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Effect of Some Medicinal Plants Extract on Aggressive Behaviour of Laboratory Mice

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ABSTRACT. The effect of the aqueous extracts of *Anchusa affinis, Fumaria parviflora* and *Peganum harmala* on social aggression behaviour of laboratory male MFI albino mice was studied, using video camera. Aqueous extracts of different concentrations were injected for 14 days intraperitoneally (i.p.) into the experimental animals. Twenty minutes after the last dose, social aggression encountered was videotaped for 10 minutes for each animal. All three plants produced a significant reduction in, at least, one of the elements of aggressiveness (latency to first bite and number of bitings) monitored. *A. affinis* was the most efficient in this respect. The effect of *P. harmala* appeared to be partially through its debilitating action on the mice. In the present study the potential hepatotoxic, nephrotoxic and cardiotoxic effects of *A. affinis, F. parviflora* and *P. harmala* on MFI mice were also investigated. At the doses tested, aqueous extract of these plants exhibited potential hepatotoxic, nephrotoxic and cardiotoxic effects. CNS stimulant effects were also demonstrated. Further studies are required for proper evaluation of these effects.

Introduction

In recent years medicinal plants are widely used as traditional folk medicine in many countries. The toxicity, pharmacological and social effects of *Anchusa affinis, Fumaria parviflora* and *Peganum harmala* medicinal plants were described in the literature[1,2,3,4]. Pharmacological studies have isolated and identified 40 pyrrolizidin alkaloids from *Anchusa affinis*[3]. Also studies on *Fumaria parviflora* were focused on the pure alkaloids from which many were isolated, namely: Protopine, parfumine, fumariline, dihydrofumariline and cryptopine[4]. Furthermore pharmacological studies on *Fumaria parviflora* have yielded al-

kaloids isolated from these plants, namely: harmine, harmaline, harmalol and hurmol[1,2]. The poor information and rare empirical or critical bioassays that have been conducted on behavioural (social aggression) effects of the medicinal plants led us to carry out a survey which might enlighten our understanding on the type of effects these medicinal plants have on behaviour. The investigation of types of aggression particularly social aggression is important simply because the use of the term "aggression" alone may imply that one can extrapolate interspecific attack (fighting between two different species) to interspecific attack (fighting between members of the same species)

Many authorities[1, 5-7] also reported that, mice proved to be better animals for studying social aggression since individually-housed members of this species will spontaneously attack conspecifics more readily than rats.

In the present study, the effects of three medicinal plants on social aggression were evaluated. Selection of plants for this particular study was based on beliefs of traditional healers and local people claiming that *F. parviflora* is considered one of the traditional healers. Another plant *A. affinis* believed to have tranquilizing properties. In addition to that *P. harmala* believed to have known effects on CNS[2].

Materials and Methods

Preparation of extracts

The dry leaves of the three medicinal plants included under the present investigation (*Anchusa affinis, Fumaria parviflora* and *Peganum harmala*) were used. *A. affinis* and *F. parviflora* were obtained from the local market, whereas *P. harmala* was collected from suburbs of Taif province. The plants were separately ground in a coffee machine for 3-4 minutes, then the powder or paste-like ground material was mixed with physiological saline solution (0.9%) and stirred thoroughly using an electromagnetic stirrer. This mixture was left undisturbed overnight at room temperature. After acid treatment, the neutral aqueous extracts were prepared following the procedure of Adaay[8]. The clear supernatant thus obtained was aspirated and used in such a manner that a constant injectable volume (0.1-ml) produces doses as follows: 50 and 100 mg extract/kg body weight of the mice of each of *A. affinis, F. parviflora* and *P. harmala*.

The experimental animals

Animals used

Ninety albino male MFI mice (12 weeks-old group-housed) (30-35 g in weight) were used in this study. They were kept under controlled breeding facil-

ity and housing conditions as described by Benton and Brain[6]; Al-Hazmi and Brain[9]. The animals were divided into three categories, each category (30 animals) were divided into three groups (10 animals each) and injected i.p. with either saline vehicle (control) or with each of the two doses *viz*. (50 mg/kg and 100 mg/kg) of each plants for two weeks.

After that, mice were housed in groups of 6 in opaque plastic cages M1 type. They received food (Pillsbury diet) and water, except during behavioural trials.

