I. P. G. T. & R., JAMNAGAR.  
PHARMACOLOGY LABORATORY  
EVALUATION OF ............................................ ON GROSS BEHAVIOUR  
IN MICE  

Date: Group No: Route:  

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Time after drug/extract admn.</th>
<th>Exitus</th>
<th>CNS DEPRESSION</th>
<th>EYE LID OPENING</th>
<th>CNS - STIMULATION</th>
<th>Other observations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypoactivity</td>
<td>Passivity</td>
<td>Relaxation</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>240 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 &amp; 72 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX-II

Source of chemicals used in the study

1. Acetic acid : (BDH)
2. Acetylcholine chloride : (Loba)
3. Adrenaline hydrochloride : (Loba)
4. Alcohol ethyl : (Alembic)
5. 4-Aminoantipyrine Puriss : (Loba)
6. Anaesthetic ether : (Nirayu Pvt.Ltd.)
7. Ascorbic acid : (Sarabhai Chemicals)
8. Betamethasone Sodium Phosphate : (Glaxo)
9. Bovine albumin fraction v : (CDH)
10. Bradykinin acetate (Creatinine sulphate) : (Sigma) (USA)
11. Carbachol chloride : (Sigma) (USA)
12. Carbon tetrachloride : (MERCK)
13. Carrageenan sodium : (Sigma) (USA)
14. Castor oil : (Metro Pharma)
15. Chlorpromazine hydrochloride : (May & Baker)
16. Cholesterol : (SDS)
17. d-Amphetamine sulphate : (BDH) (UK)
18. dl-Alanine : (Loba)
19. dl-Amphetamine : (MERCK)
20. dl-Aspartic acid : (Loba)
21. Evan's Blue : (Wilson)
22. Formaldehyde : (Sarabhai Chemicals)
23. Freund's adjuvant complete : (Difco) (USA)
24. Glucosamine hydrochloride : (Sigma) (USA)
25. Glucose Standard : (Glaxo)
26. Haemoglobin powder : (CDH)
27. Heparin sodium : (Loba)
28. Histamine diphosphate : (John Baker)
29. Hydrogen peroxide : (MERCK)
30. 5-Hydroxytryptamine (Serotonin Creatinine sulphate) : (Boehringer-Ingeleim) (W.Germany)
31. Hydroxy proline : (Loba)
32. Imipramine hydrochloride : (May & Baker)
33. Indomethacin : (Wilson)
34. Isoprenaline hydrochloride : (Boehringer-Ingeleim) (W.Germany)
35. L(-) Tyrosine : (Loba)
36. Nor-adrenaline : (Fluka)(Switzerland)
37. Nystatin : (CDH)
38. Oxotremorine sesquifumarate : (Sigma) (USA)
39. Oxytocin : (SANDOZ)
40. Paracetamol : (Burroughs-wellcome)
41. Pentobarbitone sodium : (Loba)
42. Pentylenetetrazole : (Sigma) (USA)
43. Peptone : (Qualigen-Glaxo)
44. Pethidine hydrochloride
45. Phenobarbital sodium : (May & Baker)
46. Phenol : (Sarabhai Chemicals)
47. Phenylbutazone sodium : (Wilson)
48. Procyclidine hydrochloride : (Burroughs wellcome)
49. Prostaglandin E2 : (Up John)(UK)
50. Pyruvic acid : (Johnson Chem.)
51. Reserpine : (Boehringer-Ingelheim) (W.Germany)
52. RNA & DNA : (Sigma) (USA)
53. Russell’s viper venom : (Sigma) (USA)
54. Strychnine sulphate : (Sigma) (USA)
55. Triolein : (BDH) (UK)
56. Triple antigen : (Haffkine Institute)
57. TRIS (Hydroxy-methyl) aminomethane (L.Light & Co.,U.K.)
58. Tween-80 : (CDH)
59. Urethane : (Reiedel-dehaenag) (W.Germany)
ERRATA / ADDENDUM

(1) Units have been provided in all the figures. Corrected in Fig. 18 it should be mg/100g body weight.

(2) The data were fed to computer and the figures were drawn employing "Harvard Graphics" programme for 3 dimensional figures, the figures obtained were printed in a Laser 'Printer' photocopy of which is incorporated into the thesis (See additional reference 1 - Planta Med 57-2-1991)

(3) All SEM indicated by the referee have been checked and found to be correct.

(4) Toxicity is given in the form of LD50 on page 117.

(5) Phytochemical analysis table containing results given in Page 199 and reference is given in summary page 213 and discussion parts page 117.

(6) Observation of dose independent nature of activity is quite common with plant extracts and it has been explained as due to the presence of different active principles often with opposing pharmacological activity (see addtl. references 1,2,3,4,5). Such type of activity is even reported with Prostaglandins (e.g. addtl. reference 2)

REFERENCES


(3) Bhattacharya et al., J. Pharm. Sc. 65 (1976).


