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ANTINEMATODAL AND ANTICESTODAL EFFICACY OF *MORUS ALBA* LINN. STEM BARK IN SHEEP

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Antinematodal and anticestodal efficacy of single dose treatment with *Morus alba* dried stem bark powder was studied in infected sheep. Powdered drug was administered at 1, 2 and 3 g/kg body weight and its water and methanol extracts were also tried at the highest dosage level. Pre-treatment and post-treatment faecal eggs per gram (EPG) counts on 3rd, 10th and 15th day after drug administration were calculated. The percentage reductions in EPG counts of round worms in animals treated with 1 g/kg body weight of *Morus alba* powder on 3rd, 10th and 15th day were 12 ± 4, 28 ± 27 and 72 ± 21, respectively. The percentage reductions in EPG count after the administration of 2 g/kg body weight were 27 ± 23, 54 ± 47 and 58 ± 44, respectively and the percentage reductions after the administration of 3 g/kg body weight of *Morus alba* were 46 ± 33, 67 ± 60 and 82 ± 47, respectively. Water extract equivalent to 3 g/kg body weight produced a reduction of 43 ± 23, 62 ± 49 and 79 ± 69%. Methanol extracts equivalent to 3 g/kg body weight produced 52 ± 46, 79 ± 51 and 81 ± 67% reductions, respectively, in EPG counts in this group. The percentage EPG reductions in the group infected with cestodes and treated with 1 g/kg body weight were 13 ± 13, 28 ± 27 and 42 ± 12, respectively. The respective percentage reductions in the group treated with 2 g/kg body weight were 41 ± 37, 68 ± 28 and 73 ± 14. The percentage EPG reduction with 3 g/kg body weight were 78 ± 65, 81 ± 36 and 85 ± 66, respectively. Water extracts equivalent to 3 g/kg body weight produced a percentage reduction of 12 ± 4, 34 ± 15 and 70 ± 33 while methanol extracts produced a reduction of 20 ± 14, 67 ± 39 and 79 ± 42% respectively.

INTRODUCTION

Morus alba, Linn. is a moderate size tree which is cultivated all over the Pakistan and grows wildly in hilly tracts also. Studies on the various parts of this plant have proved medically important. Its root is considered as an anthelmintic and vermifuge, whereas root bark and stem bark are reported to be vermifuge and purgative. Alcoholic extracts of this plant inhibit fungal growth. Fruit is refrigerant in fever and useful for sore throat, dyspepsia and malancholia (Chopra, 1956). In view of its medicinal importance and properties, the dried stem bark of this plant was tried for its anthelmintic action in sheep.

MATERIALS AND METHODS

Collection of Morus alba Stem Bark : The stem bark was peeled from the stem with a sharp knife in thin slices. The collection started in the month of March and continued in April also. The hard wooden part of the stem bark was removed from the bark carefully. The bark was dried in shade in a dust free hygienic area. The dried stem bark was finely powdered with Electric Grinder and stored in air tight bottles in refrigerator.

Animals Used : Eighty-four sheep naturally infected with mixed gastro-intestinal nematodal and cestodal infections were selected after checking their faeces by direct smear method as described by Soulsby (1982). The animals were randomly divided into 14 groups of six animals each (animals infected with nematodes and cestodes were kept in seperate groups). All the animals were kept under similar managemental conditions. Initial body weight of all the animals were recorded.

Sampling Procedure : The faecal samples of all the sheep were taken early in the morning direct from the rectum and processed by the method of Stoll and Hauscheer (1926), in order to determine the faecal eggs per grams (EPG) counts as described by Soulsby (1982).

Administration of Morus alba Stem Bark Powder and Control Drug : A group of sheep sufferingfrom mixed nematodal infection was kept as untreated control while another group was administered with morantal tartrate (Banminth 4%) at the dosage level of 10 mg/kg body weight. Similarly, 3 groups of sheep were treated orally with powdered *Morus alba* at the dosage levels of 1, 2 and 3 g/kg body weight. The powder was suspended in 2 per cent gum tragacanth solution.

Similarly, two groups of 6 sheep each were treated with methanol extract and water extract equivalent to 3 g/kg body weight. One group of sheep suffering from mixed nematodal infection was kept as untreated control. The total number of sheep in this group was 42. In the group of sheep suffering from mixed cestodal infection one group was kept as untreated control while another group treated with Nilzan (R) (Levamisole HCl 5 % + Oxyclozanide 3 % w/v) at a dosage level of 1 ml/5 kg body weight acted as control. Similarly, 3 groups were treated with *Morus alba* dry stem bark powder at dosage levels of 1, 2 and 3 g/kg body weight. Two groups having 6 sheep in each were treated with water and methanol extracts equivalent to 3 g/kg body weight. Post-treatment faecal samples in all the groups were taken on 3rd, 10th and 15th day after drug administration.