Preparation of aggressive mice

Adult mice were housed individually for two weeks prior to the test period. The isolation rendered them aggressive so that they would attack conspecifics when introduced into their homecages.

Standard opponent (passive) animal

The standard opponent procedure was described by Brain and Poole[10]. Mice were rendered anosmic by intranasal perfusion of 4% $ZnSO_4$ solution after which they were housed in groups of six. This treatment induces a passive attitude in mice so that they never attack the aggressive animals (*i.e.* the attack is unilateral, directed by the aggressive animals only and can easily be quantified)[10,11].

Experimental parameters

I. Neurobehavioural tests

Social aggression test

This test is a modification of what was described by Al-Maliki and Elisha[1]; Brain and Nowel[11]. Behavioural elements in the course of a ten minute encounter between isolated mice (aggressive) and the anosmic standard opponents were monitored. The encounter was conducted in the homecage of the isolate. The behavioural parameters recorded were:

1 – The proportion of mice evidencing over attacks.
2 – The latency (in seconds) from the time of introduction of the standard opponent to the first biting attacks on the mice.
3 – The number of discrete bouts of biting attacks on the standard opponent.

Thirty aggressive mice (through isolation) were randomly allocated to 3 categories (10 mice each) used for each plants extract dose. Mice were injected intraperitoneally with either the control (Physiological saline) or with low (50 mg/kg) or high (100 mg/kg) doses from each plant extract.

The treated animal groups and their control group were injected (i.p.) for 14 days. At day 14 and twenty minutes post injection (i.p.) each plant extracts a 'standard opponents[6,10,12] was introduced into the test animal's homecage and the social aggression encounters were videotaped for 10 minutes[9,12]. The tape was later analysed in terms of latency to attack (in seconds) and the number of attacks.

II. Biochemical parameters

After completing the assessment of behavioural parameters, the animals were decapitated and blood samples collected in plain and heparinized tubes were used for evaluation of the level of liver enzyme such as: Glutamic Oxalic Transaminase (GOT), Glutamic Pyruic Transaminases (GPT), Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) . The transaminases, ALP and LDH were determined by Klin and Klin[13] technique. The serum creatinine and blood urea (Bl. Urea) levels were determined by Tietz[14] technique.

III. Histopathological examination

Sections were prepared from heart, liver and kidneys of both control and animals treated by the 3 types of plant extracts at all doses and examined for detection of any histopathological changes.

Statistical Analysis

Paired comparisons of behavioural data were carried out between control and plant extract treated groups at each allocated testing time using Mann-Whitney 'U' test[15]. Biochemical data were statistically analyzed by student's "t" test for paired groups[16]. Proportion of attacking mice was compared using Fisher's exact probability test[15].

Results

Social Aggression Test

The median values (with ranges) for the behavioural tests together with statistical comparison between the behavioural elements in control and test animals are presented in Tables 1-3. Paired comparisons with Mann-Whitney 'U' test revealed that male mice given *P. harmala* extract significantly allocated more time in latencies to attack in low (50 mg/kg) dose ($U = 1.6$, $P < 0.05$) and high (100 mg/kg) dose ($U = 3.67$, $P < 0.001$) than the untreated control, but no significant difference was seen in the number of attacks. *F. parviflora* showed no significant difference in behaviour when measuring the treated doses. Male mice given *A. affinis* extract significantly allocated more time in latencies to attack in low (50 mg/kg) dose ($U = 3.7$, $P < 0.001$) and less time in high (100 mg/kg) dose ($U =$

1.9, $P < 0.05$) than control. Although male mice showed significant increase in terms of the number of attacks in low and high doses (both, $U = 3.7$ and $P < 0.05$). According to Fisher's test the proportions of animals biting the target showed no significant differences in all doses compared to the control.

TABLE 1. Effect of the *A. affinis* extract on social aggression in MFI mice.

Treatment	Proportion of biting mice	Median (with ranges) of latency to attacks (secs)	Median (with ranges) of number of attack
Control 0.0	6/10	478 (0.0 - 570.0)	2.0 (0.0 = 5.0)
Low dose 50 mg/kg	6/10	493** (591.0 - 600.0)	3.0* (0.0 - 4.0)
High dose 100 mg/kg	7/10	407* (0.0 - 453.0)	4.0* (0.0 - 6.0)

*Significantly influenced by dose on the Mann-Whitney 'U' test $P < 0.05$.
**Significantly influenced by dose on the Mann-Whitney 'U' test $P < 0001$.

