

PHARMACOLOGICAL STUDIES OF SAPONINS OF THE FRUITS OF
LUFFA ECHINATA ROXB. AND SEEDS OF TRIGONELIA
FOENUM - GRAECUM LINN.

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In view of the claims that both *Methi* and *Kukudvel* are useful in Ayurvedic system of medicine, we decided to study the pharmacological actions of both these drugs. Our studies are restricted only to saponins obtained from whole fruits and seeds of *Luffa Echinata* (L. E.) and *Trigonella Foenum-graecum* (T. F. G.) respectively.

The saponins were studied as it has been claimed in Ayurvedic literature, that infusions of these drugs were given in the treatment of dropsy, jaundice, ascites, enlargement of liver and spleen, cough and dysentery.

Methods and Material. All the experiments were performed on cats, dogs, guineapigs, rabbits, rats and frogs. Dogs were obtained from Municipal Dog Licensing Department, while the cats were supplied by the local animal dealers. Rabbits, rats and guineapigs were obtained from Haffkine Institute. The experiments were performed on animals which were kept under observation for a period of four days in our laboratory.

Effects on circulation and respiration. The saponins were studied for their effects on circulation and respiration in ten dogs and five cats. Dogs varying in weight from 10 to 15 kg. were anaesthetised by administering pantobarbitone (Nembutal) 40 mg. per kg. intraperitoneally. A few animals were anaesthetised with administration of Phenobarbitone soluble, 140 mg. Cats weighing 2.5 to 3 kg. were anaesthetised with ether and the anaesthesia was maintained by intraperitoneal Nembutal 40 mg. per kg. In some cases, anaesthesia was by intratracheal ether.

Trachea was dissected by a midline incision in the neck. Tracheal cannula was inserted and connected to a Marie's tambour, for recording the rate, rhythm and amplitude of respiration. Carotid artery was cannulated for recording the systemic blood pressure. In some experiments the blood pressure was recorded through the femoral artery.

Sodium citrate 10% solution and heparin were used as anticoagulants. Femoral vein was cannulated for injection of drugs.

Drugs were injected in various concentrations, taking care that the total volume of the injection at a time did not exceed 1 ml. Every injection was followed by 1.5 ml. normal saline for washing out the drug in the cannula. In some cases the effect of the drug was studied in spinal cats. (J. H. Burn). The animal was allowed to recover from spinal shock and the observations carried out after one hour.

Fig. 1 shows the effect of 50 mg. of saponin of T. F. G. on the blood pressure and respiration of cats. It can be seen that 50 mg. produced a fall in blood pressure coming on within 10 seconds of administration and the blood pressure came back to normal within 30 seconds. Thus the effect was evanescent. There was a rise in the amplitude in respiration, associated with this fall of blood pressure.

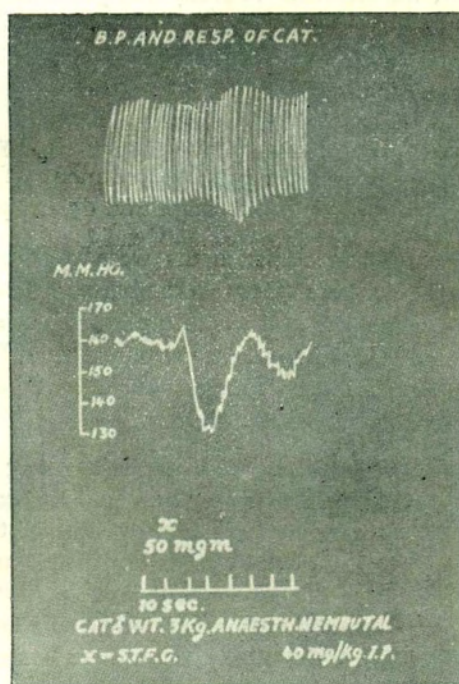


Fig. 1

Fig. 2 shows a fall in blood pressure, increasing proportionally with the increase in dose. Thus with a small dose of 5 mg. there was a definite and perceptible fall and 10, 20, 40 and 100 mg. produced a proportionally increasing fall in blood pressure.

