PHARMACOLOGICAL EVALUATION OF AN AQUEOUS EXTRACT OF ORTHOSIPHON STAMINEUS (MISAI KUCING) FOR ITS DIURETIC PROPERTY.

BY

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To my mother, my wife and my children Nurul Asyikin and Muhammad Suffian.

ACKNOWLEDGEMENT

I wish to express my gratitude to my Supervisor, Associate Professor Gan Ee Kiang, Dean of School of Pharmaceutical Sciences, for his invaluable advice and guidance throughout this project.

Many thanks to all the Pharmacology Laboratory staff members, for their wonderful cooperations and technical assistance.

ABSTRACT

The diuretic property of O. stamineus was evaluated in unanaesthetised, male albino rats. Twelve rats, divided into three groups of four were used. One representing the controls, treated with distilled water(5ml/kg), the second group, treated with Frusemide (10mg/kg) as the standard and finally the third group was treated with the O. stamineus extract, all were injected intraperitoneally.

Before drug administration the animals were deprived of food, but allowed to drink water freely, for six hours. After drug administration, urine was collected hourly for the next six hours.

The urinary sodium and potassium ions concentrations were determined by means of an Atomic Absorption Spectrophotometer, while the chloride ion level in the urine was determined by titration (Volhard's Method). The pH was measured using indicator pH paper, and the urine volume was determined by a calibrated pipette.

CONTENTS

1. INTRODUCTION

1.1 Classification of Orthosiphon stamineus

Kingdom	:	Plant
Division	:	Spermatophyte
Subdivision	:	Angiospermae
Class	:	Dicotyledonae
Order	:	Tubiflorae
Family	:	Labiatae (Lamiaceae)
Subfamily	:	Ocimoideae
Genus	:	Orthosiphon
Species	:	Orthosiphon stamineus

Orthosiphon stamineus is a shrub, sometimes subshrub, often aromatic, stems and branches four-angled. The leaves are opposite and the flowers are bisexual; inflorescences mostly fasciled and axillary, often the opposite pairs form false whorls. Flowers mostly in dense axillary (rarely terminal) clusters, often capitate stamens descending, lying down upon or enclosed in the lower lip. The flowers are white or pale blue, the nutlets are dry and minute(Hsuan Keng -1969)

Orthosiphon stamineus, Benth,(Ridley, 1923) Kumis kuching (cat's whiskers), Ruku hutan (woodland patchouli);in Java, Remuk jung; in Sudanese, kumis uching. It is found from India and China to Australia and the Pa Pacific, in the Peninsula it occurs wild in the north and in the gardens elsewhere. It is cultivated among the Javanese. Decoction of the leaves, also called Java tea is a diuretic(Uphof,J.C. -1968). Contains a glycoside orthosiphonin, an essential oil and tannin.

The active constituents present in the leaves of the plant are not constant throughout the years. The factors that have to be put into consideration are the season at which the leaves are collected, the age of the plant and also the time at which the leaves are collected may cause variations in the composition of the secondary metabolites present in the leaves(Harbone - 1973). Urea was identified (Dietzel, R. & Schmidt, E. - 1936), among the extracts of this plant and determined quantitat quantitatively in the form of ammonia (NH_3) after liberation by the action of urease. Petroleum ether extract (Di Modica, G.& Rossi, P.F - 1958) of the plant shows the presence of sitosterol and amyrin.

1.2 Medicinal Use.

Pharmacologically it has been found that the leaves act as a diuretic, and do not injury to the kidneys. It has long been used in Malaysia in cases of catarrh of the bladder(Burkill J.H - 1966). In Java it is not used alone but with other drugs which stimulate the kidneys. The high percentage of potassium salts present in the leaves, which themselves act on the kidneys, and the glycoside also may be responsible for the diuretic activity.

Extraction of the leaves of O. stamineus, Benth (Societe Pluripharm. Fr. - 1965) gives a Mg. containing product with diuretic activity. Treatment of 10 Kg. dried leaves with 100 - 125 litre ethanol and addition of CaO to pH 7 - 10 gave after filtration and evaporation at a temperature less than 40 degrees Centrigrade in a thin film evaporator, a liquid residue. Spray drying of the residue gave a solid containing Mg. 2.2 - 3.3, Ca. 0.3 - 0,5, and K 9 - 13 %. The solid products effects diuresis in rats. It was found that a simple aqueous or aqueous alcoholic extraction of the leaves gives a product with almost no diuretic activity.

1.3 Diuresis and Diuretics

Diuresis can be defined as an increase in the rate of urine formation. It may be caused by copious fluid intake (water), by pathological states, or by the action of certain drugs. Such drugs are referred to as diuretics. Regardless of the cause of urine formation the site of action of this phemomenon is centred in the kidney. The kidney plays an important role in maintaining the constancy of the internal environment of the organism. It does this by eliminating the waste products of metabolism and thus maintaining a constant volume and composition of the body fluids.

Each of the two human kidneys contain approximately one million nephrons. Each of these nephrons consists of a glomerulus, tubules (both proximal and distal), the loop of Henle, and the collecting tubule - all of which participate in the elaboration of urine. Therefore, the important factors which determine the volume and composition of urine are; (1) glomerular filtration (2) proximal and distal tubular reabsorption, and (3) tubular excretion.