TABLE 2. Effect of the *F. parviflora* extract on social aggression in MFI mice.

Treatment	Proportion of biting mice	Median (with ranges) of latency to attacks (secs)	Median (with ranges) of number of attack
Control 0.0	10/10	337.0 (225.0 - 422.0)	31.0 (14.0 - 55.0)
Low dose 50 mg/kg	10/10	357.0 (192.0 - 438.0)	33.0 (23.0 - 53.0)
High dose 100 mg/kg	10/10	277.0 (190.0 - 422.0)	38.0 (28.0 - 49.0)

N.B. No significant influences of doses are evident on the Kruskal-Wallis test.

TABLE 3. Effect of the *P. harmala* extract on social aggression in MFI mice.

Treatment	Proportion of biting mice	Median (with ranges) of latency to attacks (secs)	Median (with ranges) of number of attack
Control 0.0	10/10	337.0 (225.0 - 422.0)	31.0 (19.0 - 55.0)
Low dose 50 mg/kg	9/10	364* (450.0 - 600.0)	25.0 (0.0 - 46.0)
High dose 100 mg/kg	10/10	100.0** (11.0 - 243.0)	29.0 (3.0 - 46.0)

*Significantly influenced by dose on the Mann-Whitney 'U' test $P < 0.05$.
**Significantly influenced by dose on the Mann-Whitney 'U' test $P < 0001$.

Biochemical results

A. affinis (Table 4)

The liver transaminase (SGPT) showed highly significant increase in its level in the treated mice at low dose ($t = 8.5$, $P < 0.001$) and high dose ($t = 4.9$, $P < 0.001$). SGOT showed statistically significant increase after low and high doses ($t = 7.7$, $t = 4.7$) respectively, both at ($P < 0.001$) from the control group. Alkaline phosphatase enzyme also showed a highly significant increase in the treated mice at low and high doses ($t = 7.4$, $t = 10.1$) respectively, both at ($P < 0.001$). Serum creatinine in the treated mice exhibited only significant decrease in high dose ($t = 2.3$, $P < 0.025$). Blood urea was significantly increased in the treated mice in low and high doses ($t = 11.3$ and $t = 6.5$) respectively, both at ($P < 0.001$). Lactate dehydrogenase enzyme (LDH), showed significant increase in the treated animals in low ($t = 2.9$, $P < 0.01$), but highly significant decrease in high dose ($t = 10.6$, $P < 0.001$).

TABLE 4. Effect of aqueous extract of *A. affinis* on different biochemical values in MFI-Mice.

Biochemical parameter	0.0	Low dose 50.0 mg/kg	High dose 100.0 mg/kg
SGPT (U/L)	19.9 ± 1.0	31.8 ± 1.0***	27.1 ± 1.2***
SGOT (U/L)	20.6 ± 0.5	32.5 ± 0.3***	37.2 ± 0.7***
Alk. Phos. (U/L)	91.4 ± 13.6	192.6 ± 1.0***	228.9 ± 1.3***
Creatinine (mg/dl)	0.6 ± 0.6	0.7 ± 0.0	0.33 ± 0.0*
Bl. Urea (mg/dl)	19.3 ± 0.6	29.9 ± 0.7***	26.0 ± 0.8
LDH (U/L)	146.3 ± 0.9	164.5 ± 6.1**	115.5 ± 2.8***

*Statistically significant difference from control $P < 0.025$ (*t*-test).
**Statistically significant difference from control $P < 0.01$ (*t*-test).
***Statistically significant difference from control $P < 0.001$ (*t*-test)

F. parviflora (Table 5)

The liver transaminase (SGPT) showed no significant variation in its level in the mice treated by both doses. However, the treated mice showed a highly significant decrease in their level in SGOT at low and high doses ($t = 6.8$, $t = 9.4$) respectively, both at ($P < 0.001$) from the control group. Alkaline phosphatase enzyme showed a highly significant increase in treated mice with low and high doses ($t = 4.1$, $t = 4.2$) respectively, both at ($P < 0.001$). Serum creatinine was significantly increased in treated mice only in low dose ($t = 2.3$, $P < 0.001$).

