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PHARMACOLOGICAL STUDY AND FRACTIONATION OF PASPALUM SCROBICULATUM EXTRACT

BY

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The dried ethanol extract of the husk of the grain of *Paspalum scrobiculatum* produced tranquillization and tremors in various species of animals. It potentiated the effect of hexobarbitone in mice, produced hypothermia in mice and rats and enhanced leptazol toxicity in rats. Amphetamine group-toxicity in mice increased after injecting the extract or an emulsion containing a similar quantity of olive oil. Vomiting in pigeons and decrease of morphine rage in cats were noted. Diminution of carotid occlusion reflex and hypotension were observed in anaesthetized dogs. Tremors and sleep were experienced by a human volunteer after taking the extract orally. Stability of the extract under different conditions was studied in dogs. Fractions of the extract, resolved by solvent separation and column chromatography, were tested in dogs for tranquillization and tremors.

Paspalum scrobiculatum (Fam. Gramineae, *Harik* in Marathi language), a sturdy millet thriving in the poorest soil, has been cultivated in some provinces of India since ancient times (Charak, about 1000 B.C.). Frequently, the husk and leaves acquire poisonous character which is popularly attributed to heavy rainfalls. The grain of such crop is detoxified (by deliberately storing for about a year or by keeping overnight in buffalo dung), dehusked, boiled, and is consumed by the poor rural population after discarding the supernatant water.

A few hours after the accidental consumption of the toxic grain, which has no distinctive taste, the individual suffers from sleepiness (without hallucinations), tremors and giddiness. The symptoms last for 1 to 3 days, followed by complete recovery. Vomiting and abdominal colic occasionally occur. In cattle, tremors, clonic convulsions, coma and even death may occur.

Toxicological references are found in the works of Chanakya (321 B.C.), Narahar (about 1400), Ramdas (1636), Chevers (1870) and Swarup (1922). Apparently the plant enjoys no important place in ancient medicine or existing drug-lore.

Sundaram Ayyar & Narayanaswamy (1949) observed tremors and death (but not tranquillization) after injecting the " fatty " portion of the grain into various animal species. They described a colour reaction to identify the toxic samples. Poisoning by this grain was considered to be very similar to that produced by *Lolium temulentum* (Chopra, Chopra, Handa & Kapur, 1958). Bhide & Aimen (1959) reported that the active fraction of the husk of the grain had a tranquillizing effect.

METHODS

Preparation of the extract

Samples of unpolished grain, harvested in November, 1959 and 1960, were used within 5 months. Each sample was kept separately in ethanol or rectified spirit for 2 days and stirred occasionally. After a brief warming to 70° C the tincture was removed, filtered and evaporated to dryness under a fan, first at room temperature (14° to 37° C) and finally over a boiling water bath. The sample of the dried ethanol extract that proved most active in dogs (after intraperitoneal administration) in producing tremors and tranquillization was used in the biological experiments.

For most of the experiments, 1.0 g of the dried ethanol extract was mixed thoroughly with a few drops of ether and 1.0 ml. of Tween 80 and emulsified by adding water to make a volume of 50 ml. An emulsion prepared from comparable volumes of ether and Tween 80 in water (referred to subsequently as solvent) was always given as a control in all the experiments except those on the anaesthetized dogs. Except when stated otherwise, all substances were administered by the intraperitoneal route.

Experiments on dogs suggest that the extract loses its activity in 6 months at room temperature and in 8 days at 65° C.

Pharmacological tests

Experiments on unanaesthetized animals. Dogs, cats, rabbits, albino rats and mice, and rhesus monkeys received the extract by the oral, intraperitoneal or intramuscular route. General behaviour, gait, posture, feeding and response to handling and prodding were observed before and after giving the extract.

Acute toxicity. Five groups of adult albino mice, each consisting of 20 animals, received (intraperitoneally) 0.5, 0.6, 0.8, 0.9 and 1.0 g/kg of the extract. Twenty-four-hr mortality values were converted into probits. The LD50 was calculated from the log dose-probit graph.

Hexobarbitone sleep. Adult albino mice were injected with 100 or 300 mg/kg of the extract, followed 30 min later by 100 mg/kg of sodium hexobarbitone. The duration of loss of the righting reflex (sleeping-time) was recorded. Forty-eight hr after giving 300 mg/kg of the extract, the effect of 5 mg/kg of reserpine on the hexobarbitone sleeping-time was also recorded. The sleeping-time was studied in groups of mice receiving a subthreshold anaesthetic dose of hexobarbitone (50 mg/kg) 30 min after 50, 100 and 300 mg/kg of the extract.

