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ANTHELMINTIC EFFICACIES OF TOTAL ALKALOIDS AND GLYCOSIDES ISOLATED FROM *PUNICA GRANATUM* FRUIT-RINDS (ANAR)

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Anticestodal efficacies of total alkaloids and glycosides isolated from fruit-rinds of Punica granatum (Anar) were studied in goats infected naturally with cestode infection. Both these isolates were administered separately at the dose rates of 75, 150 and 225 mg/kg body weight orally. A standard synthetic tapeworm remedy, Nilzan(R) (Levamisole, 1.5% + Oxyclozanide, 3 %) was also given to a group. Pre-treatment and post-treatment faecal eggs per gram (EPG)counts on 3rd, 10th and 15th days of the drug administration were determined in all the groups. Total glycosides of P. granatum failed to exert a significant anticestodal effect whereas total alkaloids reduced the EPG counts highly significantly ($P<0.001$) on day 15th at the doses of 150 mg and 225 mg/kg b.wt. However, significant ($P<0.05$) EPG reductions were also recorded on days 3rd and 10th at 150 mg/kg and 225 mg/kg doses while 75 mg/kg dose level produced a similar effect on day 15th only. The percentage EPG reductions at dose rates of 150 mg and 225 mg/kg were statistically similar to that of the control drug, Nilzan(R) . Therefore, it is conceivable that alkaloids of P. granatum are as potent as Nilzan(R) against the cestode infection in goats and these data suggested that they could be at least in part responsible for the anticestodal action of this indigenous plant drug.

INTRODUCTION

Ideal curative drugs are not yet availabe for treating several common parasitic infections which continue to prevail even today. The modern drugs employed these days to treat helminthic infections have been observed to be neither completely effective against some common parasites nor they retain their efficacies on their continuous administration due to the development of resistance (Waller and Prichard, 1985). Moreover, anthelmintic efficacies of indigenous plants and herbs used empirically since centuries to treat various parasitic infections in folk medicine have not been explored so as to find a scientific use of these herbs for veterinary therapeutic purposes. Therefore, Akhtar and Riffat (1985) determined efficacy of the powdered Punica granatum fruit-rinds (Anar) against natural nematode and cestode infections in sheep. They reported that the crude drug possessed broad spectrum anthelmintic properties. However, they did not isolate active principles responsible for the antiparasitic activity of this plant drug. Thus present study was conducted to evaluate the anticestodal efficacies of total alkaloids and the glycosides isolated from fruit-rinds of Punica granatum (Anar).

MATERIALS AND METHODS

i) Isolation of total alkaloids and glycosides from P. granatum fruit-rind: The fruit-rinds of P. granatum (Anar) were purchased from herbal dealers (Dawakhanas) of Faisalabad and made free from dust and other adultrations and then completely dried in incubator at 40°C. After complete drying, they were finely powdered in an electric grinder. Then total alkaloids and glycosides were isolated by the method as described by Brain and Turner (1975).

ii) Animals used: Forty-eight goats naturally infected with cestodal infections were selected after checking their faeces by direct smear method as described by Soulsby (1982). The animals were randomly divided into 8 groups of six animals each. All the animals were kept under similar managemental conditions.

iii) Sampling procedure: The faecal samples of all the goats were taken directly from the rectum and processed by the method of Stoll and Hauscheer (1926) in order to determine the faecal eggs per gram counts as described by Soulsby (1982).

iv) Administration of isolated plant drugs and control drug: A group of goats suffering from cestodal infection was kept as untreated control while another group administered with Nilzan(R) (Levamisole,1.5% + Oxyclozanide,3 %) at dosage level of 5 ml/15 kg body weight acted as a control. Similarly, 3 groups were treated with alkaloids, another 3 groups with the glycosides isolated from P. granatum at a dose rates of 75 mg, 150 mg and 225 mg/kg body weight. Post-treatment EPG counts were made on days 3rd, 10th and 15th.

v) Interpretation of results and statistical analysis: The data obtained were expressed as mean ± SEM (standard error of mean). The percentage reductions were calculated by the formula:

$$\frac{\text{EPG reductions after treatment}}{\text{Pre-treatment EPG counts}} \times 100$$

Student 't' test was used to determine the statistical difference in groups (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Anthelmintic evaluation of the powdered P.granatum fruit-rinds against cestode and nematode infection has already been carried out (Akhtar & Riffat, 1985). In the present studies, total alkaloids and glycosides of P.granatum fruit-rinds (Anar) were administered separately in the oral dosage of 75, 150 and 225 mg/kg body weight to the goats infected naturally with the cestodes (mainly Monezia spp.). Mean faecal EPG counts in goats before and after the administration of various doses of the alkaloids and glycosides have been shown in Table 1. In the group treated with single oral dose of 75 mg/kg body weight of alkaloids, mean ± SEM pre-treatment EPG count was 2763 ± 592 which reduced to 2136 ± 204, 1440 ± 396 and 720 ± 248 on 3rd, 10th

Table 1. *Mean ± SEM faecal EPG counts and their percentage reduction in goats naturally infected with cestodal infection orally treated with glycosides and alkaloids isolated from Punica granatum (fruit-rinds) at various dosage levels*

