Materials
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A total of fifty male individuals between the age of forty and seventy five years, were divided into two groups each consisting of twenty five individuals. Out of these twenty five individuals in each group, they were further subdivided into two subgroups of ten and fifteen. The ten being in the age group of forty to fifty nine years and fifteen were in the age group of sixty to seventy five years. All care was taken to select the subjects who were not suffering from any specific disease. The first and second subgroups of ten and fifteen individuals respectively belonging to the group I were treated with extract from root of Satavari (Asparagus racemosus) and the other subgroup of ten and fifteen individuals belonging to the group II were treated with extract from seed of Alkushi (Mucuna pruriens) obtained from Institute of Postgraduate Ayurvedic Education and Research Calcutta. Each individual in each subgroup served as his own control. The respective groups were treated with the Satavari (Asparagus racemosus) and Alkushi (Mucuna pruriens) extracts in the dosage of 5 grams thrice daily for thirty days only, and the different parameters were assessed on the zero day, one month after treatment and after two months.

To study the hormonal profile, serum $T_3$, $T_3$, TSH, FSH, LH and Testosterone were estimated and for polyamine study spermine
spermidine, putrescine and cadaverine in the serum were determined while triglyceride, total cholesterol, HDL Cholesterol were mapped out for assessment of lipid profile. Serum creatinine was also measured in each individual to assess the status of renal function.

PREPARATION OF EXTRACTS FROM ROOTS OF ASPARAGUS RACEMOSUS

Fresh roots of Asparagus racemosus were procured from apothecary department of Institute of Post Graduate Ayurvedic Education and Research (IPGAE&R), Calcutta, identified pharmacognostically and authenticated. It was cleaned, cut into small pieces and dried. It was then handpowdered. Drypowder was weighed and mixed with distilled water at a ratio of 1 : 10 and boiled over a low flame for 30 minutes, cooled and decanted. Residue was then mixed with distilled water at 1 : 10 ratio and boiled for half an hour. It was then cooled and decanted. The above procedure was repeated twice. The clear supernatant obtained each time was decanted and then centrifuged at a speed of 3000 rpm for a period of five minutes. The supernatant was evaporated on low flame when a thick paste like extract was obtained, and dried in an incubator at 37°C and the dry powder was stored in a desiccator (Kiranmai et al 2001)

PREPARATION OF EXTRACTS FROM SEEDS OF MUCUNA PRURIENS

Dried seeds of Mucuna pruriens were procured from apothecary department of Institute of Post graduate Ayurvedic Education and Research
(IPGAE&R), Calcutta identified pharmacognostically and authenticated. The outer covering was removed and then shade dried. The shade dried kernel was subjected to aqueous and 50% ethanolic extraction, separately in Soxhlet apparatus for a period of sixteen hours. After filtration through whatman filter paper No. 40 the filtrates were evaporated and dried in vacuo at a temperature below 50°C. The extracts were stored in screw cap vials at 4°C until further use (Sharma et al 1997).

**BLOOD COLLECTION**

8 ml. of blood were collected from anticubital view of fifty male individuals not suffering from any specific disease in marked Tarson’s centrifuge tube and were allowed to clot. The individuals were selected from the Out door Patient Department (OPD) of Institute of Post Graduate Ayurvedic Education and Research, Calcutta.

The sera were separated from the blood samples by centrifugation at 4,000 rpm for 30 minutes and were collected by Pasteur’s pipette in specific sample vials, and stored at 26°C temperature till the analysis was over.

**DETERMINATION OF THYROID HORMONES AND THYROTROPHINS**

The concentrations of T3 and T4 hormones in the serum of male individuals were estimated by the Enzyme Linked Immunosorbent assay

The principle of this process is as follows —

The assay is based on a competitive reaction principle. After separating the hormone from its carrier proteins TBG (Thyroxine Binding Globulin), TBPA (Thyroxine Binding Pre-albumin) and albumin using 8 Aniline-1-Napthalene sulfonic acid (ANS) and sodium salicylate, the assay is performed in two steps.

(a) Immunological steps. (b) Enzymatic step.

The serum concentration of TSH hormone was estimated by the Enzyme-Linked Immunosorbent assay technique, introduced and developed by Azais TL (1985) and Kaplan MM (1985).

The Principle of this process is based on a sandwich technique using two monoclonal antibodies. The process is also performed into two steps -

(a) Immunological steps (b) Enzymatic steps.

The brief of the above two steps as follows —

(i) Monoclonal anti-hormone coated tubes.

(ii) Hormone present in the sample, standards and in Control.

(iii) Enzyme-conjugate : horseradish peroxidase labelled monoclonal anti-hormone.
Determination of Gonadotrophins and Gonadal Hormones

The serum concentrations of FSH and LH hormones were estimated by the Enzyme Linked Immunosorbent assay technique introduced and developed by Widel et. al (1976) and Butt et al. (1983). Finally the serum testosterone concentration was estimated by the Enzyme Linked Immunosorbent assay technique introduced and developed by Trietz N.W. (1986).

The principle is as follows –

The assay is based on a Sandwich technique using two monoclonal antibodies. It is performed in two steps.

(a) Immunological steps (b) Enzymatic steps.

(i) Monoclonal anti-hormone coated tubes.

(ii) Hormone present in the sample, standards and in Control.

(iii) Enzyme-conjugate : horseradish peroxidase labelled monoclonal anti-hormone.

(iv) Chromogen substrate : Orthophenylene-Diamine (OPD)/H₂O₂

(v) The reading taken at 492 nm at ELISA reader.