Additional References - 1


Hypoglycemic Activity of Triterpenes and Tannins from Sarcopoterium spinosum and two Sanguisorba Species

G. Reher¹, M. Slijepcevic², and Lj. Kraus³

¹ Lehrstuhl für Pharmakognosie der Universität Hamburg, Bundesstraße 43, D-2000 Hamburg 13, Federal Republic of Germany
² R. Boskovic Institute. Bijenicka 54. YU-41001 Zagreb, Yugoslavia

The root bark of Sarcopoterium spinosum Spach = Uerium spinosum L. (Rosaceae) is used to treat diabetes mellitus in the traditional medicine of the eastern Mediterranean region (1). In order to evaluate its pharmacological activity, experiments on hyperglycemic mice were undertaken. Hyperglycemia was provoked by i.p. injection with alloxan (65 mg/kg).

Three isolated triterpenes were tested: substances 1 (23-hydroxytormentic acid ester glucoside) and 2 (23-hydroxytormentic acid) have been isolated from the root bark of Sarcopoterium spinosum and also from the root bark and the aerial parts of Sanguisorba minor Scop. (Rosaceae) (2). Substance 3 (3-O-α-arabinopyranosylpomolic acid ester glucoside) (3) has been isolated from the root bark and the aerial parts of Sanguisorba officinalis L., but was absent in Sarcopoterium and Sanguisorba minor. The pharmacological results are summarized in Figs. 1 – 3. Statistical significance is marked.

The tannins of the root bark of Sarcopoterium spinosum were investigated concerning the proanthocyanidins in 1987 (4). For the pharmacological test three different tannin extracts were prepared: a fraction consisting of only polymers as well as a fraction of di-, tri-, and oligomer proanthocyanidins and (+)-catechin have not shown any activity. The fraction 5 – 6, however, a combination of tannins and triterpenes (ca. 95 : 5), lowered the blood sugar level statistically significant in a concentration of 300 mg/kg (Fig. 4).

Experimental conditions:

Experimental animals: groups of 6 – 10 male mice (Stamm CBA/IR Zg) weighting 26 ± 3 g. Glycemia was measured by the glucose-oxidase method with o-toluidine (5). Only mice with a blood sugar level ≥ 300 mg/dl were used for the test. The triterpenes and tannines dissolved in a little DMSO were applied per os as aqueous solutions. 15. 45. and 75 min after application the glucose level was measured. Control mice were given DMSO-H₂O mixtures.
PROSTAGLANDINS

Physiology, Pharmacology &
Clinical Significance

DAVID F HORROBIN

CHURCHILL LIVINGSTONE
Study of Isolated Organs, Tissues and Cells

The use of isolated preparations allows study without anaesthesia and the use of media whose composition is strictly defined and controlled. It also enables careful dose/response studies to be carried out using precise amounts of PGs and drugs like NSAID. Many studies have been carried out of PG actions in such situations but unfortunately in a high proportion of these the potential advantages have been thrown away. It is routine, for example, to add to perfusing fluids concentrations of PGs 100,000 or a million times higher than the concentrations likely to be found in vivo. Such concentrations are justifiable if employed as part of a complete dose/response study but all too often they are tried alone with no attempt to study lower concentrations. The rationale seems to be that many substances have plateau-type dose response curves and that therefore the application of a high concentration will simply enable one to see the maximal physiological response. There are unfortunately two fallacies in this concept:

1. Many PGs in many circumstances have not plateau-type but bell-type dose response curves (Horrobin, 1977). There are now large numbers of observations to this effect. The response often seems to become apparent at a concentration below $10^{-11}$M, to peak between $10^{-11}$ and $10^{-8}$M and either to disappear or to reverse at higher concentrations. Since investigators commonly report work in which the minimum concentration used has been $10^{-7}$M or higher it is clear that the literature is full of misleading reports. Some of these claim that a PG has no effect when in fact a reduction of the concentration used by a 1000 fold would have demonstrated a profound effect. Second the reported action of a PG in a particular situation may be diametrically opposite to its action at physiological levels. A good example of this concerns PG actions on the heart. The literature is full of claims that PGs are anti-arrhythmic and that PGI2 dilates the coronary circulation. These claims are true at concentrations above $10^{-7}$M. But at lower concentrations likely to be present in vivo PGs consistently enhance susceptibility to arrhythmia development (Swift et al, 1978) and PGI2 is a potent coronary vasoconstrictor (Karmazyn et al, 1977).

2. Many PG actions appear at concentrations of $10^{-7}$M and above which are not present at all at lower concentrations. It is therefore very uncertain as to how relevant these are to the understanding of normal physiology.