Treatment	Dosage	Pre-treatment EPG counts (zero day)	Post-treatment EPG counts			EPG reductions (%)		
			3rd day	10th day	15th day	3rd day	10th day	15 day
Untreated control	-	2304±672	2400±784	2460±460	2448±560	-	-	-
Treated control Nilzan (R)	5 mg/kg b.wt.	2244±512	1284±256*	576±120*	0±0**	43±3	74±7	100±0
Glycosides of Punica granatum	75 mg/kg b.wt.	2588±387	2480±396	2540±260	2590±160	4±3	2±1	10±3
-do-	150 mg/kg b.wt.	2174±396	2016±432	2082±284	2084±84	5±2	2±1	2±1
-do-	225 mg/kg b.wt.	2066±456	1859±244	198±184	1942±24	10±4	4±2	6±2
Alkaloids of Punica granatum	75 mg/kg b.wt.	2761±592	2136±204	1440±396	720±248*	23±5	47±2	74±9
-do-	150 mg/kg b.wt.	3745±470	2322±312*	1236±248	408±36**	38±6Ø	67±11Ø	89±7Ø
-do-	225 mg/kg b.wt.	3264±310	1600±327*	880±136*	164±26**	51±4Ø	73±9Ø	95±12Ø

Number of sheep in each group = 6.

* = Significantly ($P < 0.05$) less than that of pre-treatment value.

** = Highly significantly ($P < 0.001$) less than that of pre-treatment value.

Ø = Non-significantly ($P > 0.05$) different than that of respective values of Nilzan (R).

Table 2. *Side effects observed in goats treated with total alkaloida (75, 150 and 225 mg/kg) and total glycosides (75, 150 and 225 mg/kg) isolated from Punica granatum fruit-rinds (Anar)*

Side effects	Total alkaloids/kg			Total glycosides/kg			Nilzan (R)
	75 mg	150 mg	225 mg	75 mg	150 mg	225 mg	5 ml/15 kg
Dullness	0	0	0	0	0	1	0
Restlessness	0	1	1	0	0	1	0
Diarrhoea	0	1	1	0	0	1	0
Abdominal pain	0	0	0	0	0	0	0
Tremors and shivering	0	0	0	0	0	1	0
Goats showing side effects	0	1	1	0	0	1	0
Percentage of side effects	0	16.6	16.6	0	0	16.6	0

Number of goats in each group = 6

and 15th days after treatment respectively, showing respective percentage reductions of 23 ± 5, 47 ± 2 and 74 ± 9 in the EPG counts. These reductions were significant ($P < 0.05$) on the day 15th. In the group treated with a single dose of 150 mg/kg body weight, pre-treatment EPG count was 3745 ± 420 which reduced to 2322 ± 312, 1236 ± 248 and 408 ±36 respectively on days 3rd, 10th and 15th and the respective percentage reductions were 38 ± 6,67 ± 11 and 89 ± 7. The reductions were highly significant ($P < 0.001$) on day 15th. Similarly, the group treated with alkaloids of P.granatum at dosage level of 225 mg/kg body weight had pre-treatment EPG counts of 3264 ± 310 which reduced to 1600 ± 327, 880 ± 136 and 164 ±26 with the percentage reductions of 51 ± 4, 73 ± 9 and 95 ± 12 on days 3rd, 10th and 15th. The EPG counts were found to be highly significantly ($P <0.001$) reduced on day 15th but only significantly ($P <0.05$) on days 3rd and 10th. However,the total glycosides of P. granatum did not show any significant anticestodal activity in the dosages checked. In the group treated with Nilzan(R) (5 ml/ 15 kg body weight), the EPG counts were reduced from 2244 ± 512 to 1284 ± 256,576 ± 120 and 0 ± 0, showing percentage reductions of 43 ± 3, 74 ± 7 and 100 ± 0 on days 3rd, 10th and 15th respectively. The EPG counts were highly significantly ($P <0.001$) reduced on day 15th. The percentage EPG reductions at 150 and 225 mg/kg doses of the alkaloids of P.granatum are non-significantly ($P<0.05$) different from that of the control drug,whereas the percentage EPG reductions of group treated with the glycosides are highly significantly lower than the control drug.

In conclusion, these data suggest that total alkaloids isolated from fruit-rinds of P.granatum possess significant activity against cestodal infection in goats. Interestingly, their anti-tapeworm efficacy has been found to be statistically similar to a standard anticestodal drug,Nilzan(R). In addition, it has also been observed that the total glycosides of P.granatum might have been responsible for the efficacy of the powdered plant drug against cestodes in sheep as reported earlier by Akhtar and Riffat (1985). It may also be suggested that the active principles of this

indigenous plant drug could be any one or all of the previously reported alkaloids like pelletierine, methyl-pelletierine, iso-pelletierine, pseudo-pelletierine (Chopra et al., 1957). The calculated dose of alkaloids of P.granatum was 30 mg/kg which was expected to produce peak response but the actual dose producing this effect was 225 mg/kg; it became evident that a higher dose than the expected dose produced a response similar to 3 g /kg of the powdered drug. Thus it is conceivable that P.granatum powder contains some additional compounds which exert synergetic action.

Moreover, 16.6 % of the goats treated with 150 mg and 225 mg/kg of alkaloids were observed to temporarily suffer from some minor side effects like diarrhoea and restlessness (Table 2). This further supports our contention that in addition to the synergistic active principles, some corrective ingredients are present in the natural crude form of most of the time honoured plant drugs which prove more safe and show greater potency than their isolated chemical entities (Akhtar, 1987). Further phyto-chemical and pharmacological studies are, however, still needed to isolate the exact active principle (s) of this indigenous plant drug and to know its mechanism of anthelmintic action.

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