The filtrate passes into Bowman's capsule before going into the tubules. The filtered components are water, electrolytes (NaCl, KCl, $(NH_4)_2HPO_4$, $NaHCO_3$, etc.) and low molecular weight organic substances such as glucose, essential nutrients, and urea. These are highly reabsorbed to maintain the volume and composition of the extracellular fluid. As a matter of fact, in the act of tubular reabsorption 98 - 99 % of the water of the glomerularfiltrate plus electrolytes and organic compounds are reabsorbed through the cell walls of the tubules and back into the bloodstream (Breuner,B.M. and Berliner, R.W.).

Congestive heart failure is a disease process characterised by sodium retention resulting in expanded extracellular fluid volume or edema. The same process of renal tubular reabsorption may accompany cirrhosis of the liver, renal disease, toxaemia of pregnancy, the side - effects of drugs, and other states of fluid retention. In all these situations, treatment directed at the cause is desirable, but treatment of the sodium retention must often include inhibiting renal tubular function to decrease reabsorption of sodium(Frederick H.M. et al). The usefulness of diuretics in the treatment of essential hypertension is correlated with their ability to increase the excretion of sodium. It is, therefore, sodium diuresis (natriusis) rather than merely an increase in urine volume that is the important therapeutic effect of most of the drugs.

The most widely used group of diuretics and their effects are:-

(1) Mercurial diuretics: The organic mercurials act st strongly on the proximal tubule to produce sodium diuresis, with only a minor depletion of potassium. However, the mercurial diuretics can be used only by injection.

(2) Carbonic anhydrase inhibitors: Inhibition of carbonic anhydrase by certain sulphonamides interferes with the ion exchange mechanisms of the distal tubule responsible for the acidification of the urine. The resulting sodium diuresis is transient and accompanied by a disproportionate loss of potassium.

(3) Thiazide diuretics: The most widely used class of diuretics are orally active sulphonamides that have an action similar to that of the mercurials on the proximal tubules but retain some of the properties of the carbonic anhydrase inhibitors from which they were derived.

(4) Two very potent diuretics: Frusemide (Lasix) and ethacrynic acid have a greater effect on the loop of Henle than the thiazides.

(5) Miscellaneous potassium-sparing diuretics: The thiazides cause increased excretion of sodium as well as potassium and may cause potassium depletion as a toxic effect. Diuretics that do not cause a loss of potassium retention are the aldosterone antagonists and some miscellaneous compounds that act directly on the renal tubules.

(6) Acidifying salts: These are the weak diuretics. An example is ammonium chloride.

(7) Osmotic diuretics: These are not used to increase sodium loss but to maintain a high volume of urine or to withdraw water from overhydrated cells.

1.4. Objective of Study

It is the intention of this graduation project to do a pharmacological evaluation of an aqueous extract of the leaves of Orthosiphon stamineus, Benth for its diuretic property. The various factors to be considered in evaluating the diuretic property of the leaves of the above plant is by comparing the sodium ion (Na^+), the potassium ion (K^+) , and the chloride ion (Cl^-) concentrations and also the pH of the urine of the rats treated with distilled water (Control), Frusemide (Standard), and O. stamineus extract. The concentrations of the r respective ions were measured using Atomic Absorption Spectrophotometer except for Cl^- where it was determined by titration by Volhard's method.

1.5 Drug Evaluation and Screening Methods:

1.5 Drug Evaluation and Screening Methods:

When a new drug or a specified type is sought, or when a series of compounds is to be investigated for some pharmacological effects, a program of testing is required to provide information about the compound. The aim of the screening is more limited than for blind screening, and greater precision in the results is often expected. The programme may include the use of quantitative assay, or for comparison with drugs recognised to be quite active representatives of their pharmacological class.

For initial screening of diuretics, the test may be made quite accurate if the number of animals in the group is made sufficiently large. For initial screening, groups of 2, 3 or 4 rats can suffice (Turner - 1965). The animals placed in metabolic cages after dosing, for the collection of a six-hour sample of urine. The effects on urinary volume and on sodium, potassium and chloride contents are evaluated in terms of the response of another group receiving a standard dose of a standard diuretic and a control receiving only water.

Drug evaluation and scanning constitute a tactical problem which usually begins with animal studies and for selected compounds, extrapolated to man. In order to screen effectively the investigator should impose numerous limitations as to the strain, sex and species, route of administration and procedures employed. But, at times there are still factors that may be overlooked.

2. EXPERIMENTAL PROCEDURES

2.1 Materials

2.1.1. Drugs

(a) Frusemide (Lasix) - Farbwerke Hoechst A. G. Frankfurt (Main) Germany.

(b) Extract from Orthosiphon stamineus leaves.

2.1.2. Chemicals

(a) Potassium Chloride - Merk Germany

(b) Sodium Chloride - BDH Chemical Ltd. England.

(c) Concentrated Nitric acid 70 % - May and Baker Ltd. England.

(d) Nitrobenzene - Merck, Germany.

(e) Potassium Chromate - May and Baker Ltd., England.

(f) Ammonium Thiocyanate - Riedel -de Haen ag Seelze, Hanover.

(g) Ferric Ammonium Sulphate - Hayashi Pure Chemical Industries Ltd., Japan.

2.2 Apparatus

(a) Unicam Atomic Sp 191 Absorption Spectrophotometer - Pye Unicam (England).

(b) Metabolic cages (England).

(c) Rotary Vacuum Evaporator - Division of Searle Diagnostics Inc. Fort Lee, New Jersey, U.S.A.

