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Anti oxidant activity of a classical siddha drug Kiranthimega Chooranam

Praveena R[1], Muthukumar N. J[2], Banumathi V[3]
[1]Yoga expert, National Institute of Siddha, Chennai
[2]HOD, Sirappu Maruthuvam Dept, National Institute of Siddha, Chennai
[3]Director, National Institute of Siddha, Chennai

Abstract

The SIDDHA system of medicine, a branch of Indian system of medicine laid emphasis on eternal happiness. SIDDHA literature contains boundless medicinal preparations to alleviate all unwelcome sufferings that the physical body attains due to deceptive MAYA.

Kiranthi Mega Chooranam (KMC), a poly herbal preparation is used to treat skin & venereal diseases. In the present study KMC has been evaluated for anti-oxidant property by the estimation of Lipid peroxidation, Superoxide dismutase and Catalase in animal models. This study is still a basic one, which lends evidence for further studies.

Keywords: SIDDHA, Kiranthi Mega Chooranam, Anti-Oxidant

Introduction

In this world, everyone wants to be happy in their own way and this differs from person to person. Happiness is of two kinds; Ephemeral & Eternal.

Ephemeral or temporary happiness pertains to mundane life which is enjoyable for short period. Eternal happiness pertains to spiritual life that is enjoyable forever. i.e.,perpetual happiness.

The word SIDDHA is derived from the word SIDDHI (trans-consciousness). SIDDHI in turn is interpreted in two ways vide attaining perfection in life & heavenly bliss.

According to SIDDHARS, the purpose of life is to attain salvation. In order to achieve spiritual heights, SIDDHARS believe, maintaining a healthy body is essential as it holds a healthy mind which is required to attain salvation. They have tested all possible avenues for preserving their body. The result was amazing, which paved way to the evolution of NATURE BASED HEALTHCARE system. And we call it as SIDDHA SYSTEM.

According to SIDDHAR's augmenting the process of decay in human health system leads to body's longevity which in turn leads to spirit's longevity.

Activated oxygen is thought to be a major factor in Ageing, Hardening of the Arteries, Diabetes, Cancer & Tissue injury of skin. Anti-oxidants play major role in scavenging the activated oxygen species. In reference to this, below is the trial drug which has been tested for Anti-Oxidant property

Materials and Methods

Preparation of trial drug (internal)
Kiranthi mega chooranam

(Ref: Anubogha Vaidhya Navaneetham – Part 10)

Ingredients:

- Nilaavarai Ilai
- Thavasu Murungai Samoolam
- Shivanar Vembu Samoolam
- Velarugu Samoolam
- Thoothuvalai Samoolam
- Kottaikaranthai Samoolam

Preparation:

The above ingredients are dried in shade and powdered separately. These ingredients are mixed in equal proportion and again powdered for an hour and purified before preserving in a peangon vessel. The purification process is as follows,

An earthen pot which contains equal volume of water and milk is taken and covered fully with a cloth. The above ingredients are placed over the cloth and the pot has to be covered by another pot and heated till the vapour comes out of the pot.

Dosage : 1.5 to 2 varagan (6-8 gms)

Vehicle : Hot water

Indications : Kiranthi, Megam, Kadi vidam, Kuttam, Mega vayu, Mega soolai.

Estimation of anti oxidant activity of kiranthi mega choornam

Materials and Methods

Test Drugs

The medicinal formulation used in the study was processed by the methods prescribed in standard text books of siddha medicines.

Kiranthimega Chooranam - Referece: Anoboga Vaithiya Navaneetham

Preparation of drug for dosing

KMC was not soluble in water and made into a suspension in sodium carboxy methyl cellulose before administration. The drug suspension was administered at the dose of 2000 mg/kg/po for acute toxicity study and at the dose of 900 mg/kg/po for 15 days repeated oral toxicity and other pharmacological studies.

In Vivo Anti-Oxidant study

Samples of centrifuged exudate from the cotton pellet implants collected from positive control rats and rats treated with test drugs were assayed for LPO (Okhawa et.al.1979), SOD (Manelund et.al.1974),Catalase (Sinha,1972and the results were compared with negative and positive control.

