bark[10] of neem contain nimbin, nimbinin, nimbidin, nimbosterol etc., but not nimbidol. Since nimbidol is the active antipyretic agent, the absence of nimbidol in the bark (either from the root or the trunk) probably explains the lack of antipyretic action with decoction of the bark. Similarly, the absence of effective antipyretic action by the oil can probably be explained by the insufficient concentration of Nimbidol in the quantity of the oil administered.

SUMMARY

Neither the neem oil nor the bitter fractions nimbidin and nimbidol exhibit any estrogenic activity.

Amongst the neem oil and its fractionates, nimbidol, in 50 mg/kg dose, caused an immediate reduction in temperature equipotent to that of acetanilide, the effect being more lasting in duration than that of acetanilide.

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