Interpretation of Results and Statistical Analysis : The data 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's t-test was used to determine the statistical differences in groups (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The sheep used for anti-nematodal trials were naturally infected with Haemonchus, Oesteragia, Bunostomum and Trichostrongylus species and for anti-cestodal studies, sheep infected with Moniezia and Avitellina species were selected. Most of the animals were reported to have reduced weights with anaemia. The history data revealed most characteristic symptoms of the parasitic infection like poor growth, rough body coat, diarrhoea and occasional abdominal colic, Some sheep were too weak to walk and showed no response even when disturbed. Sunken eyes, dullness and depression were other additional symptoms observed.

1) *Antinematodal Evaluation :* Mean faecal EPG counts in the sheep before and after the administration of various doses of the powdered *Morus alba* stem bark have been shown in Table 1. In the group treated with a single dose of 1 g/kg body weight of *Morus alba* mean±SEM pre-treatment EPG counts was 18000± 1720 which reduced to 15000 ± 1600, 9000 ± 1525 and 8000 ± 1480 on 3rd,

Table 1. *Average faecal EPG counts and percentage EPG reductions in sheep suffering from mixed nematodal infections before and after treatment with powdered water and methanol extracts of stem bark of Morus alba and the control drug Morantal*

Treatment	Dosage	Pre-treatment EPG counts	Post-treatment EPG counts			Percentage EPG reductions		
			3rd day	10th day	15th day	3rd day	10th day	15th day
		Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean± SEM	Mean± SEM	Mean± SEM
Untreated control	—	13400±4900	14100±8800	14400±7200	14500±6600	—	—	—
Morus alba dry powder	1 g/kg b.wt.	18000±1720	15000±1600	9000±1525	8000±1480	12±4	28±27	72±21
— do —	2 g/kg b.wt.	18500±3000	13500±2300	8500±1575*	1250± 900*	27±23	54±47	58±44
— do —	3 g/kg b.wt,	14400±4500	7800±2100	4700±1300*	2500± 800**	46±33	67±60@	82±47@
Water extract of *alba* eq. to :	3 g/kg b.wt.	15300±4170	8700±3200	5700±2100	3200±1300*	43±23	62±49	79±69@
Methanol extract of *M. alba* eq. to :	3 g/kg b.wt.	13900±4300	6700±4100	2900±2100*	2700±1400*	52±46	79±51@	81±67@
Morantal tartrate	1ml/kg b.wt,	16000±4000	8000±3000*	400± 90**	250± 70**	50±42	97±57	98±32

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

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

@ = Non-significantly ($P<0.05$) different than that of respective value of Morantel tartrate group,

10th and 15th day after treatment respectively, showing a respective percentage reduction of 12 ± 4, 28 ± 27 and 72 ± 21 in the EPG counts. In the group treated with a single dose of 2 g/kg body weight, pre-treatment EPG count was 18500 ± 3000 which reduced to 13500 ± 2300, 8500 ± 1575 and 1250 ± 900 respectively on days 3rd, 10th and 15th with a respective reduction on 27 ± 23, 54 ± 47 and 58 ± 44%. The reductions were significant ($P<0.05$) on 10th and 15th day. The group treated with powdered *Morus alba* stem bark at a dosage level of 3 g/kg body weight, the pre-treatment EPG count had a mean ± SEM value of 14400 ± 4500, which reduced to 7800 ± 2100, 4700 1300 and 2500 ± 800 on days 3rd, 10th and 15th respectively with a percentage reduction of 46 ± 33, 67 ± 60 and 82 ± 47 respectively. The reductions were highly significant ($P<0,001$) on days 10th and 15th.

Water extract equivalent to 3 g/kg body weight was also tried against natural nematodal infection in sheep. The pre-treatment EPG count in this group was 15300 ± 4170, which reduced to 8700 ± 3200, 5700 ± 2100 and 3200 ± 1300, on days 3rd, 10th and 15th respectively with a percentage reduction of 43 ± 23, 62 ± 49 and 79 ± 69. Results were significant ($P<0.05$) on the day 15th. Methanol extract equivalent to 3 g/kg body weight was also tried against nematodal infection. Pre-treatment EPG count had a mean ± SEM of 13900 ± 4300, which reduced to 6700 ± 4100, 2900 ± 2100 and 2700 ± 1400 respectively with a percentage reduction of 52 ± 46, 79 ± 51 and 81 ± 67. Reductions were significant ($P<0.05$) on 10th and 15th day. The control drug, Morantel, was administered to the last group. Pre-treatment EPG count had mean ± SEM of 16000 ± 4000 which reduced to 8000 ± 3000, 400 ± 90, and 250 ± 70 showing a percentage reduction of 50 ± 42, 97 ± 57 and 98 ± 32 respectively. These reductions were highly significant ($P<0.001$) on 10th and 15th day. The results of the treated group were non-significant ($P<0.005$) as compared to the control drug treated group. The reductions of 2 g/kg of *M. alba* treated group and water extract equivalent to 3 g/kg body weight treated group were non-significantly different with the morantel treated group. The reductions due to *Morus alba* stem bark drug powder at a dosage level of 3 g/kg body weight were non-significantly different from those of the Morantel treated group od 10th and 15th day.