Ether anaesthesia. One animal of a pair of adult albino rats of the same sex and comparable weights received 150 mg/kg of the extract or 5 mg/kg of chlorpromazine; the uninjected animal served as a control. (Some pairs consisted of 2 uninjected control animals.) Half an hour after injection the pair were introduced into a 5-litre stoppered glass jar, in which 2.0 ml. of ether was allowed to vaporize. After 7 min the pair were removed quickly and kept in the supine position. The time in sec required for regaining the righting reflex was noted. The experiment was repeated 8 days later, after crossing over the 2 groups.

Rectal temperature. The effect of the extract on the rectal temperature of adult mice and rats, recorded with a thermometer, was studied for at least 5 hr.

Leptazol toxicity. Groups of adult albino rats received different doses of the extract. After 1 hr, 70 mg/kg of leptazol was injected subcutaneously. Mortality in 24 hr was recorded.

Pigeon emesis. About 2 hr after feeding, adult pigeons (average weight about 260 g) received 4 mg of the extract (diluted to 1 ml. vol.) in the thigh muscles. Each pigeon was observed under a bell jar for at least 1 hr. Control birds received the solvent. After 4 to 6 days the 2 groups of birds were crossed over. Thirty pigeons were used in these experiments.

Amphetamine toxicity. Adult albino mice in groups of 8 (Dandiya & Cullumbine, 1959) were placed in cubical wire mesh cages (16 cm) after receiving subcutaneously 20, 25 or 30 mg/kg of amphetamine sulphate. Other groups received, in addition, the extract, or a comparable volume of solvent, or an aqueous emulsion of Tween 80 and ether containing 2.5% olive oil (v/v). Deaths were counted after 24 hr.

Morphine rage in cats. Cats were observed for at least 8 hr after receiving various substances intramuscularly. In all, 28 cats were used; some recovered completely and were used again after 10 to 15 days.

After conducting 27 experiments, the effective but sublethal dose of morphine sulphate was found to be 4 to 6 mg/kg.

The effect of chlorpromazine, reserpine, or the extract, on the course of morphine rage was studied in 11, 5 and 20 experiments, respectively. Chlorpromazine hydrochloride (4 to 6 mg/kg) or the extract (0.5 to 70.0 mg/kg) was injected 1 hr before or after the effective dose of morphine. Reserpine acetate (0.2 to 1.0 mg/kg) was injected 4 hr before morphine.

Dog blood pressure. In these experiments Tween 80, gum acacia and propylene glycol were found unsuitable as emulsifying agents. Sodium alginate was therefore used for preparing a stable emulsion. The extract (10 to 200 mg/kg of the dog's weight) was mixed with ¼ its weight of sodium alginate, a few drops of olive oil, and 5 ml. of 0.9% sodium chloride solution to get a jelly-like mass, which was homogenized in a Waring blender and further diluted to 50 to 100 ml. by adding 0.9% sodium chloride solution. These comparatively large volumes of 0.9% sodium chloride solution were considered necessary to reduce the viscosity of the jelly. However, no critical criteria were followed for the final dilution of the jelly. Emulsion of olive oil and alginate in 0.9% sodium chloride solution was used for control experiments.

In anaesthetized dogs (intraperitoneal pentobarbitone sodium 30 mg/kg) the carotid or femoral artery blood pressure was recorded with a mercury manometer for 3 to 6 hr. Electrocardiogram records (lead II) and blood pressure change after 10 sec occlusion of the common carotids were also studied in some animals. The emulsions in 50 to 100 ml. vol. were slowly administered through the cannulated femoral vein.

Six anaesthetized dogs were used for control experiments. They received 35 to 125 mg/kg of sodium alginate (as olive oil emulsion in 50 to 100 ml. 0.9% sodium chloride solution), and were then observed for changes in blood pressure for 3 to 6 hr. The carotid occlusion reflex (2 dogs) and the electrocardiogram (2 dogs) were also recorded.