(d) Soxhlet Apparatus

2.3 Methods

2.3.1. Preparation and Standardization of Silver Nitrate Solution (0.01 N).

A 0.01 N Silver Nitrate solution was prepared by dissolving 0.425 g of crystalline Silver Nitrate in distilled water and making the final volume up to 250 ml. in a volumetric flask. It was then standardised using Mohr's method (Day and Underwood, 1974), by titrating with 0.01 N Sodium Chloride solution and using Potassium Chromate solution (0.5 %) as indicator.

2.3.2. Preparation and Standardization of Ammonium Thiocyanate Solution (0.01 N).

A 0.01 N Ammonium Thiocyanate solution was prepared by dissolving 0.76 g. Ammonium thiocyanate in distilled water and making the final volume to 1000 ml. It was then standardised by titration with 0.01 N Silver Nitrate solution in the presence of concentrated Nitric acid 70 % and Ferric ammonium Sulphate solution (10 %) as indicator (Day and Underwood, 1974).

2.3.3. Preparation of acidified Silver Nitrate soln. (0.07N)

This solution was prepared by dissolving 2.97 g Silver Nitrate in a 250 ml. volumetric flask. 60 ml. concentrated Nitric acid was added and followed by sufficient distilled water to final volume, 250 ml.

2.3.4. Preparation of Standard solutions of sodium ion (Na^+) and Potassium ion (K^+) for Atomic Absorption Spectrophotometer calibration.

2.3.4.a. Standard solutions of Na^+ at concentrations 10, 20, 40 and 80 parts per million (p.p.m.) respectively were prepared by dilution using deionised water, from a solution containing 100 p.p.m. Na^+ (i.e. 0.2543 g Sodium Chloride of analytical grade dissolved in deionised water then making it to 1000 ml.

2.3.4.b The same procedure was adopted for the preparation of 10, 20, 40 and 80p.p.m. respectively of K^+ standards from a 100 p.p.m. K^+ solution (i.e, by dissolving 0.1907 g Potassium Chloride of extrapure grade in sufficient deionised water to make 1000 ml.)

The above Standard solutions were contained in plastic bottles to avoid interference by Na^+ and K^+ from glass containers.

2.3.5. Preparation of the leaves of O. stamineus for extraction.

Leaves of the plants were collected from the Botanical Gardens, Penang, at about 11.00 a.m. Only leaves of the mature plants were collected. The leaves were dried in an oven at a fixed te temperature of 45° C to ensure that thermolabile substances present in the leaves were not affected. The drying lasted for 3 days. After the leaves were completely dried they were crushed and powdered using a mortar and pestle and stored in an amber glass bottle to overcome any possible decomposition that might take place.

2.3.6. Extraction of the leaves of O. stamineus.

100 g. of the powdered leaves was weighed out and extracted using a Soxhlet apparatus in 300ml. of ethanol. The extraction was allowed to proceed for 3 hours to ensure complete extraction had taken place. The dark green residue was collected and treated with $Ca(OH)_2$ powder and the pH was adjusted to 9. After achieving this the suspension was filtered and the filtrate collected, dried and evaporated to dryness using a Rotary Evaporator. A thin film was seen and scraped off and 1 g was dissolved in 10 ml deionised water. Not all of it dissolved. It was leftfor sometime shaking occassionally to allow maximum disintegration and dissolution to take place. A suspension was observed. This was filtered off and the residue dried and weighed. The total amount of the O. stamineus extract dissolved was only 0.74 g. Therefore, the solution obtained and used was a 7.4 % O. stamineus extract.

2.3.7. Determination of most suitable dose.

12 male albino rats weighing 200 - 350 g were placed in metabolic cages, divided into groups of four. They were deprived of food but allowed to take water freely. After every hour urine was collected, until six hours. After the sixth hour each group was treated with 4ml/kg., 6ml/kg., and 8ml/kg.water intraperitoneally. It was found that rats treated with 8ml/kg. excreted urine at every hour, and this dose was selected as the control.

After one week the same rats were used to determine the most suitable dose to be used of rats for the Standard. The above method was again employed. The

rats in each group were treated with 10mg., 20mg., and 40mg./kg respectively. It was found that rats treated with 10mg/kg Frusemide produced a reasonable urine at every hour. This dose was selected since the upper doses produced high volumes of urine, as Frusemide is a known powerful diuretic.

For the extract, each group of rats were treated with 2ml/kg, 4ml/kg and 6ml/kg of the extract solution. It was found that rats treated with 4ml/kg of extract solution excreted urine at every hour. Thus this dose was selected.

2.3.8. The Screening Procedure

12 male albino rats weighing 200 - 350 g were placed in metabolic cages. They were divided into 3 groups of four, deprived of food but allowed to drink water freely. At every hour intervals urine samples were co collected for six hours. This was to stabilise the rats and to take the urine flow rate. After the sixth hour each group of rats were treated with 6ml/kg water - control; 10mg/kg Frusemide - Standard and 4ml/kg extract solution intraperitoneally. Urine samples were again collected at hourly intervals for the six hours. The volumes and pH of each sample were measured and recorded. They were then stored in Bijou bottles for the analysis of Na^+, K^+ and Cl^-.

2.4 Analysis of urine samples

The urine samples were diluted suitably.

2.4.1. Measurement of Urine Volume

The volumes of urine collected at each hour interval

were measured by means of a graduated pipette with a a rubber teat attached to its tip. From the volumes obtained the urine flow rate was determined and calculated in terms of ml/kg/min.

4.2 Measurement of urine pH

A pH paper was used to measure the pH of the urine samples since the volumes excreted were not high enough to use a pH meter.