Blood urea in treated mice exhibited highly significant increase in low and high doses ($t = 4.9$, $P < 0.001$ and $t = 2.7$, $P < 0.01$). Lactate dehydrogenase enzyme (LDH), showed highly significant increase in treated mice in low and high doses (both, $t=4.02$, $P<0.001$, respectively).

TABLE 5. Effect of aqueous extract of *F. parviflora* on different biochemical values in MFI-Mice.

Biochemical parameter	0.0	Low dose 50.0 mg/kg	High dose 100.0 mg/kg
SGPT (U/L)	19.9 ± 1.0	18.77 ± 0.3	18.9 ± 0.3
SGOT (U/L)	20.6 ± 0.5	16.46 ± 0.4***	14.3 ± 0.4***
Alk. Phos. (U/L)	91.4 ± 13.6	157.9 ± 9.5***	162.4 ± 10.5***
Creatinine (mg/dl)	0.6 ± 0.6	0.7 ± 0.0*	0.55 ± 0.0
Bl. Urea (mg/dl)	19.3 ± 0.6	26.2 ± 1.3***	22.6 ± 1.2**
LDH (U/L)	146.3 ± 0.9	185.5 ± 0.8***	167.0 ± 0.3***

*Statistically significant difference from control $P < 0.025$ (*t*-test).
**Statistically significant difference from control $P < 0.01$ (*t*-test).
***Statistically significant difference from control $P < 0.001$ (*t*-test).

P. harmala (Table 6)

SGPT showed a highly significant increase in its level in the *P. harmala* treated mice; by low ($t = 7.7$, $P < 0.001$) and high dose ($t = 4.67$, $P < 0.001$). SGOT showed no statistically significant variation from the control group. Alkaline phosphatase enzyme also showed a highly significant decrease in the *P. harmala* treated mice after low and high doses ($t = 4.2$, $t = 7.2$) respectively, both at ($P < 0.001$). Serum creatinine in the *P. harmala* treated mice exhibited a highly significant decrease after low and high doses ($t = 6.3$, $t = 7.2$) respectively, both at ($P < 0.001$). Blood urea was significantly elevated in the *P. harmala* treated mice only with high dose ($t = 2.7$, $P < 0.01$). Lactate dehydrogenase enzyme (LDH), showed highly significant elevation in the *P. harmala* treated animals after low ($t = 32.6$, $P < 0.01$) and high doses ($t = 21.2$, $P < 0.025$).

Histopathological changes

Sections from hearts, livers and kidneys injected with A. *affinis, F. parviflora* and *P. harmala* extract showed no significant pathological alteration from the normal.

TABLE 6. Effect of aqueous extract of *P. harmala* on different biochemical values in MFI-Mice.

Biochemical parameter	0.0	Low dose 50.0 mg/kg	High dose 100.0 mg/kg
SGPT (U/L)	19.9 ± 1.0	56.2 ± 4.2***	69.4 ± 9.8***
SGOT (U/L)	20.6 ± 0.5	25.0 ± 7.9	24.3 ± 8.6
Alk. Phos. (U/L)	91.4 ± 13.6	30.9 ± 9.5***	28.4 ± 8.7***
Creatinine (mg/dl)	0.6 ± 0.6	0.34 ± 0.1***	0.32 ± 0.1***
Bl. Urea (mg/dl)	19.3 ± 0.6	54.8 ± 5.5	91.7 ± 7.5**
LDH (U/L)	146.3 ± 0.9	284.4 ± 7.4**	203.8 ± 3.2***

*Statistically significant difference from control P < 0.025 (*t*-test).
**Statistically significant difference from control P < 0.01 (*t*-test).
***Statistically significant difference from control P < 0.001 (*t*-test).

Discussion

The present results showed that the administration of low dose resulted in increase in number of attacks and increase in latency of attacks in high dose by *A. affinis, P. harmala* (Tables, 1-3). Similar results have been reported in mice[1,2,7]. High doses of these two plants were associated with an increase in number of attacks with a decrease in latency to attacks. The effects of these plants on social aggression is difficult to interpret. In this respect, it is well established that pituitary, adrenocortical and gonadal hormones may play important roles[1,7]. Overactivity of the amygdala, possibly due to inadequate inhibition by serotonin, excessive noradrenaline activity in the forebrain and acetylcholine activity at the level of hypothalamus have also been implicated as the motivators of rage and aggression[17]. Male odour pheromones also appear to be primary eliciting stimuli for these reactions in many mammals[12, 18]. Moreover, each type of aggression has a different physiological basis and the physiological substrate underlying each type is not yet known.