In another series of 6 anaesthetized dogs, 10 to 200 mg/kg of the extract was injected (as olive oil-alginate emulsion in 0.9% sodium chloride solution, volume 50 to 100 ml.) for changes in blood pressure and carotid occlusion reflex. Electrocardiograms were obtained in 4 dogs, and splenic volume changes and adrenaline effect in one each.

Isolated guinea-pig ileum. Eight experiments were performed on the isolated guinea-pig ileum suspended in oxygenated Tyrode solution at 37° C. To minimize the interference of naturally occurring electrolytes, the petroleum-ether-soluble fraction (Fig. 1) of the dried ethanol extract was used. This fraction (16 to 300 μg/ml. as Tween 80 emulsion) was allowed to act for 0.5 to 5 min. Effects of acetylcholine chloride (0.04 μg/ml.), histamine hydrochloride (0.04 μg/ml.) and barium chloride (150 μg/ml.) were also studied before and after adding the extract.

Experiment in man. Blood pressure, axillary temperature and pulse rate were measured repeatedly for 5 days in the subject (N.K.B., body weight 172 lb., teetotaller). Forty mg of the extract (in Tween 80 emulsion) was then taken orally in 3 divided doses every day for 3 days. After 12 days 70 mg of the extract was taken orally in 5 divided doses every day for 3 days. Electroencephalographic records were taken with surface scalp electrodes before and after the second trial.

Fractionation of the extract. The dried ethanol extract could be separated into water-soluble and ether-soluble portions. The ether-soluble portion partly dissolved in petroleum ether (boiling point 60° to 80° C). About 400 mg of the dried petroleum-ether-soluble portion was dissolved in petroleum ether and the resulting solution applied to the top of a chromatography column. This column was prepared by packing the slurry of alumina (about 60 g of chromatographic grade, Merck) and petroleum ether into a 1 in. diameter glass burette with a cotton plug at the bottom. The column was successively developed with the following solvents: petroleum ether, benzene, chloroform, ether-ethanol (1:1, v/v), absolute ethanol, and 1% glacial acetic acid in ethanol. The chloroform eluate was evaporated to dryness and