(vi) The hormone concentration of each sample was determined by using a calibration curve.
Determination of Cholesterol in Serum:

Principle: Cholesterol estimation is a fully enzymatic procedure with colorimetric determination at 500 nm (Allain et al 1974).

The reaction takes place in three stages:

\[
\text{Esterified cholesterol} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol + fatty acids}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest - 4 en - 3 one} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{Phenol} + 4 \text{ aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{quinone-mine} + \text{H}_2\text{O}
\]

The optical density is read at 500 nm is proportional to the concentration of total cholesterol.

Reagents: The reagents consist of Enzymes and buffer

1. Enzymes: (a) Sodium cholate, (b) 4 aminoantipyrine, (c) Peroxidase, (d) Cholesterol oxidase, (e) Cholesterol esterase

2. Buffer: Phosphate buffer (pH 7.0) and phenol.

Preparation of Reagent:

A vial of Enzymes (Reagent I) was reconstituted with one bottle of buffer (Reagent II) and mixed gently until completely dissolved, but not shaken as shaking might cause enzyme denaturation.
Procedure: 0.03 ml of serum was taken to which 3 ml of reagent was added to constitute the “sample”. Similarly 0.03 ml of standard was taken to which 3 ml of reagent was added to constitute the “Standard”. Again 3 ml of reagent was taken to which 0.03 ml of distilled water was added to constitute the R. Blank.

\[
\text{Cholesterol mg/100ml} = \frac{\text{O.D. of specimen}}{\text{O.D. of standard}} \times \text{Concentration of standard (mg/100 ml)}
\]

Determination of Serum Triglyceride


\[
\text{Triglyceride} \xrightarrow{\text{Lipase}} \text{Fatty acid + glycerol}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-PO4} + \text{ADP}
\]

\[
\text{Glycerol-3-PO4} + \text{O2} \xrightarrow{\text{GPO}} \text{hydroxyacetone phosphate + H$_2$O$_2$}
\]

\[
\text{H$_2$O$_2$} + \text{3-OH, 2,4,6, Tri-iodobenzoic acid} \xrightarrow{\text{Peroxidase}} \text{Chinoeimmine + H$_2$O + 4 amino antipyrine}
\]

The optical density read at 500 nm is directly proportional to the concentration of triglyceride.

Preparation of Regent: A vial of Enzymes (Reagent I) was reconstituted with one bottle of buffer (Reagent II) and mixed gently until
completely dissolved, but not shaken as shaking might cause enzyme denaturation.

**Procedure**: 0.02 ml of serum was taken to which 2 ml of reagent was added to constitute the "sample". Similarly 0.02 ml of standard was taken to which 2 ml of reagent was added to constitute the "Standard". Again 2 ml of the reagent was taken to which 0.02 ml of distilled water was added to make the R. Blank.

\[
\text{Triglyceride (mg/100 ml)} = \frac{\text{O.D. of specimen}}{\text{O.D. of standard}} \times \text{concentration of standard (mg/100 ml)}
\]

**Determination of Serum HDL-Cholesterol**

**Principle**: HDL-Cholesterol is determined after precipitation of LDL and VLDL fractions with phosphotungstic acid and magnesium chloride (Burnstein et al 1970, Lopes Virella et al 1977 and Grove 1979).

**Procedure**:

In a centrifuge test tube 1 ml of serum was taken to which 0.1 ml of precipitating reagent was added. Then it was mixed and let stand for ten minutes at 18-25°C. Then it was centrifuged at 5000-6000 rpm. The supernatant was removed and HDL-Cholesterol was measured following the procedure of cholesterol estimation.
Determination of Putrescine, Cadaverine, Spermine and Spermidine Concentrations in Serum

Paper chromatographic method

From the serum of both benign and malignant cases, putrescine, cadaverine, spermine and spermidine were separated by the paper chromatographic method of Herbst et al (1958). From the serum of normal healthy individuals, polyamines and diamines were separated similarly as control.

The equipment for paper chromatography was similar to that described by Woodruff and Foster (J Biol. Chem. 183, 569, 1950). Whatman filter paper No. 3 was used. The descending method was used routinely and the development of chromatograms was complete by 16 hours at room temperature. The samples were applied 4 inches (10 cm) from the end on the one side of the chromatographic paper strip by streaking with a 25 ml micropipette. The chromatographic chamber was saturated with the solvent (Ethylene-glycol mono-methyl ether, propionic acid and double distilled water in the ratio of 70 : 15 : 15) saturated with sodium chloride (NaCl) prior to placement of chromatographic papers in the trough filled with solvent.

After 16 hours of the run at room temperature, the chromatographic paper was dried in air at room temperature. In the preliminary screening of solvents for the separation of polyamines, the compounds were detected by spraying the papers with 0.25% ninhydrin in acetone
solution (Anal Chem 23, 823, 1951), followed by brief heating at 100 °C. The colour developed were eluted in 71% ethanol and the absorbance was read at 570 mm in a Beckman spectrophotometer and quantitative values were obtained from the standard curves of the respective polyamines and expressed in n mol/ml serum.

Putrescine, cadaverine, spermine and spermidine were obtained from the Sigma Chemical Company, St. Louis, MO, USA. All the chemicals used were of analytical grade.

DETERMINATION OF CREATININE CONCENTRATION IN SERUM

Creatinine forms a yellow-orange compound in alkaline solution with picric acid. At the low picric acid concentration used in the method a precipitation of protein does not take place. The concentration of the dye-Stuff formed over a certain reaction time is a measure of creatinine concentration.

As a result of the rapid reaction between creatinine and picric acid later Secondary reactions do not cause an interference, (Helger et al 1974). This method distinguishes itself by its high specificity (Bartels et al 1972; Ulman et al 1976).