Anti-Cestodal Evaluation : The animals in this group were mostly infected with Moniezia and Avitellina species. Mean faecal EPG counts and the percentage

Table 11. *Average faecal EPG count and percentage EPG reduction in faeces of sheep suffering from Cestodal infection after oral treatment with Morus alba Linn. stem bark powder and its water and methanol extracts equivalent to 3 g/kg body weight and Nilzan (R)*

Treatment	Dosage	Pre-treatment EPG counts	Post-treatment EPG counts			Percentage EPG reductions		
		Zero day	3rd day	10th day	15th day	3rd day	10th day	15th day
Untreated control	—	14000±1500	14500±1800	15000±1700	14000±1800	—	—	—
Morus alba	1 g/kg b.wt.	1583± 443	1385± 385	1177± 318	925± 340	13±13	28±27	42±12
— do —	2 g/kg b.wt.	2588± 720	1533± 165*	826± 124**	697± 61**	41±37	68±28@	73±14@
— do —	3 g/kg b.wt,	1616± 281	342± 139*	307± 102**	242± 76**	78±65@	81±36@	85±66@
Water extract of *M. alba* eq. to :	3 g/kg b.wt.	1400± 755	1225± 630	921± 412	419± 121	12±04	34±15	70±33
Methanol extract of *M. alba* eq. to :	3 g/kg b.wt.	1200± 435	865± 270	395± 132	252± 45	20±14	67±39	79±42@
Nilzan (R)	5 ml/15 kg	2000± 260	280± 150	55± 38	15± 7	86±42	97±37	99±29

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

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

@ = Non-significantly ($P<0.05$) different than that of respective values of Nilzan grup.

ruductions are given in Table 2. In the group treated with *Morus alba* at 1 g/kg body weight, the pre-treatment EPG count was 1583 ± 443 which reduced to 1385 ± 385, 1177 ± 318, 925 ± 340 on days 3rd, 10th and 15th respectively with a percentage reduction of 13 ± 13, 28 ± 27 and 42 ± 12. These values were non-significantly ($P<0,05$) different on all the days checked. The group traeted with 2 g/kg body weight of powdered *Morus alba* showed the pre-treatment EPG count of 2588 ± 720 which reduced to 1533±165, 826±124 and 697±61 respectively on days 3rd, 10th and 15th. The reductions were highly significant ($P<0.001$) on 10th and 15th days, and the respective percentage reductions were 41 ± 37, 68 ± 28, and 73 ± 14. In the group treated with 3 g/kg body weight of powdered *Morus alba*, the pre-treatment EPG count was 1616 ± 281 which reduced to 342 ± 139, 307 ± 102 and 242 ± 76 respectively, on day 3rd, 10th and 15th. These reductions were significant ($P<0.05$) on the 3rd day after treatment and highly significant ($P<0.001$) on 10th and 15th days after treatment with respective percentage reductions of 78±65, 81±36, 85±66. No adverse effects were recorded at this dosage level. Water extract of powdered *Morus alba* equivalent to 3 g/kg body weight was also tried. Pre-treatment EPG count was 1400 ± 755 which reduced to 1225 ± 630, 921 ± 412 and 419±121 on day 3rd, 10th and 15th respectively. These values were found non-significant statistically ($P>0.05$) on all the sampling days. The respective percentage reductions were 12 ± 04, 34 ± 15 and 70 ± 33. Similarly, methanol extract of *Morus alba* equivalent to 3 g/kg body weight was also tried. Pre-treatment EPG count had a mean ± SEM of 1200 ± 435 which reduced to 865 ± 270, 395 ± 132 and 252 ± 45 respectively on days 3rd, 10th and 15th. These reduction were found non-significantly ($P>0.05$) different from the pre-reatment count on all the sampling days. Percentage reductions in this group were 20 ± 14, 67 ± 39 and 79 ± 42, respectively.

The control drug, Nilzan(R) was administered at a dosage level of 5 ml/15 kg body weight. The pre-treatement EPG count had mean ± SEM of 2000 ± 260 which reduced to 280 ± 150, 55 ± 38 and 15 ± 7 on days 3rd, 10th and 15th respectively. The percentage reductions were 86 ± 42, 97 ± 37 and 99 ± 29 showing a maximum reduction of 99 per cent in the EPG count. The percentage EPG reduetions of all the treated groups were also compared with the percentage reductions of Nilzan (R) treated group. The percentage reductions were non-significantly different from these of the control group at the dosage levels of 2 and 3 g/kg body weight on 10th and 15th days. Similar trend was found with methanol extract on 15th day post-treatment.

After treatment with *Morus alba* and Nilzan (R), the animals regained quickly in the convalesence period. Before administering the drug the animals were suffering from diarrhoea with unthriftiness and lazy body reflexes which are suggestive of severe parasitic infestation (Soulsby, 1982). Although in the present trials the enthelmintic efficacy of *Morus alba* has been found at par with that of Nilzan (R) yet the mechanism of action of the former is not clear which indicates the need to conduct further investigations on this aspect in order to elucidate the exact mechanism of anthelmentic activity.

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