4.3. Measurement of the Na^+ and K^+ concentration in urine.

A Pye Unicam Atomic Absorption spectrophotometer was used to analyse the ions. The machine was operated according to the instructions given in the manual. The monochromotor bandpass was adjusted at 0.8 nm. The operating current for both Na^+ and K^+ was 5.6 mA. The absorbance of K^+ was taken at wavelength 766.5 nm and that for Na^+ was at 589 nm. The lamp current was within the range of 5.5 - 20.0 mA. From the absorbance values obtained for both the ions they were converted to mEq/l. after which the excretion rate of the respective ions were calculated. Necessary dilutions were made.

4.4 Measurement of Chloride (Cl^-) concentration in urine.

The Volhard's method was used in the determination, where an excess of silver nitrate solution was added to the urine, and then back titrating the amount of silver ion (Ag^+) left unreacted with the Cl^-, using thiocyanate (SCN^-) solution in the presence of acid and with ferric ion as indicator.

$$Cl^- \text{ (urine)} + Ag \text{ (excess)} = AgCl + Ag \text{ (unreacted)}$$
$$Ag^+ \text{ (unreacted)} + SCN^- = AgCN$$

The difference between the volumes of SCN^- solution needed to titrate a known volume of Ag^+ solution in the absence of urine, and that same volume of Ag^+ in the presence of urine, gave an equivalency between the conce concentrations of SCN^- and Cl^- in the titration. The concentration of Cl^-, and hence its excretion rate in the urine was then calculated. (Appendix

Note: To obtain a sharper endpoint in the titration of Cl^-, nitrobenzene was added (Day and Underwood, 1974).

3. RESULTS

From Table I, it is seen that the urine flow rate/kg./min. for rats treated with Frusemide 10mg/kg. was the highest compared to those treated with distilled water and the Orthosiphon stamineus extract. This is followed by the urine flow rate of the extract treated rats.

For the excretion rates of electrolytes, it was found that Frusemide treated rats have the highest rate of Na^+ excretion/kg./min. This is followed by the rats treated with distilled water, and finally those rats treated with the extract. For K^+ excretion rate, the Frus Frusemide treated rats had the highest rate. O. stamineus extract treated rats came next, Finally, it is followed by rats treated with distilled water. The chloride (Cl^-) excretion rates were almost the same for rats treated with distilled water and O. stamineus extract, whereas the Cl^- excretion rate for rats treated with Frusemide were markedly high.

Table 1.

Data for urine flow rate of rats treated with control, standard and extract.

Time (Hours)	Mean + S . E . M . (ml/kg/min.)		
	Control	Standard	Extract
-6	0.02 ± 0.03	0.018 ± 0.19	0.126 ± 0.02
0	D R U G	A D M I N I S T R A T I O N	
1	0.106 ± 0.03	0.498 ± 0.19	0.126 ± 0.02
2	0.094 ± 0.03	0.126 ± 0.02	0.076 ± 0.01
3	0.101 ± 0.02	0.125 ± 0.02	0.076 ± 0.01
4	0.074 ± 0.01	0.326 ± 0.37	0.068 ± 0.02
5	0.095 ± 0.01	0.104 ± 0.01	0.056 ± 0.01
6	0.084 ± 0.02	0.081 ± 0.01	0.058 ± 0.02

Dose administered:

Control (Distilled water) - 8ml/kg.
Standard (Frusemide) - 10 mg/kg.
Extract (O. stamineus) - 296 mg/kg.

Table 2

Data for Sodium ion (Na^+) excretion rate of rats treated with control, standard and extract.

Time (Hours)	Mean ± S.E.M. (mEq/kg/min.)		
	Control	Standard	Extract
-6	1.610 ± 0.37	2.175 ± 0.39	1.53 ± 0.25
0	D R U G A D M I N I S T R A T I O N		
1	0.182 ± 0.05	63.67 ± 20.7	0.11 ± 0.04
2	0.108 ± 0.02	20.41 ± 11.2	0.06 ± 0.04
3	0.153 ± 0.04	15.01 ± 6.56	0.036 ± 0.02
4	0.077 ± 0.02	8.42 ± 2.50	0.04 ± 0.02
5	0.153 ± 0.13	9.39 ± 4.02	0.07 ± 0.03
6	0.411 ± 0.30	9.24 ± 7.60	0.06 ± 0.03

Dose administered:

Control: (Distilled water) - 5ml/kg.
Standard: (Frusemide) - 10 mg/kg.
Extract: (O. stamineus) - 296 mg/kg.

Table 3

Data for Potassium ion (K^+) excretion rate of rats treated with control, standard and extract.

Time (Hours)	Mean + S.E.M. (mEq/kg/min,)		
	Control	Standard	Extract
-6	1.67 ± 0.31	1.55 ± 0.14	1.62 ± 0.15
0	D R U G A D M I N I S T R A T I O N		
1	8.9 ± 5.52	10.33 ± 2.73	14.1 ± 9.36
2	5.99 ± 1.90	13.75 ± 3.60	11.68 ± 3.89
3	9.91 ± 8.91	12.15 ± 6.02	7.19 ± 0.34
4	7.96 ± 5.34	11.41 ± 10.03	7.63 ± 2.49
5	9.16 ± 4.87	11.38 ± 3.20	5.94 ± 1.55
6	8.28 ± 6.20	6.77 ± 2.37	3.60 ± 0.73

Dose sdministered:

Control: (Distilled water) - 8 ml/kg.
Standard: (Frusemide) - 10 mg/kg.
Extract: (O. stamineus) - 295 mg/kg.

Table 4

Data for Chloride ion (Cl^-) excretion rate of rats treated with control, standard and extract.