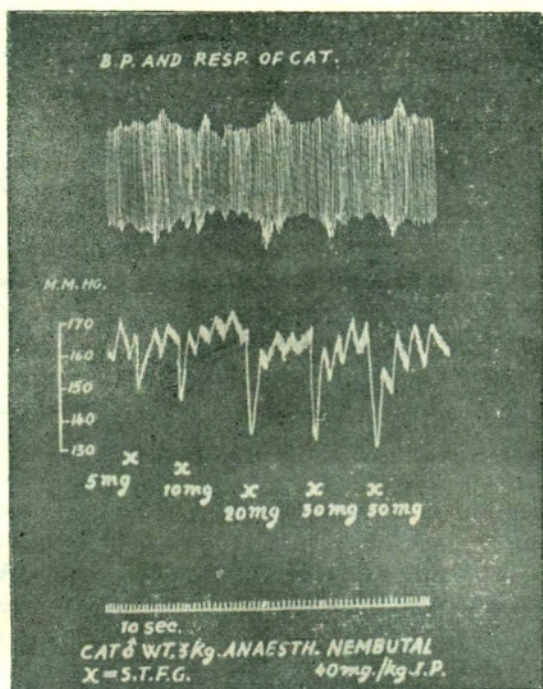


Fig. 2

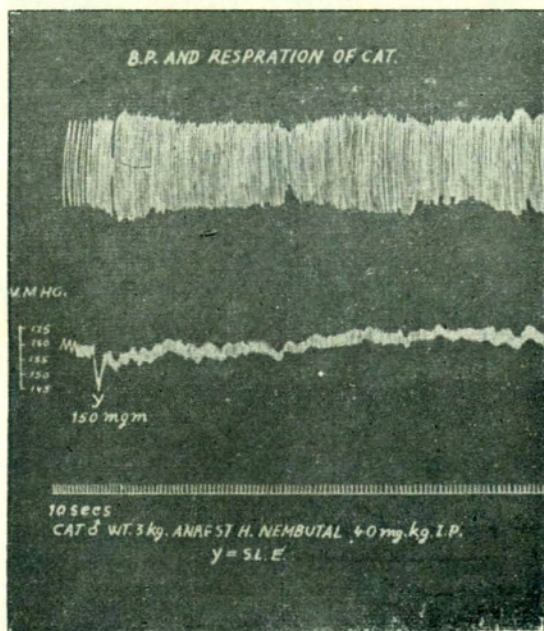


Fig. 3

Fig. 3 shows the effect of L. E. Saponin on cat's blood pressure. As compared to the saponins of T. F. G., it produced much smaller effect. With a large dose of 100 mg. there was a double fall in blood pressure. It was interesting to note that associated with the blood pressure effect there was a fall in the rate of the amplitude of respiration and the animal subsequently went into a state of apnoea from which it recovered slowly. The same phenomenon was repeated after another dose of 100 mg. of saponin of L. E. injected in the same cat. When 150 mg. of the drug was given third time in the same cat, the effect on the blood pressure was evident, but the apnoea could not be observed. There was a slight diminution in the amplitude of respiration.

Fig. 4 shows the effect of both saponins on blood pressure and respiration in dog. Twenty mg. of Saponin of T. F. G. produced a fall of blood pressure comparable to 10 mcg. of ACh. The fall came on within 10 seconds and was evanescent. 10 and 20 mg. of Saponin of L. E. produced a similar fall but less marked in its effect. Associated with this fall, there was an increase in the amplitude of respiration and this effect also lasted for the same length of time. 40 mg. per kg. of Saponin of T. F. G., produced an initial transient fall of blood pressure equivalent to that produced by 10 mcg. of ACh and second fall followed immediately the first one and was gradual in onset and more prolonged. Associated with this there were changes in depth and rate of respiration.