Estimation of Lipid Peroxidation (LPO)

Lipid peroxidation in the exudate was determined by measuring the amounts of malondialdehyde produced primarily by the following method

Reagents:

1. 20% Acetic acid
2. 8.1% Sodium dodecyl sulphate (SDS)
3. 0.8 % Thiobarbituric acid (TBA)
4. N-Butanol-Pyridine mixture (15:1v/v)

Procedure:

0.2 ml of exudate, 1.5 ml of 20% acetic acid, 0.2 ml of sodium dodecyl sulphate and 1.5 ml of thiobarbituric acid were added. The volume of the mixture was made up to 4.0 ml with distilled water and then heated at 95°C in a water bath for 60 min. After incubation the tubes were cooled to room temperature and final volume was made to 5.0 ml in each tube. 5.0 ml n-butanol pyridine (15: 1) mixture was added and the contents were vortexes thoroughly for 2 minutes. After centrifugation at 3000 rpm for 10 min, the organic upper layer was taken and its optical density was measured at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as n moles of Malon dialdehyde (MDA)/mg protein in liver homogenate.

Assay of Superoxide Dismutase (SOD)

Superoxide dismutase was assayed by following method

Reagents:

1. 0.05M, Carbonate buffer, pH 10.2
2. 0.49M, Ethlene diamine tetra acetic acid (EDTA)
3. 3mM Pyrogallol solution
4. Absolute ethanol
5. Chloroform

Procedure:

0.5ml of exudate was diluted with 0.5ml of distilled water. To this 0.25ml of ethanol and 0.15ml of chloroform (all reagents chilled) were added. The mixture was shaken for 1 minute and centrifuged at 2000rpm. The enzyme activity in the supernatant was determined.

To 0.5ml of the exudate , 1.5ml of buffer was added. The reaction was initiated by the addition of 0.4ml of pyrogallol and the changes in optical density per minute was measured at 480nm using a photochem colorimeter. SOD activity was expressed as units/min/mg protein. Change in optical density per minute at 50% inhibition of pyrogallol to adenochrome transition by the enzyme was taken as one enzyme unit.

Estimation of Catalase (CAT)

Catalase activity was assayed by the following method.

Reagents:

1. 0.01M, Phosphate buffer, pH 7.0
2. Dichromate-acetic acid reagent: 5% potassium dichromate was prepared with dilute acetic acid (1.3 v/v in distilled water)
3. 0.2M, Hydrogen peroxide

Procedure

To 0.1ml of the exudate with 1.0ml of buffer, 1ml of hydrogen peroxide was added and the timer was started. The reaction was arrested by the addition of 2.0ml of dichromate-acetic acid reagent. Standard hydrogen peroxide in the range of 4 to 20 µmoles was taken and treated similarly. The tubes were heated in a boiling water bath for 10minutes. The green colour development was read at 570nm using a photochem colorimeter.

The catalase activity was expressed as nmoles of H_2O_2 consumed/minute/mg protein under incubation conditions

Results

Anti – oxidant activity

Lipid Peroxidation (LPO)

LPO level in KMC (180mg/kg/p.o) treated rats showed significant decrease (P<0.01) when compared to carrageenan+ egg albumin cotton pellet implantation model of chronic inflammation in rats. Diclofenac also showed significant (p<0.001) decrease in LPO level when compared to carrageenan+ eggalbumin induced chronic inflaaamtory model.

Superoxide Dismutase (SOD)

A significant decrease (P<0.01) in the serum Superoxide dismutase (SOD) was observed in group-2 animals when compared to control animals. The SOD level in KMC (18mg/kg/p.o) treated rats showed significant(p<0.05) increase when compared to group-2 animals Diclofenac (5 mg/kg b.w/p.o) showed significant (p<0.001) increase in SOD levels when compared to group-2 animals.

Catalase (CAT)

A significant (p<0.001) decrease in the exudate of Catalase (CAT) was observed in group-2 animals.The exudate CAT levels in KMC(180mg/kg/p.o) treated animals showed significant increase(p<0.01) when compared to group-2 rats Diclofenac(5mg/kg b.w/p.o) showed significant (p<0.01) increase in exudate CAT levels when compared to group-2 animals.

Discussion

Oral administration of (KMC) for 7 days at the dose of 180mg/kg/po showed an decrease in LPO in inflammatory exudates significant difference was showed in the levels of SOD and Catalase in animals treated with KMC when compared to positive control group of animals. The inhibition of lipid peroxidation,

increase in SOD and Catalase antioxidant enzyme levels in the inflammatory exudate may be attributed to the antioxidant activity of the test drug against free radicals

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