EFFECT OF SOME MEDICINAL PLANT PREPARATIONS OF ADIPOSE TISSUE METABOLISM

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ABSTRACT: Powder in fine suspension, water and alcoholic extract preparations of Cyperus Rotundus (Mustak), Iris versicolor (Haimavati) and Holoptelai integrifolia (Chirubilva) were used in adipose cell suspension and also administered orally to evaluate the effect of these plant preparations on adipose tissue metabolism in rats. The result, showed that the preparations from these medicinal plants exhibited lipolytic action to mobilize fat from adipose tissues in rats and consequently helped in the reduction of obesity.

Introduction
It is accepted that the lipolytic response to hormone or lipolytic agent is the result of activation of adenylate cyclase at the membrane with a subsequent increase in the intracellular levels of 3’, 5’ – adenosine monophosphate (Cyclic AMP)⁴. Cyclic AMP then activates a protein kinase which in turn activates a triglyceride lipase ⁵,⁷⁻³¹. Any weight related to changes in hormone or lipolytic agents stimulated lipolysis could occur through alterations in any or all of these processes.

Cyperus rotundus (C. rotundus), Iris versicolor, and Holoptelia integrifolia (H. integrifolia) were claimed in Ayurvedic literature to possess an antiobesity property ⁶⁻⁸. This claim has been proved to be true by our previous experimental work on human and rats ⁹⁻¹¹. Our recent experimental work showed that these medicinal plant preparations were effective in reducing obesity in rats and humans by releasing enhanced concentration of biogenic amines from nerve terminals of the brain in rats which suppresses an appetite centre to reduce food in-take with the consequence of reduction of obesity ⁹,¹⁰,¹¹. Our earlier experimental work also showed that these plant preparations after treatment decreased cholesterol, Triglyceride, Lipo-proteins, Lipoprotein lipase in tissue, liver and plasma in rats and humans ³². It is well established that catecholamines apart from their action on appetite centre induce lipolysis in adipose tissue.

¹²⁻¹⁴--- Our trial of C. rotundus on human subjects for the evaluation of its effect or obesity in human showed that some subjects experienced an anorexia but some did not but all the obese subjects lost
weight. From these results it might be possible that those who did not experience anorexia, and still lost weight, might have a direct lipolytic effect of C. rotundus on adipose tissue so that FFA liberated from triglyceride might be instead of reesterified, used in other tissues to produce energy.

These observations encouraged us to undertake in these series of the present experiments to evaluate the direct lipolytic effect of these indigenous plant preparations on adipose tissues in rats. 

Materials and Methods

Animals

Albino rats weighing 190-220 g, fed with Gold Mohar rat feed were kept in a room maintained at 24 ± 4°C with 12 – 12 dark-light cycle.

Drugs

Cyperous rotundus, 1. Versicolor and Holopetlia integrifolia were purchased from local market. Tubers of C. rotundus and roots of 1. Versicolor were ground into fine powder mixed some crystals of sugar and transformed into a fine suspension in saline. Water extract of bark of H. integrifolia was prepared as described in literature. The dosage of these indigenous plant preparations were calculated as described by the author.

Alcoholic extract of C. rotundus, 1. Versicolor and H. integrifolia was prepared as follows:

Tubers of C. rotundus, rhizomes of 1. Versicolor and barks of H. integrifolia was ground to a fine powder. The powder of these plants was mixed with alcohol (1:90) and was kept for 8 days. The alcoholic mixture of these various plants was filtered and the filtered were evaporated to dryness at the room temperature. The dried extracts obtained were taken for experiments to see lipolytic effect of these plant preparations. Collagenase and Alkalline phosphate were of analytical grade.

In vitro study

Minced adipose tissue obtained from the epididymal and subscapular fat pads from the head blow stunned rats were incubated with 1 mg/ml of collagenase (sigma) in Kreb’s – Ringer bicarbonate buffer containing 4% bovine albumin at 37°C for 60 min for the separation of fat cells according to the method of Rodbell. After incubation the medium was centrifuged at low speed and the cells were separated and washed with saline. (40 – 50 cells approximately were used in the incubation medium). Eight rats were used for each cell preparation.

Lipolysis was determined in isolated fat cells by measuring glycerol and free fatty acids released into the medium containing 2ml of kreb’s – Ringer bicarbonate buffer (7.4 Ph) with 4% bovine albumin. Concentrations of 0.32 mg, 0.64mg – C. rotundus; 0.30 mg, 0.60mg ---1. Versicolor and 0.36 mg, 0.72 mg of H. integraifolia were added to the medium containing 40-50 fat cells, without norepinephrine or with norepinephrine. The cell samples were
incubated in triplicate at 37\(^0\) for 30 min. Glycerol and FFA released into the medium were determined by al\(^{17}\) and Trout et al\(^{18}\) respectively.