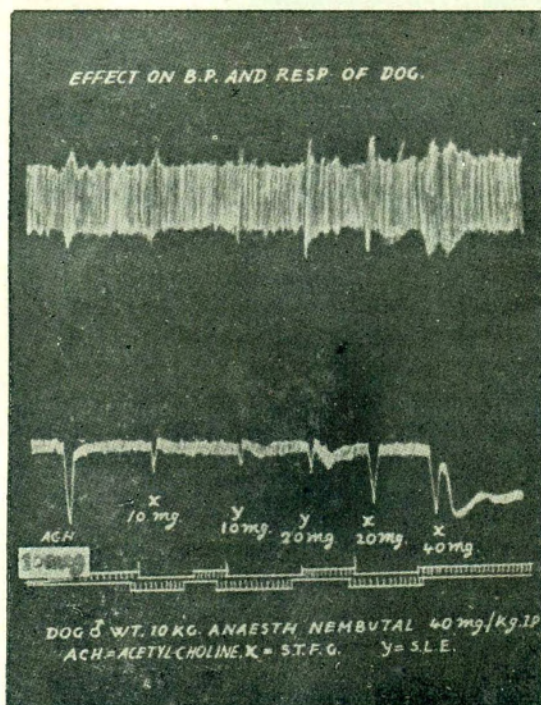


Fig 4

Fig. 5 shows the effect of the drugs in the same dog after atropinisation (2 mg. of atropine per kg. given intravenously). It was found that 10 mg. of Saponin of *T. F. G.*, per kg. failed to produce any perceptible effect on blood pressure and respiration, 20 mg. per kg. of Saponin of *T. F. G.* produced an effect similar to 1 mg. of ACh. But it was noted in this case that the initial fall was much smaller than what was recorded before while drug produced a second fall which was not seen before at this dosage level.

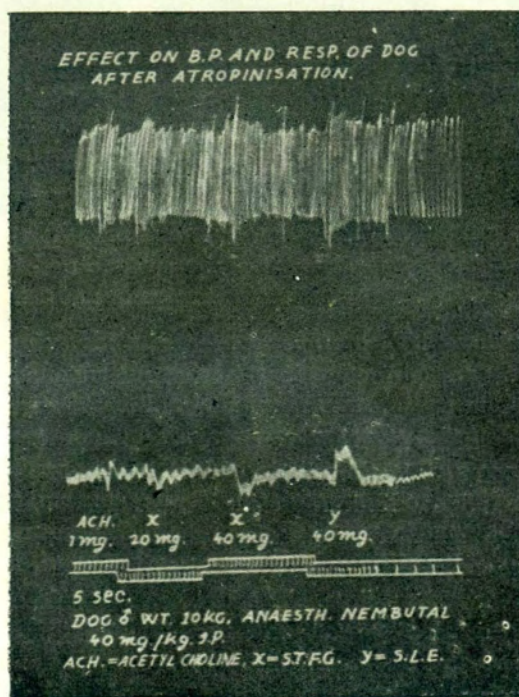


Fig. 5

Forty mg. per kg. of Saponin of *T. F. G.*, produced a single gradual fall of blood pressure which was prolonged, with an associated rise in respiration. Forty mg. per kg. of Saponin of *L. E.* now produced an abrupt rise instead of fall in blood pressure and this effect was maintained for some time and then came to normal. Associated with this there was an increase in depth of respiration which was irregular and slow.

Figure 6 shows the effect on the respiration in a female pregnant cat. Five mg. per kg. and 10 mg. per kg. of Saponin of *T. F. G.*, did not produce any effect on rate, rhythm or amplitude of respiration.

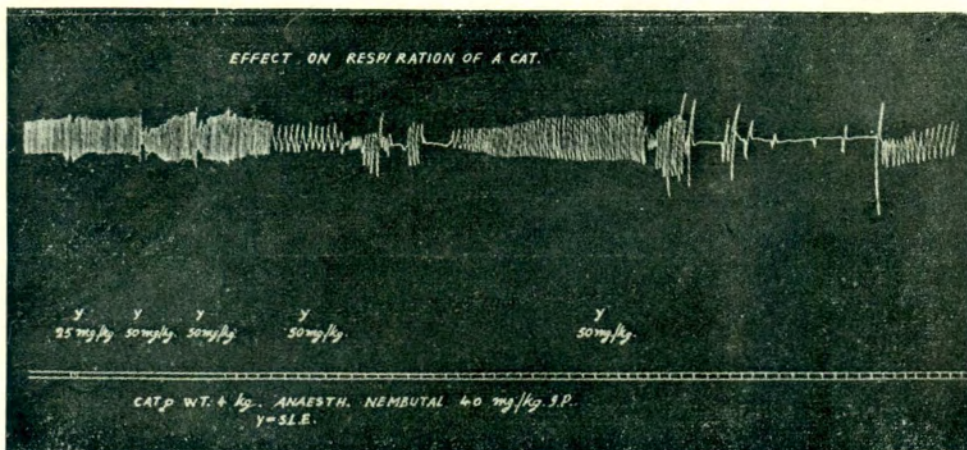


Fig. 6

Twenty mg. per kg. of Saponin of T.F.G., produced a slight increase in the amplitude of respiration. Twentyfive mg. per kg. of Saponin of T.F.G., produced a depression in the amplitude of respiration which came back to normal in a short time.