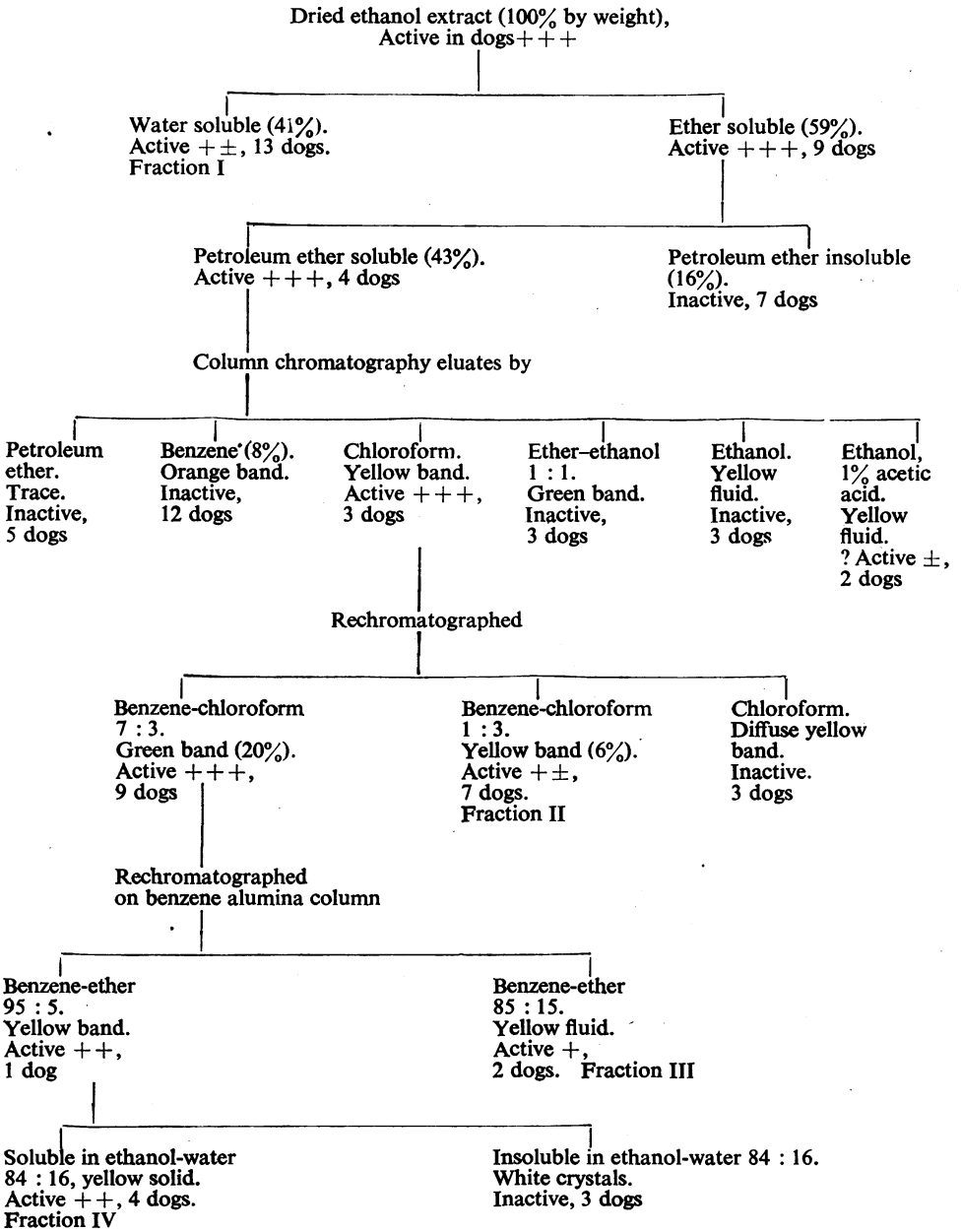

Fig. 1. Fractionation of dried ethanol extract of *Paspalum scrobiculatum*.

the residue dissolved in petroleum ether and again chromatographed on an alumina-petroleum ether column and the column was successively developed with two benzene-chloroform mixtures (7:3 and 1:3, v/v, respectively) and chloroform. The eluate obtained with the 7:3 benzene-chloroform mixture was evaporated to dryness and the residue dissolved in benzene and chromatographed on an alumina-benzene column which was successively developed by two

benzene-ether mixtures (95:5 and 85:15, v/v). After evaporating the first eluate to dryness, the residue could be separated into a yellow substance soluble in 84% ethanol, and a white crystalline substance insoluble in the same solvent (Fig. 1).

Elution with each solvent was continued until the eluates were colourless, except for petroleum ether, which did not appear to elute any substance even with 200 ml. volumes.

The entire process was repeated with dried ethanol extracts from 4 different samples of the grain. Evaporation, when required, was carried under a fan at room temperature or for a minimum period over a hot water bath.

Every fraction obtained in the above procedure was mixed with a few drops of ether and 0.04 ml. of Tween 80, emulsified in 5 to 10 ml. of water and injected intraperitoneally into dogs.

RESULTS

Unanaesthetized animals. All the animals studied showed tranquillization and intention tremors (detectable after muscular exertion) with small doses of the extract. Severe tremors, clonic convulsions and death occurred after larger doses. Ether, pentobarbitone, hexobarbitone or mephenesin abolished the tremors and clonic convulsions.

Within half an hour after injection, the animals were usually quiet, relaxed and easy to handle; body movements and aggressiveness decreased and resistance to restriction (caging, chaining) often disappeared completely. A cataleptic state was occasionally observed. If undisturbed, the animals would go into a sleep-like condition but still responded to external stimuli. Feeding, micturition, defaecation and stools were normal. In dogs, there was asynchronous blinking of the two eyes, but no change occurred in pupil size or tone of the nictitating membrane.

Vomiting was occasionally observed in dogs. In 5 dogs, an effective dose of the extract did not prevent vomiting induced by apomorphine (0.2 mg/kg given intramuscularly). After injecting 5 to 10 mg/kg, a full effect was elicited in dogs; the tremors subsided within 2 to 3 days and tranquillization subsided within 4 to 6 days after a single injection.

The cats were found to be the most sensitive and mice the most resistant species, the effective doses being approximately 1 and 400 mg/kg, respectively.

Two to twelve hours after injecting the extract, the surviving rabbits were sacrificed, the livers removed and fed to dogs. In 13 out of 20 experiments, dogs showed tranquillization and tremors. This suggests that the active principle is present in considerable amounts in the liver.