Phosphodiesterase was assayed by the method of Butcher Suther—land\(^{19}\), modified for adipose tissue. In this procedure, phosphodiesterase activity is determined from the rats at which 3', 5'. AMP is converted to 5’ – AMP. The later compound is measured from in organic Phosphate liberated by the action of bacterial alkaline phosphatase. Fat pads, which were removed after the rats sacrificed were homogenized in 0.33 M sucrose in glass to glass pestle and mortar for 2 min. The supernatant solution was adjusted to 0.2 saturation with respect to ammonium sulphate by addition of 11.4 g of the solid salt/100 ml, the precipitate was separated by centrifugation and discarded. The supernatant was then adjusted to 0.4 saturation by adding 12.3 g of solid (HN\(_4\))\(_2\) SO\(_4\)/100 ml, the precipitate was separated by centrifugation as above and dissolved in 1mm tris buffer, p\(^H\) 7.5, and 0.5 mm MgSO\(_4\). This preparation was fractionated with amm. Sulphate as above, and the fraction that precipitated between 0.25 and 0.50 saturation was dissolved in 1mm tris, p\(^H\) 7.5 and 1mm MgSO\(_4\). The incubation mixture for enzyme assay consisted of cyclic 3’ -5’ – AMP (0.5 MM), MgSO\(_4\) (3.5 mm), alkaline phosphatase (20 mg) and a suitable dilution of phosphodiesterase in tris buffer (0.04 MO,p\(^H\) 8.00, in a final volume of 1 ml). The reaction mixture was incubated for 30 min.at 37\(^0\) C (with alkaline phosphatase present only during the final 10min) and stopped by addition of 0.1 ml. of 55% TCA. An aliquot of the supernatant solution was taken for measurement of inorganic phosphate by the method of Goldenberg and Fernandez\(^{20}\).

In Vivo Study
C. rotundus, I. Versicolor and H. integrifolia were administered orally to three groups consisting of 6 rats in each group in dosage of 60 mg/kg, 20 mg/kg, and 1.66 g/kg respectively. Fourth group of 6 rats was treated as control. At the end of 4 hours the blood was collected from the sacrificed rats. Plasma glycerol and FFA were determined as described previously. P values were calculated using student’s test. AP of < 0.05 was considered to be significant.

Result and Discussion
In Vitro Study
C. rotundus, I.versicolor and H.integrifolia have significant lipolytic action on adipose tissue in rats as evidenced from the data presented in table I. C. rotundus in the concentration of 0.32 mg, and 0.64 mg; I. Versicolor in the concentration of 0.03 mg and 0.60 mg ; and H.integrifolia in the concentration of 0.36 and 0.72 when added to the cell suspension caused the release of glycerol and FFA in increased quantities as compared to the control.

\((A_\text{P}<0.001, \ B_\text{P}<0.02, \ C_\text{P}<0.01)\)
The addition of C. ratundus, I. Versicolor and H.integrifolia in the same concentration as above to the prestimulated fat cells with noradrenaline also further increased the release of glycerol and FFA (A P <0.001, B <0.05, C P <0.02)

As shown in table IV.

The inhibition of AMP phosphodiesterase in the isolated fat cells after addition of C. rotundus, I.versicolor and H. integrifolia is presented in TABLE III. It is seen from the table II.

Table 1: Effect of C. rotundus, I. Versicolor and H.integrifolia on Glycerol and FFA release in adipose tissue.

| Experiment Number and additions | Lipolysis | FFA |
|-------------------------------|-----------|-----|
|                               | Lipolysis |     |
|                               | Glycerol  | FFA |
|                               | Moles/g±Se | Moles/g±Se |
| 1) Control                     | 0.699 ± 0.014 | 1.04 ± 0.18 |
| 2) C. rotundus                 | 2.733 ± 0.164^A | 2.91 ± 0.41^C |
| 0.32                           |            |     |
| 0.64                           | 4.381 ± 0.426^A | 5.15 ± 0.47^A |
| 3) I. versicolor               | 2.724 ± 0.130^A | 2.92 ± 0.130^A |
| 0.30mg                         |            |     |
| 0.60 mg                        | 5.354 ± 1.476^A | 5.08 ± 0.01^A |
| 4) H. integrifolia             | 2.846 ± 0.54^A | 3.05 ± 0.27c |
| 0.36mg                         | 4.660 ± 0.130^A | 5.14 ± 0.35^A |
| 0.72mg                         |            |     |

FFA Free Fatty acids. A P <0.001, B P <0.02, C P <0.01.