Time (Hours)	Mean + S.E.M. (mEq/kg/min.)		
	Control	Standard	Extract
-6	0.793 ± 0.40	±·01 ± 0.56	0.62 ± 0.32
0	D R U G A D M I N I S T R A T I O N		
1	1.97 ± 0.20	59.19 ± 19.20	1.53 ± 0.04
2	1.25 ± 0.32	19.03 ± 10.70	1.50 ± 0.33
3	1.90 ± 0.59	12.74 ± 6.10	1.20 ± 0.35
4	1.33 ± 0.24	7.36 ± 2.38	1.27 ± 0.31
5	2.0 ± 0.91	8.44 ± 4.20	1.37 ± 0.30
6	1.75 ± 0.32	8.04 ± 6.85	1.85 ± 1.00

Dose administered:

Control: (Distilled water) - 6 ml/kg.
Standard: (Frusemide) - 10 mg/kg.
Extract: (O. stamineus) - 296 mg/kg.

Fig. 1a.

Graph showing the urine flow rate of rats after administration of distilled water (8 ml/kg).

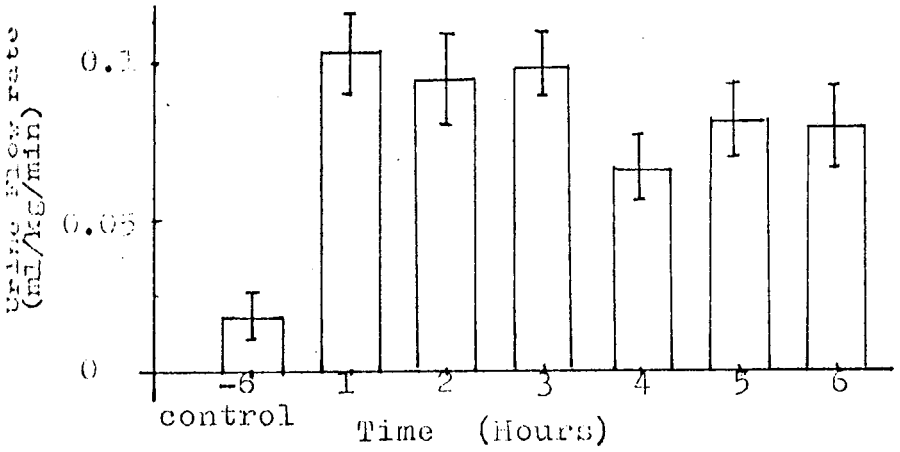


Fig. 1b.

Graph showing the urine flow rate of rats after administration of Frusemide (10 mg/kg).

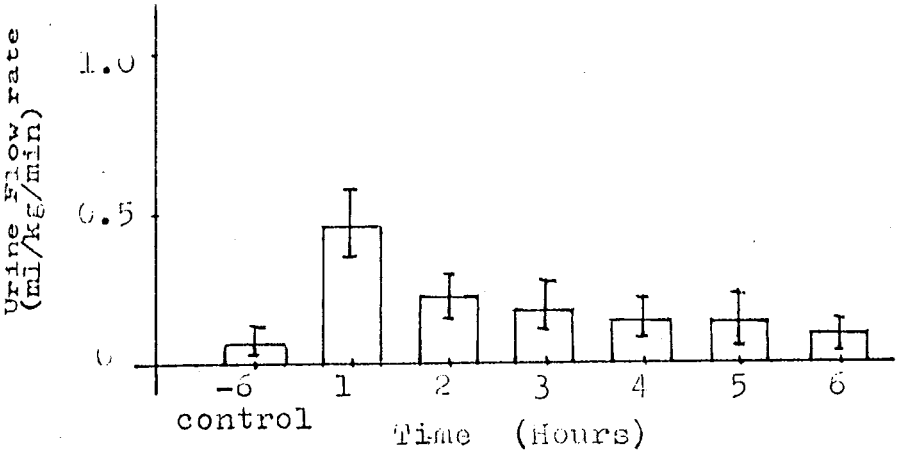


Fig. 1 c.

Graph showing the urine flow rate of rats after administration of O.stamineus extract (296mg/kg).

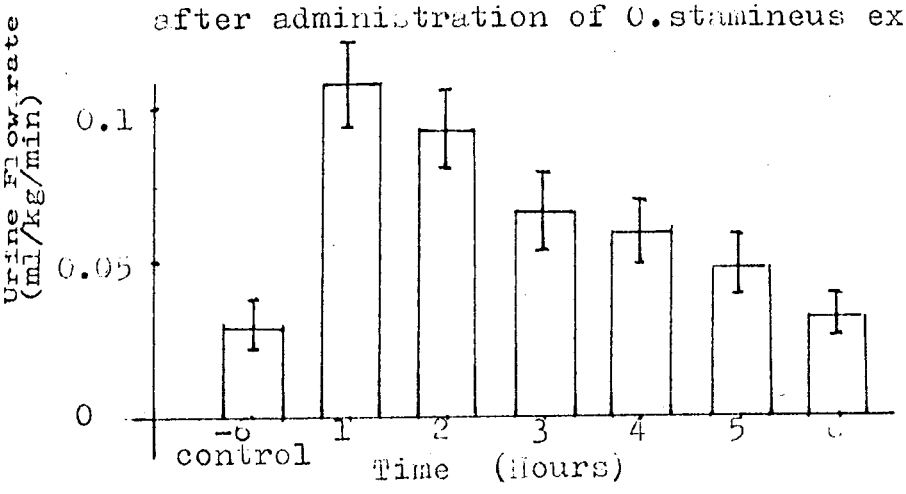


Fig. 2a.