In all these experiments a mild degree of tranquillization without intention tremors was not taken into consideration to avoid subjective error. Tween 80, the emulsifying agent, exerted little action of its own, except that it occasionally produced, because of histamine release, erythema and itching in conscious dogs and marked hypotension, following intravenous administration, in anaesthetized dogs.

Acute toxicity. The LD50 in mice by intraperitoneal injection was found to be 0.88 g/kg. Control mice receiving the solvent were entirely unaffected. In another experiment, 15 mice out of 24 died within 24 hr after receiving a certain amount of the extract as against 3 out of 24 receiving the identical amount of extract as well as 50 mg/kg of sodium pentobarbitone.

Hexobarbitone sleep. In proportion to the dose, the extract potentiated the effect of anaesthetic and sub-anaesthetic doses of hexobarbitone (Table 1). This effect was still detectable 18 hr but not 2 days after a single injection.

TABLE 1

THE EFFECT OF THE EXTRACT ON SLEEPING-TIME DUE TO HEXOBARBITONE

Hexobarbitone sodium solution 10 mg/ml. *P* (probability) calculated by " t " test

Extract mg/kg	Hexobarbitone mg/kg	No. of mice	Sleeping-time ±s.e. (min)	Remarks
—	100	17	19±6·2	
100	100	18	40±6·9	$P<0·01$
300	100	16	57±13·0	$P<0·01$
300	100	10	38±10·0	$P<0·01$, hexobarb. 18 hr after extract
300	100	10	28±10·0	Hexobarb. 24 hr after extract
300	100	10	16±5·7	Hexobarb. 48 hr after extract
—	50	32	3	3 mice slept
50	50	20	8±4·1	9 mice slept
100	50	19	17±4·6	14 mice slept
300	50	18	19±4·8	18 mice slept
Reserpine 5 mg/kg	100	13	42±10·2	$P<0·01$. Hexobarb. 1 hr after reserpine and 48 hr after 300 mg/kg extract

Ether anaesthesia. Chlorpromazine as well as the extract clearly prolonged the ether sleeping-time (Table 2). However, as in the work of Mensch & DeJongh (1959), there was wide variation in each group of values.

TABLE 2

THE EFFECT OF THE EXTRACT AND CHLORPROMAZINE ON ETHER SLEEPING-TIME IN RATS

Group	No. of rats	% rats not sleeping after ether exposure	Average (and range) of sleeping-time in sec	% rats sleeping longer than 210 sec
Control	84	21	107 (5–450)	8·3
Chlorpromazine 5 mg/kg	34	0	422 (60–900)	88·2
Extract 150 mg/kg	24	0	408 (150–1,020)	79·3

Rectal temperature. The fall in rectal temperature was maximal within 30 min of injection. This was followed by a slow and incomplete recovery during the remaining period (Fig. 2). In some experiments, rats, but not mice, had a subnormal temperature 24 hr after injecting the extract. The degree of tremor could not be correlated with that of hypothermia. Keeping the mice at 35° C or injecting 100 mg/kg of atropine sulphate had no apparent effect on the tremors.