Twentyfive mg. per kg. of Saponin of L.E. produced an increase in rate of respiration with a slight diminution in its amplitude. Fifty mg. per kg. of Saponin of L. E., produced a marked irregularity of respiration characterised by increase in rate and diminution of amplitude and irregularity of rhythm. This effect lasted for a short time. It could be seen on a slow moving drum that the rate of respiration slowed and the cat went into a state of apnoea. Respiration then became cheyene stoke's type. This effect could be repeated several times in the same cat by administration of 50 mg. per kg. of Saponin of L. E.

Spinal preparation. Five, 10 and 20 mg. of Saponin of T. F. G., did not produce any marked effect on blood pressure. Five, 10 and 50 mg. of Saponin of L. E. produced a slight fall in blood pressure not comparable to what was seen in normal cat.

Action on isolated rabbit heart. Heart was excised from a rabbit killed by a blow on head. It was mounted in tyrode solution maintained at 37°C and perfused through its coronaries at a pressure of 40 cm. of water. Changes in rate and amplitude of contractions were recorded kamographically and alterations in coronary flow were noted by collecting and measuring at the end of each minute the volume of fluid which had perfused through coronaries.

One mg. of Saponin of L. E. injected by the side cannula did not produce any effect. Two mg. of Saponin of T. F. G., did not have any marked effect on amplitude or on force of contraction but it produced a change in rhythm characterised by occasional extra systoles. Five mg. of Saponin of T. F. G., produced an increase in the force of contraction. With larger doses of Saponin of T. F. G., heart stopped in systole.

There was no change in coronary flow as measured by above method.

Perfusion of frog's heart with 1/4 calcium Ringer. Frog's heart was perfused in situ. Cannula was inserted in the sinus venosus and was connected to a reservoir on an adjustable stand. Both the aorta were cut and heart was perfused with frog's ringer at constant hydrostatic pressure and contractions were recorded on a moving drum. After taking normal record of contraction, heart was then perfused with Ringer containing $\frac{1}{4}$ calcium. There was diminution in the force of contraction as compared to normal.

One mg. of Saponin of T. F. G. when injected at this stage brought about an increase in the force of contraction. While 1 mg. of Saponin of L. E., diminished the force and heart action became feeble. When the dose of Saponin of L. E. was increased to 10 mg. there was marked diminution in force of contraction and heart stopped in diastole. At this stage 10 mg. of Saponin of T. F. G., was injected and this revived the heart.

Action on isolated rabbits auricles. Rabbit was killed by a blow on the head and heart was quickly excised. Ventricles were cut off from the auricles at the atrioventricular area. Auricles were cleaned of all the adherent tissues and horse-shape auricles were mounted in well oxygenated tyrode bath maintained at 37°C. (Burn and Bulbring).

100 mcg. of Saponin of T. F. G., or Saponin of L. E. when allowed to act for 30 seconds did not produce any change in the rate of amplitude of contraction of auricles. One mg. of Saponin of T. F. G. or of Saponin of L. E., did not alter the rate, rhythm, or amplitude of contraction. If a dose of 100 mcg. of either Saponin was allowed to remain in the bath for 1 minute a definite effect was observed on the contracting auricles. Thus it was seen that 100 mcg. of Saponin of T. F. G., produced a slowing of auricles without much alteration in the amplitude or force of contraction. 100 mcg. Saponin of L. E., on the contrary produced a marked effect on the amplitude of contraction. Auricular rate diminished and at times there was a complete auricular stoppage.

Action on tracheal rings. Tracheal rings isolated from dog were mounted in an isolated organ bath containing tyrode solution at a temperature maintained at 37°C. A record of contraction was taken by a light straw lever with an attached frontal writing point.

Ten mcg. of ACh and Histamine base produced a normal contraction. In concentration of 1 mg. of either Saponin added to 25 ml. bath failed to produce any effect on the bronchial muscle. It was also noted that they did not bring about potentiation or inhibition of action of 10 mcg. of ACh or 10 mcg. of Histamine base in the bath.