Graph showing the Sodium ion (Na^+) excretion rate of rats after administration of distilled water (8 ml/kg).

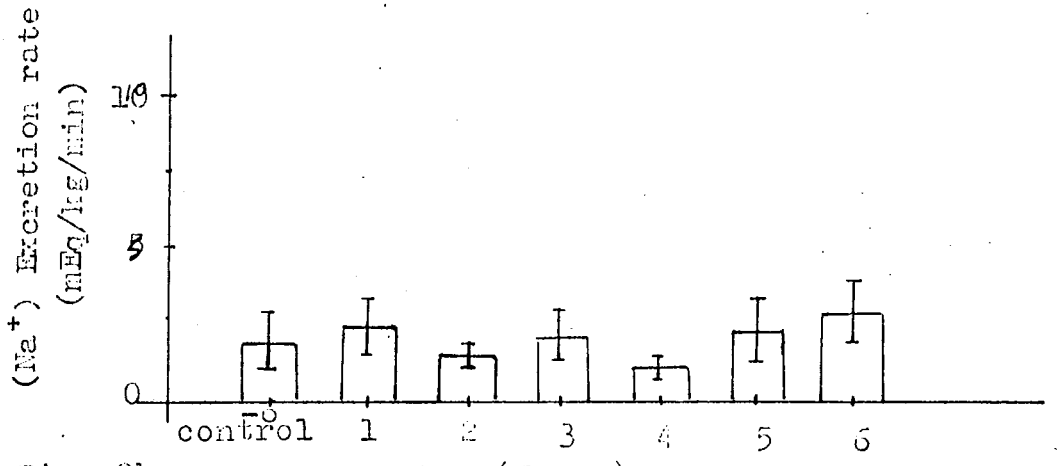


Fig. 2b.

Graph showing the Sodium ion (Na^+) excretion rate of rats after administration of Frusemide (10 mg/kg).

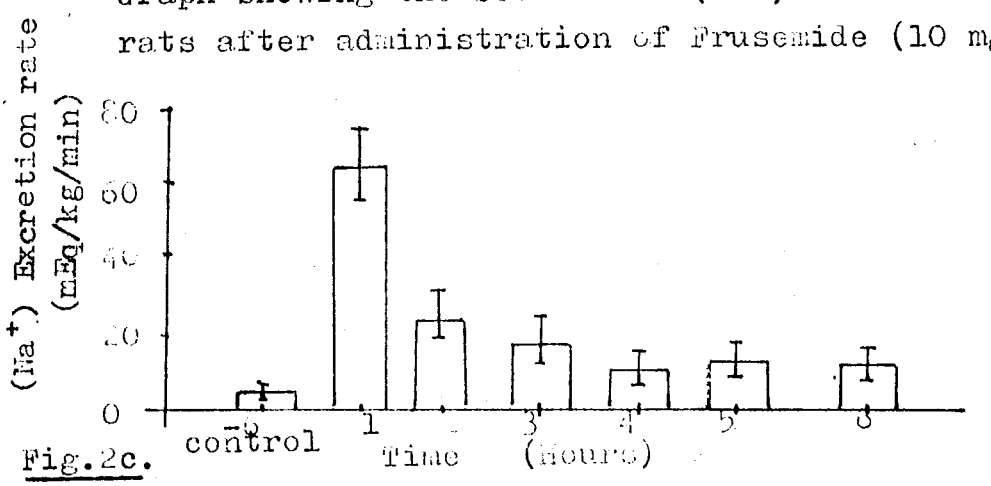


Fig.2c.

Graph showing the Sodium ion (Na^+) excretion rate of rats after administration of O. stamineus extract(296mg/kg)

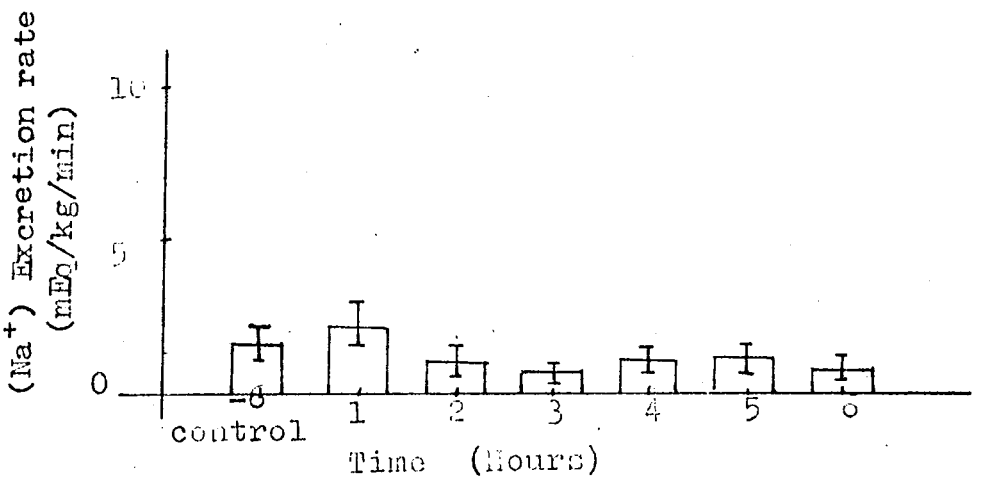


Fig. 3a.

Graph showing the Potassium ion (K^+) excretion rate of rats after administration of distilled water (8 ml/kg).