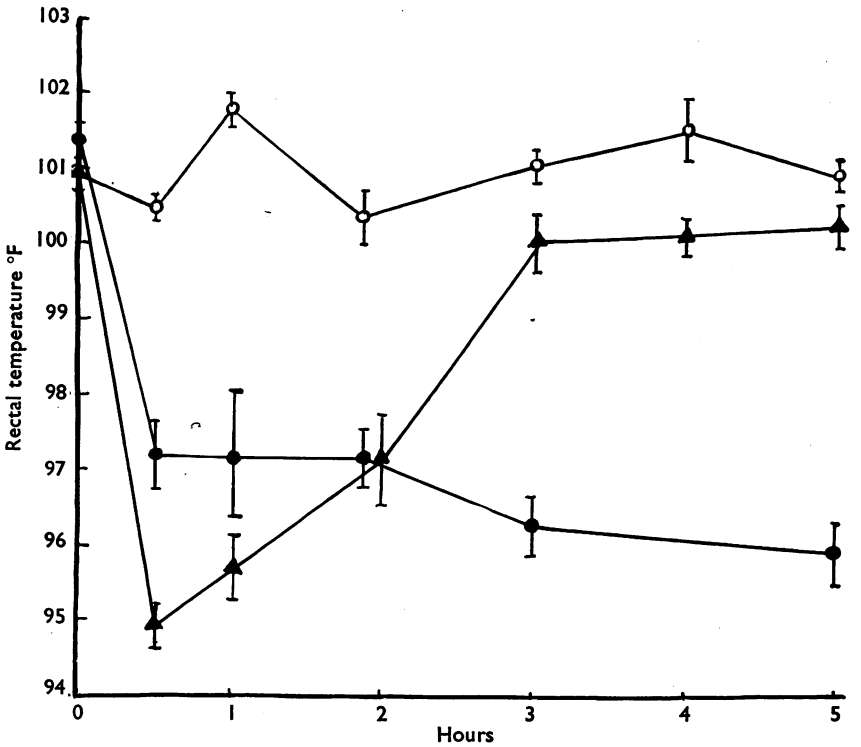

Fig. 2. Effect of extract on rectal temperature of rats and mice. Room temperature, 25° C. ○——○, Rats, Tween 80 in water. ●——●, Rats, 150 mg/kg extract. ▲——▲, Mice, 300 mg/kg extract. Mice receiving Tween 80 had a line similar to ○——○. Each group contained 10 animals. Vertical lines indicate standard error.

Leptazol toxicity. At and above 120 mg/kg, the extract enhanced leptazol toxicity; most of the deaths occurred during severe tonic convulsions within 2 hr of injecting leptazol (Table 3).

TABLE 3

EFFECT OF THE EXTRACT ON THE LEPTAZOL TOXICITY IN RATS

Leptazol (14 mg/ml.) was injected subcutaneously

Drugs and dose mg/kg	Leptazol 70 mg/kg	No. of rats	% mortality
—	Injected	25	0
Extract 180	—	13	0
Extract 360	—	15	40
Solvent	Injected	15	0
Extract 70	Injected	9	0
Extract 120	Injected	9	44
Extract 180	Injected	20	60
Extract 360	Injected	13	100

Pigeon emesis. Within 1 hr of injecting the extract, pigeons vomited in 53 out of 60 experiments; the remaining 7 promptly vomited after repeating the dose. In control experiments pigeons vomited in 3 out of 60 experiments.

Amphetamine toxicity. The extract did not protect the mice in groups against amphetamine. If at all, it slightly increased the toxicity of amphetamine. Olive oil emulsion was also found to increase the toxicity, although it had no detectable action of its own in control mice (Table 4).

TABLE 4

THE EFFECT OF THE EXTRACT ON AMPHETAMINE TOXICITY

Other substances intraperitoneally (2 ml./100 g) 30 min before subcutaneous amphetamine sulphate (2 mg/ml.). Solvent=control emulsion of Tween 80-ether in water. *P*=probability by the chi-square method

Amphetamine mg/kg	Other substances mg/kg	No. of mice	% mortality in 24 hr	*P*
20	—	40	5	
20	400, extract	16	19	<0·1
20	Solvent	16	6	
20	Solvent containing 2·5% of olive oil	24	54	<0·01
25	—	16	50	
25	400, extract	16	81	<0·01
30	—	16	88	
30	400, extract	24	94	<0·5

Morphine rage in cats. After injecting 4 to 6 mg/kg of morphine, mydriasis, anxiety, restlessness and frantic bouts of dashing against the cage wall appeared within 1 hr in that sequence and lasted for at least 5 hr. Chlorpromazine reduced the signs of rage though its effect tended to decline after about 2 hr. Reserpine completely suppressed all the signs; its effect lasted throughout the experiment. Mydriasis was more effectively reversed (to miosis) by reserpine than by chlorpromazine. The extract (in 1 to 4 mg/kg) abolished restlessness and maniacal bouts, but mydriasis persisted. However, with doses higher than 4 mg/kg of the extract, morphine rapidly produced unconsciousness, convulsions and death.

Dog blood pressure. In 6 control experiments on anaesthetized dogs, olive oil-sodium alginate emulsion had little effect on the blood pressure for at least 3 to 6 hr, except for a slight initial rise. Electrocardiographic activity and the carotid occlusion reflex were unaffected, although the quantity of sodium alginate greatly exceeded that employed in experiments with the extract.