Action on guineapig ileum. Guineapig was killed by a blow on the head. Abdomen was opened and ileum was separated. Mesentric attachments were carefully dissected and a small piece of intestine was washed with tyrode solution. It was then mounted in oxygenated tyrode at 37°C. It was found that both Saponin of T. F. G., and Saponin of L. E., did not produce any action on the intestinal strip in concentrations ranging from 1 mg. to 10 mg. in 25 ml. bath. It was also noted that previous exposure of the tissue to 10mg. of Saponin of T.F.G., or Saponin of L.E., for a period of one minute failed to modify the contraction produced by 100 mcg. of histamine or 5 mcg. of Ach.

Action on rabbit's duodenum: Duodenum was isolated from a rabbit, killed by a blow on the head. It was cleaned and washed with tyrode solution and mounted in 25 ml. oxygenated tyrode bath maintained at 37°C. Both the Saponin of T.F.G., and Saponin of L.E., in concentration of 100 mcg. to 1 mg. in the bath failed to have any action on the rhythmic activity or on the tone of the tissue. Ten mg. of either Saponin produced marked diminution in the tone and rhythmic movements (Fig. 7.)

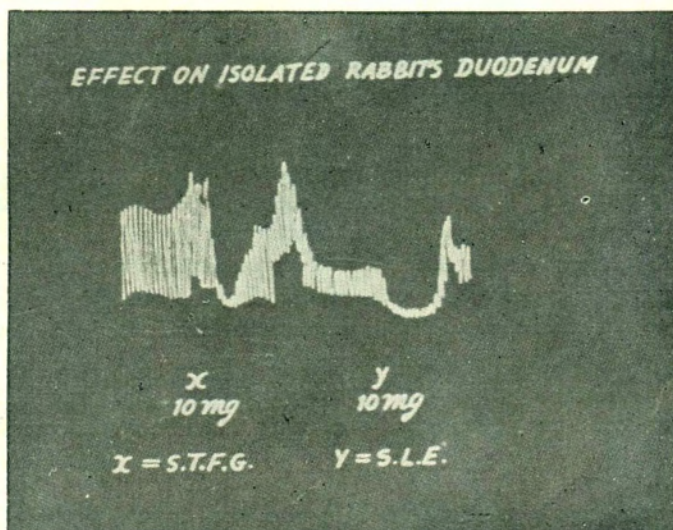


Fig. 7

Actions on rat's uterus: Rat's uterus was isolated, cleaned and mounted in an oxygenated modified Lock's solution maintained at 37°C. Saponin of T.F.G. or Saponin of L.E., did not cause contraction of uterus in doses from 100 mcg. to 1 mg. in bath. Ten mg. of Saponin of T.F.G., caused slight relaxation without much interference with the rhythmic activity of the tissue. Saponin of L.E. in concentration of 10 mg. had practically no action. In presence of 10 mg. of either Saponin, pituitary action persisted.