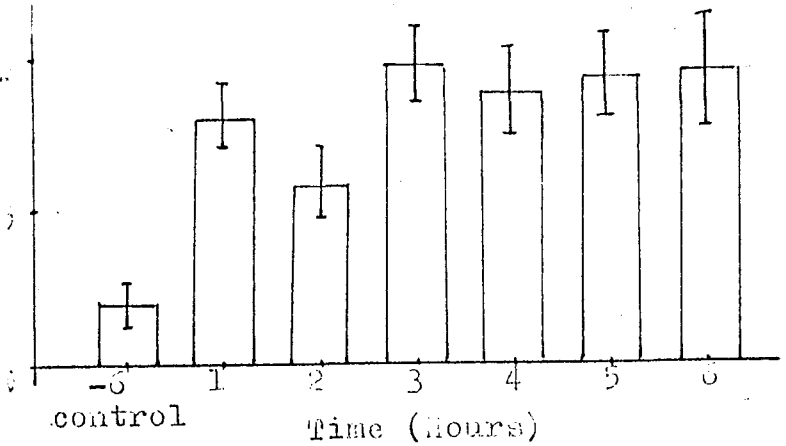


Fig. 3b.

Graph showing the Potassium ion (K^+) excretion rate of rats after administration of Frusemide (10 mg/kg).

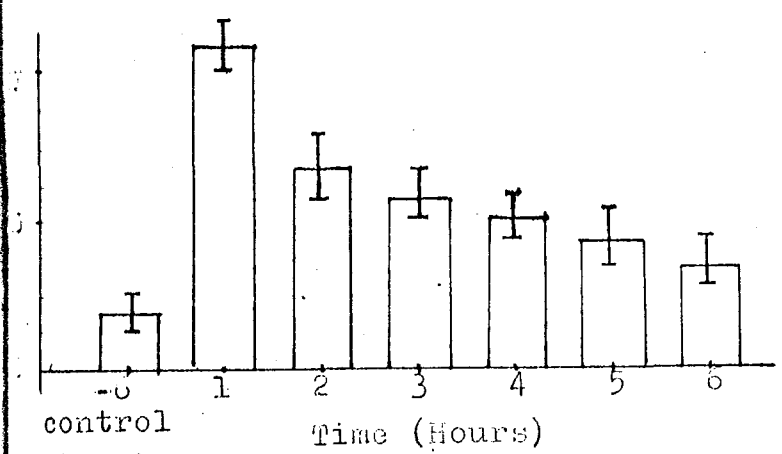


Fig. 3c.

Graph showing the Potassium ion (K^+) excretion rate of rats after administration of O. stamineus extract(296mg/kg).

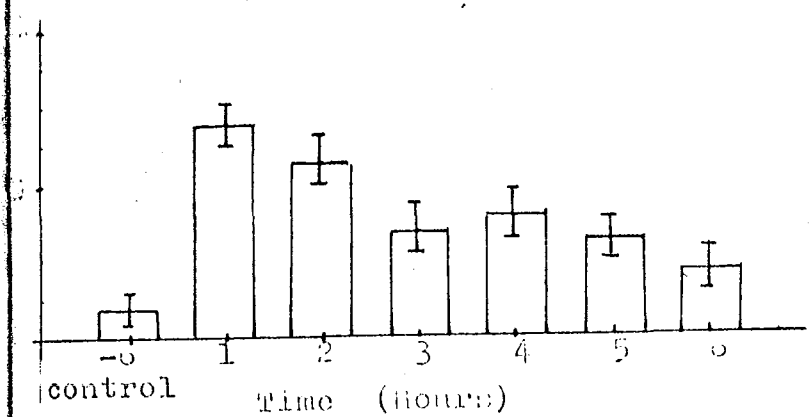


Fig. 4a.

Graph showing the chloride ion (Cl^-) excretion rate of rats after administration of distilled water(8 ml/kg).

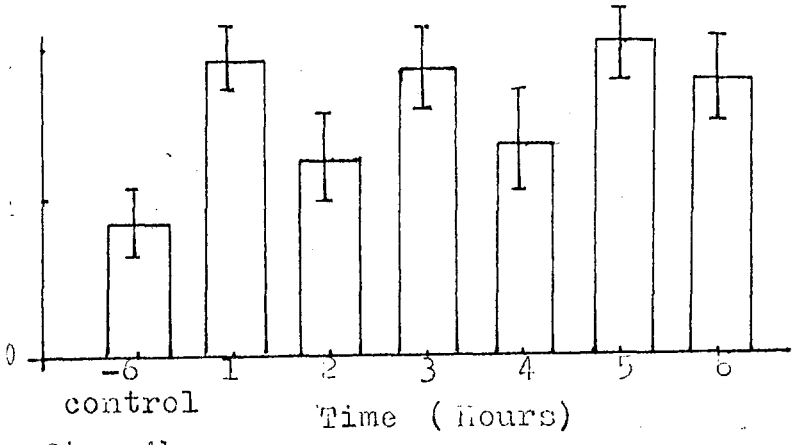


Fig. 4b.

Graph showing the Chloride ion (Cl^-) excretion rate of rats after administration of Frusemide (10 mg/kg).