In the second group of 6 anaesthetized dogs, 10 to 25 mg/kg of the extract (with olive oil-alginate in 0.9% sodium chloride solution) depressed the carotid occlusion reflex without significantly affecting the blood pressure (Fig. 3). This action appeared within 1 hr and lasted for at least 4 hr. Doses of the extract exceeding 75 mg/kg lowered the blood pressure within 2 hr to about half of the initial value. At this steady low level, the carotid occlusion reflex was greatly decreased but the electrocardiogram remained unaffected. The effect of adrenaline was unaltered after giving the extract. In 2 dogs 200 mg/kg of the extract produced a rapid and fatal fall of blood pressure.

Isolated guinea-pig ileum. Even larger amounts of the extract had no effect on the actions of acetylcholine, histamine or barium chloride. However, it is possible

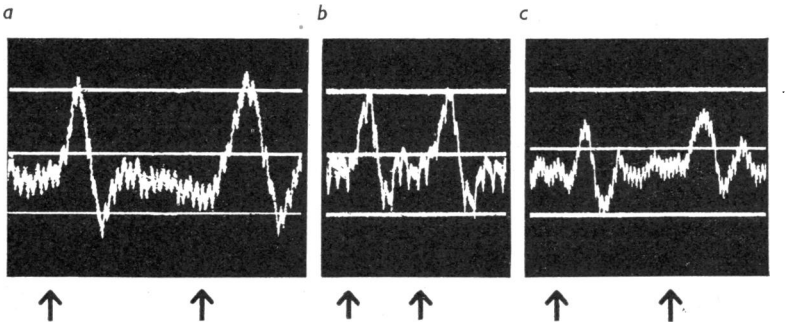

Fig. 3. Femoral artery blood pressure of a dog. (*a*) Initial blood pressure, 112 mm of mercury. (*b*) and (*c*) 1 and 3 hr respectively after 25 mg/kg of the extract. Arrows approximately indicate start of 10 sec carotid occlusion. Distance between horizontal lines=20 mm of mercury.

that in the emulsion form (with Tween 80) the extract might not have readily come in contact with the tissue even after 5 min exposure.

Experiment in man. Forty mg/day for 3 days produced, on the third day, considerable tranquillization as well as flushing of the face, yawning, feeling of tiredness, fine intention tremors of the extremities and (as judged objectively) " thick speech." On the fourth day of taking 70 mg/day there was, in addition to the exaggeration of the above-mentioned symptoms, marked orthostatic giddiness and nausea, followed by a deep sleep (not unconsciousness) from 9.30 a.m. to about 6 p.m. The electroencephalographic record, pulse rate and axillary temperature remained unaffected. Blood pressure generally tended to fall on the second and third day of the experiment (normal variation of blood pressure—116 to 136/78 to 96 mm mercury ; blood pressure on the second and third day of taking the extract 100 to 124/64 to 88 mm mercury).

Fractionation. Of all the fractions of the extract, only 4 were found to be effective in dogs (numbered I, II, III, IV in Fig. 1). Fraction I, soluble in water, acetone and ethanol but not in ether, chloroform, petroleum ether or benzene, gave a blue colour with phosphomolybdic acid and ammonia solution and blue-green colour with ferric chloride solution. It could be adsorbed on alumina from neutral but not acidified water. These findings suggest that the structure contains a catechol group. It was more unstable than and about 1/10th as active as the petroleum-ether-soluble fraction. Fractions II, III and IV were amorphous, waxy, yellow solids soluble in ethanol, acetone, petroleum ether, benzene and chloroform. Fraction IV gave an absorption peak at 228 mμ in the ultra-violet range. Unlike fractions III and IV, fractions I and II apparently produced more catalepsy than tremors.

DISCUSSION

Considerable variation in the % yield and activity of the extract was observed with different samples of the grain. The available evidence (Bonavia, 1867 ; Abdus Sattar, 1930) does not clearly indicate whether the active principle is derived from the plant or its parasitic fungus. The dried ethanol extract was found to be much

more effective than the active fraction prepared by the previous method (Bhide & Aimen, 1959).

Vomiting, present in human poisoning, was seen in some conscious dogs, irrespective of the route of administration. However, it was induced more promptly after parenteral than after oral administration of the extract. The possibility that vomiting is secondary to hypotension is not ruled out. In the experiment in man, nausea occurred only after assuming the erect posture, and this suggests that the active substance exerts a central emetic action.

Tremor and rigidity of a parkinsonian type are seen after large doses of major tranquillizers. The tremor produced by the extract appeared to be of central origin, since depressants of the central nervous system always abolished it.