Therefore it is difficult to assess how specific and selective these medicinal plants are in altering the elements of social aggression. Changes in the aggressive behaviour might be attributed to the deleterious effects of a drug on motor coordination, changes in general activity, memory dysfunction or possibly to changes in non-drugged opponent's behaviour[12, 19-22]. The apparent anti-aggressive effects showed by high dose of *P. harmala* extract seem to be partially non-specific; mediated through its general debilitating and deleterious action on motor coordination[7]. This plant contains potentially active alkaloids, the dominant ones being harmine and harmaline[7], which possess various phar-

macological effects notably on the central nervous system causing stimulation or depression depending on the dose[1,2,7,23].

The absence of any significant alteration in the main parameters of social aggression test is contrary to stimulant effects of *F. parviflora*[1]. The lack of any depressant effect of *F. parviflora* extract on aggressive behaviour activity is contrary to the findings in the earlier study[1], which reported motor dysfunction in mice treated with extract of dry *F. parviflora* plant. This was attributed to non-specific general debilitating and deleterious action on motor coordination.

The bizarre behavioural results obtained with *A. affinis* should be revaluated by further studies, since the number of attacks increased by both doses, the latency to attacks increased with low dose and markedly decreased in animals given the high dose. This plant has been considered as a tranquilizer with, presumably, some side effects since a long time ago. Hence, priority must be given to this plant for any further detailed investigations, specially as no work on isolation and identification of the active components has ever been done.

SGPT and SGOT enzymes are excellent markers of parenchymal liver damage caused by toxic substances[19,20]. The highly significant increase in the level of transaminase enzymes, and alkaline phosphatase in the *A. affinis* and *F. parviflora* treated mice demonstrates the possible parenchymal hepatotoxic effects of these three plants although the decrease in alkaline phosphatase level in the *P. harmala* was difficult to explain[7,20]. Furthermore, nephrotoxic effects of *A. affinis, F. parviflora* and *P. harmala* extract on MFI-mice were denoted by the statistically significant alterations in serum creatinine and blood urea[7]. The statistically significant elevation of LDH, points to myocardial affection (necrosis or ischemia)[7,21]. Similar results have been reported by another study However, the absence of any significant histopathological changes may be due to low doses of plant extracts, short duration of administration or lower active ingredient content in these plants[7].

Conclusion

Herbal self-medical procedures may carry potential risks to the users. Aqueous extracts of these three plants proved to have a potential toxic effects. Further intensive animal studies are required before extrapolation to human use. Until then it is recommend that herbs employed in therapy should be controlled until their safety and efficacy are assured after critical experimentation.

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تأثير مستخلص بعض النباتات الطبية على السلوك العدائي في الفئران المعملية

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جـــــدة – المملكة العربية السعودية

المستخلص. درس تأثير المستخلص المائي لكل من نبات ورد لسان الثور (*Anchusa affinis*) والباقلاء (*Fumaria parviflora*) والحرمل (*Peganum harmala*) على السلوك العدائي في الفئران المعملية البيضاء MFI باستخدام كاميرا فيديو . لقد تم حقن المستخلص المائي في التجويف البريتوني لحيوانات التجارب لمدة ١٤ يومًا ، وبعد مضي ٢٠ دقيقة من الحقن في اليوم ١٤ تم تسجيل السلوك العدائي الاجتماعي ولمدة ١٠ دقائق لكل حيوان . وجد أن لجميع هذه النباتات تأثيرًا معنويًا مثبطًا لعنصر واحد على الأقل من العناصر السلوكية العدوانية . وكان ورد لسان الثور النبات الأكثر تأثيرًا في هذا الصدد ويبدو أن الخمول والضعف العام المتسبب عن نبتة الحرمل له علاقة أيضًا بالتأثيرات المثبطة للسلوك العدواني . كما تمت دراسة التأثيرات السمية لهذه النباتات على أنزيمات كل من الكبد والكلى والقلب والتي دلت على وجود تأثيرات سامة بدلالات إحصائية للمستخلصات المائية لهذه النباتات في الفئران المعملية البيضاء من نوع MFI ، وكذلك تشير الدراسة إلى وجود تأثير محفز لهذه المستخلصات النباتية على الجهاز العصبي المركزي .