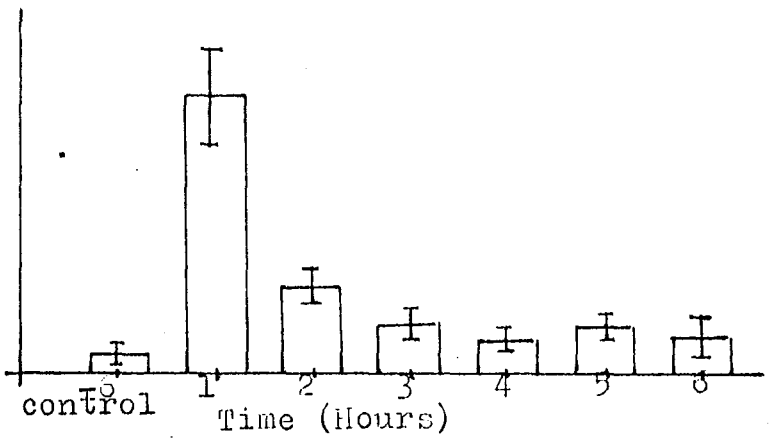
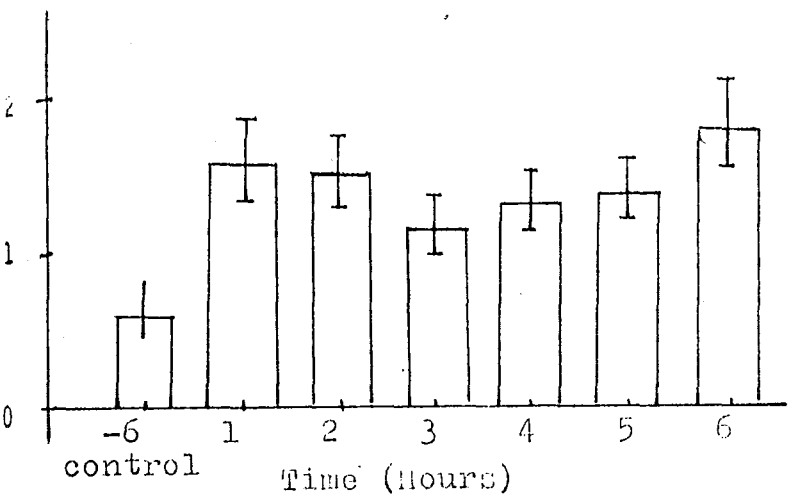


Fig. 4c.

Graph showing the Chloride ion (Cl^-) excretion rate of rats after administration of C. stamineus extract(296 mg/kg)



Data for urine flow rate in Control experiment

Time (Hours)	Urine flow rate (ml/kg/min.)				Mean + S.E
	Rat I	Rat II	Rat III	Rat IV	
-6	0.02	0.018	0.026	0.022	0.022 ± 0.003
0	Distilled water administration (6 ml/kg)				
1	0.0624	0.0966	0.1250	0.1212	0.1008 ± 0.017
2	0.0459	0.1434	0.0910	0.0833	0.0910 ± 0.034
3	0.1282	0.0627	0.1063	0.1060	0.1013 ± 0.024
4	0.0624	0.0806	0.0660	0.0680	0.0743 ± 0.007
5	0.1007	0.0966	0.0916	0.0909	0.0897 ± 0.013
6	0.1098	0.0716	0.0750	0.0606	0.0793 ± 0.018

Data for urine flow rate in Frusemide experiment.

Time (Hours)	Urine flow rate (ml/kg/min.)				Mean + S.E
	Rat I	Rat II	Rat III	Rat IV	
-6	0.0210	0.0180	0.0170	0.0270	0.021 ± 0.003
0	Frusemide administration (10 mg/kg)				
1	0.7424	0.4116	0.3317	0.8066	0.4981 ± 0.194
2	0.1960	0.1706	0.1248	0.1933	0.1712 ± 0.028
3	0.1060	0.1204	0.1059	0.1733	0.1267 ± 0.023
4	0.0910	0.1405	0.0981	0.0933	0.1057 ± 0.021
5	0.1212	0.1104	0.0802	0.1067	0.1046 ± 0.015
6	0.0909	0.0803	0.0700	0.0800	0.0803 ± 0.007

Data for urine flow rate in O. stamineus extract experiment.

Time (Hours)	Urine flow rate (ml/kg/min.)				Mean + S.E.
	Rat I	Rat II	Rat III	Rat IV	
-6	0.031	0.024	0.026	0.027	0.027 ± 0.026
0	O. stamineus extract administration (296mg/kg)				
1	0.1457	0.1026	0.1616	0.0968	0.1266 ± 0.028
2	0.0991	0.0940	0.0959	0.0829	0.0929 ± 0.006
3	0.0641	0.0769	0.0808	0.0829	0.0762 ± 0.007
4	0.0932	0.0512	0.0454	0.0429	0.0618 ± 0.020
5	0.0699	0.0598	0.0404	0.0553	0.0564 ± 0.011
6	0.0460	0.0340	0.0303	0.0484	0.0398 ± 0.008

Data for sodium ion excretion rate in control experiment.

Time (Hours)	Urine flow rate (mEq./kg/min.)				Mean + S.E.
	Rat I	Rat II	Rat III	Rat IV	
-6	1.23	1.31	2.16	1.72	1.61 ± 0.37
0	Distilled water administration (8 ml/kg)				
1	1.62	1.72	3.26	2.11	2.17 ± 0.65
2	0.99	1.56	1.39	1.27	1.30 ± 0.21
3	2.30	1.17	2.47	1.38	1.81 ± 0.54
4	1.06	0.70	1.16	0.88	0.95 ± 0.18
5	0.88	4.29	0.79	0.98	1.73 ± 1.47
6	2.38	2.80	2.44	1.30	2.23 ± 0.56