In acute toxicity studies, mice receiving pentobarbitone were clearly protected against fatal doses of the extract. Furthermore, anaesthetized dogs could tolerate about 10 times the dose fatal to conscious ones. These findings indicate that death in acute experiments is due to asphyxia during convulsions. They may be of some value in the treatment of cattle poisoning.

Both cholesterol and oil are known to alter the effects of different anaesthetics in animals (Foldes & Beecher, 1943 ; Farson, Carr & Krantz, 1947 ; Hong & Cho, 1959). However, potentiation of the effect of hexobarbitone in mice could not be due entirely to lipoid material in the extract, because it was seen previously in mice receiving the water-soluble active fraction (Bhide & Aimen, 1959). Since potentiation also occurred 18 hr after giving the extract (Table 1), when the rectal temperature was normal, it could not be due exclusively to hypothermia (Dandiya, Cullumbine & Sellers, 1959).

Tranquillizers which produce prolonged lowering of amine levels in the brain fail, if reinjected after 48 hr, to prolong hexobarbitone sleeping-time in mice (Bhide & Aimen, 1959 ; Bhide, unpublished work). Reserpine could still prolong the hexobarbitone sleeping-time, when given 48 hr after the effective doses of the extract (Table 1). This suggests that the extract and reserpine may not be acting by an identical mechanism.

Substances interfering with the metabolic inactivation of barbiturates also prolong their sleeping-time. Since ether undergoes no change in the body, prolongation of its effect by a substance would more clearly indicate true potentiation. The simplicity of technique and shorter duration of anaesthesia with ether would seem additional advantages over the barbiturate sleeping-time methods. However, wider variation in values and lack of control over the ether intake by individual animals would tend to permit only qualitative interpretation of the results.

The extract, like many other agents including major tranquillizers, produced hypothermia in rabbits, mice and rats. Burn (1956) reviewed the literature and suggested that hypothermia results from a decrease of skeletal muscle tone due to a peripheral action of some of these drugs. This would not agree with the present results, as hypothermia was observed in animals showing considerable tremor. It is possible that the hypothermia induced by tranquillizers is due to a depressant action on hypothalamic heat regulation centres.

Increase in leptazol toxicity could be due, in part, to hypothermia (Swinyard & Toman, 1948 ; Hahn, 1960) produced by the extract. Direct sensitization of the central nervous system by the extract to the convulsants is also possible. The potentiating effect of reserpine on electroshock and drug-induced seizures is well known (Bein, 1956).

Like reserpine (Earl, Winters & Schneider, 1955), the extract and one of its purified fractions (IV) induced vomiting in pigeons. The interpretation of pigeon-emesis is difficult, as it is also evoked by mepacrine, digitalis and veratrum alkaloids.

Unlike reserpine and chlorpromazine (Burn & Hobbs, 1958), the extract did not reduce mortality in the groups of amphetamine-stimulated mice. There was a slight paradoxical increase in mortality after the extract. Increased mortality was also seen in groups receiving olive oil emulsion. Similarly, mice receiving the tranquillizing volatile oil of *Acorus calamus* (Dandiya & Cullumbine, 1959) were more susceptible to amphetamine. It is possible that the above substances, because of their lipoid character, sensitize the nervous system to amphetamine. However, interference with hepatic detoxication of amphetamine cannot be excluded.

Since morphine rage appears in cats after complete removal of both the cerebral hemispheres but not after removal of the hypothalamus, the site of action appears to be on the subcortical centres in the hypothalamus (Sollmann, 1957). Tranquillizers presumably act at comparable levels. This may explain the suppression of morphine rage by reserpine and chlorpromazine (Sturtevant & Drill, 1957) and of " shame rage " by reserpine (Schneider, 1955). The extract produced suppression of rage (but not of mydriasis) ; this might be due, to some extent, to the increased muscle tone and tremor.

Sundaram Ayyar & Narayanaswamy reported that the " fatty " portion of the grain was insoluble in 90% ethanol (1948b) and was ineffective in cats and pigeons (1946, 1948a). There is no reference to the tranquillizing effect of the extract in any of their reports.

As the dose of the extract in the experiment in man was about 5 times that which produced tranquillization in acutely disturbed schizophrenics after 3 to 4 days of administration (Deo & Bhide, 1961), it is possible that the effects experienced were not due entirely to " suggestion." However, the final assessment of the extract will be possible only after further chemical and clinical investigations.

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