Table 11: Effect of Oral dosage of C. rotundus, I. versicolor and H.integrifolia on plasma Glycerol and FFA.

| Treatment                | No.of Animals | Time | Plasma Moles/dl±Se | Glyceroplasma FFA moles/dl± Se |
|--------------------------|---------------|------|---------------------|-------------------------------|
| Control                  | 6             | 4    | 9.25 ± 0.85         | 16.58 ± 1.82                 |
| C. rotundus 60mg/kg      | 6             | 4    | 15.65 ± 0.92^A      | 25.28 ± 1.66^B               |
| I. versicolor 20mg/kg    | 6             | 4    | 16.36 ± 0.72^A      | 24.70 ± 1.18^B               |
| H. integrifolia 1.66 mg/kg | 6           | 4    | 14.99 ± 0.68         | 26.67 ± 1.80^B               |

FFA Free Fatty acids. A P <0.01. B P <0.02.

Table 111: Inhibition of Cycle 3’, 5’ – AMP phosphodiesterase by C. rotundus, I.Versicolor and H.integrifolia in adipose tissue.
Phosphodiesterase was prepared as assayed as described in the text.

Table IV: Effect of C. rotundus, I. versicolor and H. integrifolia on Nor- adrenaline stimulated lipolysis in adipose tissue.

| Experiment and Additions | Phosphate released Moles/30min. | % inhibition |
|--------------------------|-------------------------------|--------------|
| None                     | 0.30                          | ----         |
| C. rotundus              | 0.30 mg 0.20 33.4             |              |
|                          | 0.64 mg 0.10 66.7             |              |
| I. Versicolor            | 0.03 mg 0.15 50               |              |
|                          | 0.60 mg 0.075 75              |              |
| H. integrifolia          | 0.36mg 0.20 33.7              | 50           |
|                          | 0.72mg 0.15                   |              |

Phosphodiesterase was prepared as assayed as described in the text.

That inorganic phosphate liberated from 3’, 5’ – AMP by the alkaline phosphatase decreased after the addition of these indigenous plant drugs.
In Vivo Study
The lipolytic activities of these indigenous plants were further evaluated in vivo. Oral administration of C. rotundus, I. Versicolor and H. integrifolia in doses of 60 mg/kg, 20mg/kg and 1.66 g/kg respectively increased the plasma glycerol and FFA concentrations (A \( P < 0.01\), B \( P < 0.02 \)) when measured after 4 hours in rats. These data are shown in table 11. The stimulating effect of C. rotundus, I. Versicolor and H. integrifolia on basal and norepinephrine stimulated glycerol and FFA release from adipose tissue is firmly established from the data of the present experiments. (table 1 & IV ). It is observed from the present experimental results that these indigenous plant drugs inhibit cyclic AMP --- Phospho—diesterase and stimulate lipolysis by increasing tissue concentrations of cyclic AMP (Table 111).

The effect of these indigenous plant preparations on norepinephrine stimulated fat cells showed that these indigenous plant preparations increased the concentrations of glycerol and FFA further. (table IV ) and the further increase in FFA can be accounted for by the reduction in reesterification. It is known that reesterification occurs concurrently with liberation of FFA during the process of lipolysis, and L. glycerophosphate is required \(^{21-22}\). This result indicates that these indigenous plant drugs have independent lipolytic effect on adipose tissue to mobilize fat. The in vivo study of these indigenous plant drugs have independent lipolytic effect on adipose tissue to mobilize fat. The in vivo study of these indigenous plant preparations on lipolysis showed that these indigenous plant drugs increased the plasma concentration of glycerol and FFA (table 11). It is well know that some lipolytic agents when administered raised the concentrations of FFA in plasma of human subjects \(^{23,31}\). In the present experiments also the plasma FFA were increased by the administration of these indigenous plant drugs. These results indicate that these indigenous plant drugs may have lipolytic effect on adipose tissue directly and/or through catacholamines. There are evidences that central neuron system had ability to specifically control lipid mobilization without affecting glucose homeostasis. As for the neurochemistry is concerned, norepinephrine, released from brain neurons (by the action of drugs) mobilizes FFA in rats by activation of B-receptors in the adipocyte plasma membrane\(^{24}\). The same type of action of these indigenous plant preparations was observed in our recent experiments to see their effect on weight and food intake in rats\(^{25}\). Therefore the present experimental results suggest that these indigenous plant preparations have two type of action on adipose tissue lipolysis:

1) Lipolytic action on adipose tissues through the release of catecholamines from the brain.

2) The direct lypolytic action on adipose tissue by increasing concentration of cyclic AMP in the
cell membrane, at the same time inhibiting reesterification by inhibiting AMP-phosphodiesterase without the intervention of insulin. A major function of adipose tissue is to store and requirements of the body. It is well established that the rate of lipolysis is correlated to the fat cell size and consequently to the weight of the body.

Therefore from the experimental results presented in this study it is concluded that these indigenous plant preparations reduce weight (obesity) by mobilizing depot fat from adipose tissue, through the action of these indigenous plant preparations directly as well as mediated through the catecholamines.

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