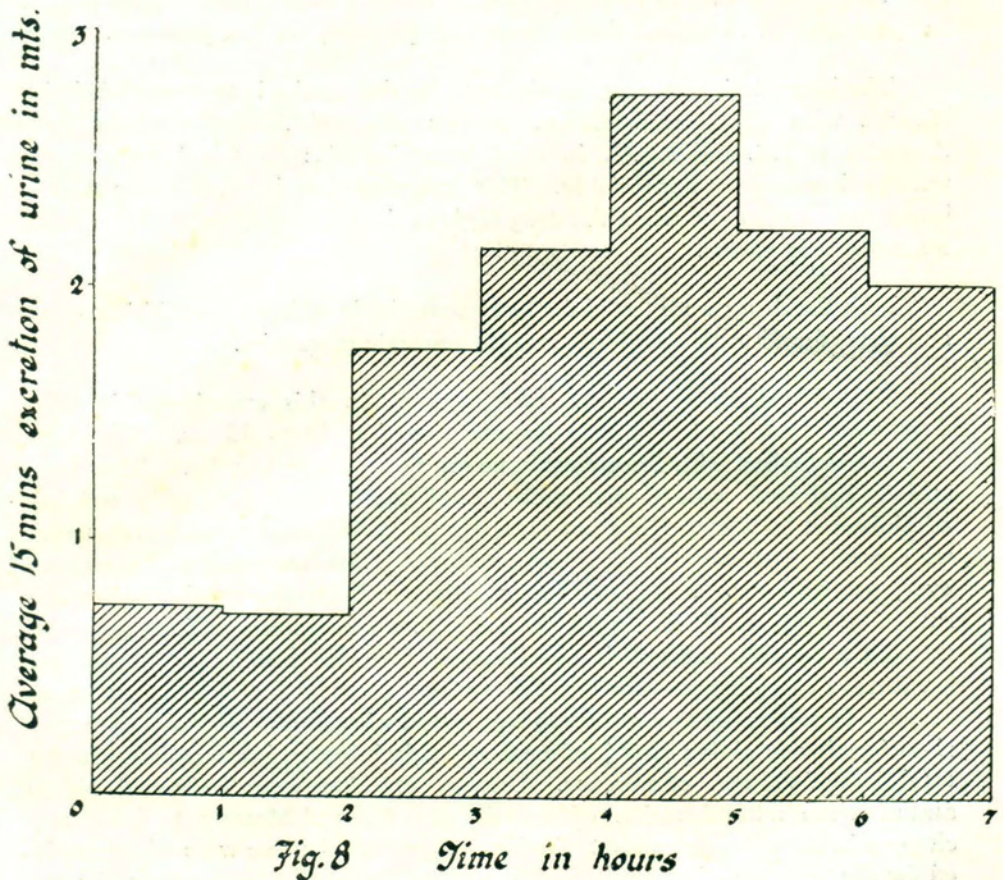
The haemolysis test was carried out by the method recommended by Hanzlik with slight modification. It was observed from the results that Saponins of L.E. and T.F.G. in a concentration of 1: 10 failed to lysis the red blood corpuscles appreciably. This concentration is much above, what is normally obtainable when the drug is given intravenously to produce pharmacodynamic effects.

Both the drugs were tested for local anaesthetic effect on rabbit's cornea and were found to be devoid of local anaesthetic properties.

Action of Saponin of L. E. on urinary output: In this experiment in all 5 dogs were used, 4 male and 1 female weighing 10 to 15 kg. The dogs were anaesthetised with Phenobarbitone soluble 140 mg. per kg. They were initially hydrated with normal saline equal to 2.5% of their body weight,, administered by a stomach tube. Ureters on both the sides were cannulated with polythelene tubings and the amount of urine excreted was measured every half an hour. After taking four initial half hourly readings when the rate of flow had declined, the Saponin of L.E. was administered intravenously, through a cannula inserted in the femoral vein, the dose being 10 mg. per kg. Rate of excretion was measured every half hour for the subsequent three hours. In some dogs the effect was compared with 2 ml. Mersalyl (Mercurial diuretic containing 39.6% of mercury) administered intravenously in the total dose of 400 mg. of mercury. Urinary chlorides were estimated before and after the administration of Saponin of L.E., and Mersalyl to detect any changes in the chloride content of urine. Samples of urine were also examined for the presence of albumin and examined under the microscope for red blood cells.

Fig. 8 shows a graphical record of urinary output in four hours before and after the administration of the drug. It can be seen from the graph that after the administration of 10 mg. per kg. of Saponin of L.E., there was a definite increase in the rate of urinary output. This effect lasted in some dogs for more than 2 hours. We have compared the effect of Mersalyl and that of Saponin of L.E. on the urinary excretion in the same dog. It was noted that Mersalyl in equimolecular doses is a more powerful diuretic than Saponin of L.E. Incidentally it was also observed that marked chloride

excretion seen in the case of Mersalyl was not seen in the case of Saponin of L.E. Further studies on the diuretic properties are reported in a separate paper. Saponin of T.F.G. was not found to produce any diuresis.



DISCUSSION

Saponins isolated from T.F.G. and L.E. have been studied for the pharmacological actions to verify the claims in the Ayurvedic literature. Since watery decoctions and infusions were used by the ancient physicians, it is but logical to expect that the water soluble ingredients may be pharmacologically more active. Saponins which have thus been isolated in purified form, have been studied for their actions on various organs and systems.

In general it may be seen that Saponins obtained from these indigenous plants, do not show a marked pharmacodynamic properties. But still they affect various organs and systems in a manner that needs careful study and evaluation.

The effect of these Saponins on blood pressure has been found to be more interesting. It was observed that both the Saponins produce a fall of Blood pressure when administered intravenously in cats and dogs. This fall was more marked with Saponin isolated from *T. F. G.*, even small dose as 5 mg. per kg. produces a definite fall of B. P. This fall was abrupt and came on within ten seconds of administration. The effect was evanescent. It might be inferred that the fall was due to the action of Saponin per se rather than due to the liberation of any hypotensive substance in the body; the fall did not seem to be non-specific but appeared to be a definite action on certain receptors which when stimulated would normally bring down the systemic blood pressure. This effect was very similar to that of acetylcholine and Histamine. This transient fall was blocked by atropinisation. Therefore it could be reasonably inferred that the effect was due to the parasympathetic stimulation. But more interesting situation could be observed if the dose of the drug is increased to 40 mg. per kg. At this higher dose there was a double fall of blood pressure, initial fall being abrupt and evanescent and second fall being gradual in onset and prolonged.

As the second effect was seen only after atropinisation of the animal, it was clear that the first effect was due to the parasympathetic stimulation while the second was probably due to the direct action of the Saponin on the blood vessels. Besides, it appeared that there was probably a central component of action which contributed to this effect on blood pressure as the effect was considerably modified in a spinal cat.

Action of Saponin of *L. E.* on blood pressure was much less marked as compared to that of Saponin of *T. F. G.* But in case of this drug an interesting phenomenon was observed. In a normal animal, it produced a slight fall in blood pressure. But after atropinisation of 40 mg. of this drug produced a rise instead of a fall. This effect was very similar to a large dose of acetylcholine given after atropinisation. This might be due to the stimulation of autonomic ganglion, like nicotine; the sympathetic action predominating due to a blocking of the post ganglionic parasympathetic fibres by atropinisation.

It has been noted by the Ayurvedic physician's studies that both the drugs have an effect on respiratory disorders. In the present studies little evidence was found to substantiate the claim. Smaller doses of Saponin of *T. F. G.* produced insignificant action on respiration; whatever effect was observed was secondary to the action on blood pressure. A large dose depressed the respiration by direct depressant action on the respiratory centre.

Saponin of L. E. appeared to exert a primary action on respiration. Thus it was observed that the drug produced a depression of respiration. There was marked respiratory irregularity and in higher doses it produced apnoea. This effect was present inspite of denervation of carotid sinus and body. Besides, it was noted that both the drugs failed to produce an action on the isolated tracheal rings, and did not even modify the normal response of this tissue to acetyl choline, histamine and adrenaline. It seems therefore that Saponin of L. E. altered the physiological reactions of the respiratory centre. Nevertheless, the possibility of secondary effect due to its action on blood pressure especially in larger doses cannot be ruled out.

It might be incidentally stated that no marked increase in the respiratory secretions was observed during these experiments. In an experiment to observe the effect on the ciliary movement on frog (Burn), both these drugs were found to be ineffective.

That the effect on the circulatory system was due predominantly to peripheral action, was strengthened by observation on isolated preparation. Saponin of T. F. G., increased the force of contraction of isolated rabbit's heart. It also increased the contraction impaired by perfusion of 1/4 calcium Ringer. This action appeared to be similar to that of digitalis glycosides. It was noted that the drug produced extra-systoles and heart-block, the effect resembling digitalis on junctional tissues.

Saponin of L. E. was found to have diuretic property in a small dose of 10 mg. per kg. In comparison with mercurials, Saponin of L. E. was found to be less potent but the effect was more prolonged especially in dogs. There was a slight reduction in urinary output initially; and this was followed by diuresis. The diuretic action thus is probably due to the effect on the kidney troubles. It may be mentioned incidentally that the drug did not cause an increased chloride excretion as is seen with mercurials. This effect on renal tubules requires further evaluation.

SUMMARY

(1) Saponins of T. F. G. & L. E. were studied for their pharmacodynamic properties.

(2) Saponin of T. F. G. produced a fall of blood pressure in cats and dogs due to parasympathomimetic action. Partly, it has also a direct action on blood vessels.

(3) Saponin of L.E. produced a fall due to parasympathomimetic action. After atropinisation it produced a rise of blood pressure.

(4) Saponin of T. F. G. has a direct stimulant action on the myocardium.

(5) Saponin of L. E. produces a diuretic effect probably affecting the glomerular and tubular function.

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