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ANTIOXIDANT ACTIVITY OF *"KRISHNA TILA"*

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ABSTRACT

Antioxidant is a property of the drug that slows down the oxidation of hydrocarbons, oils, fats etc & thus it helps to check the deterioration. Antioxidants work by significantly slowing down the oxidation by the substance called 'Free Radicals'. *Krishna tila* (Sesamum indicum, Linn) is one of the plant origin drugs which had been mentioned for its rasayana properties in classical literature of Ayurveda. So study was undertaken to evaluate the efficacy of *Krishna Tila* in the form of oil , in the experimental study on healthy human blood serum regarding Antioxidant activity.

Key words: *Krishna tila* , Antioxidant activity

INTRODUCTION:

Oxygen water and food are of fundamental importance of life. Among these deprivation of oxygen leads to sudden death. Oxygen is most essential part of functions like metabolism, respiration, phagocytosis etc, but oxygen also plays critical role in cellular injury by toxic activated oxygen species which are called as "Free radicals". Free radicals are molecules or fractions of molecule having unpaired electron in their outer most orbit .Free radicals produced continuously in cells during metabolism, respiration and deliberately during phagocytosis. These cytotoxic free radicals play an important role in immune system dysfunction which is responsible for many disorders like arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer and cardiovascular disease.

Anti-oxidants are molecules that are useful in preventing early oxidation of body cells.. Antioxidants are the agents which stimulates immunity of an individual either in healthy or with immune deficiency.

Ayurveda is one among secular biomedical sciences. It is based on biophysical, biochemical, physiological and biopharmacodynamic principles. So it is said as 'Rasa Rasayana chikitsa'. Rasayanas are the groups herbal and herbo-mineral preparations explained in ayurvedic classics to prevent the process of aging and to prevent the diseases. These will help for the nourishment of dhatus from rasa to shukra ultimately the ojas, which is responsible for physical and psycho-intellectual performances. In this way rasayana promotes long span of youthful life full of vigor and free from diseases and adverse effects of ageing. Therefore it can be postulated that rasayana drugs may possess Free radical scavenging property.

Hence to provide a scientific data and statistical validation a study on 'Screening of free radical scavenging activity effect of '*Tila*' was under taken.

AIMS AND OBJECTIVES:

To evaluate antioxidant activity of *Krishna tila beeja* (Sesamum indicum , Linn) through lipid per oxidation (TBARS method).

REVIEW:

CONCEPT OF FREE RADICLE: [1,2]

Definition: A molecule or molecular fragment containing unpaired electron in valence shell and capable of existing independently is called 'free radical. The molecule of a compound is usually made up of two parts which are known as radicals. For example, the radicals present in sodium chloride molecule are sodium and chloride. These posses electric charge. So these are highly reactive.

Types of free radicals: Free radicals may be

- Oxygen derived (Reactive Oxygen Species, ROS)
- Nitrogen derived (Reactive Nitrogen species, RNS)
- Sulphur derived (Thinly / Thiele free radicals) and others.

Exogenous sources of ROS : Electromagnetic radiation, cosmic rays, cigarette smoking, car exhaust, UV light, Ozone, ionizing radiation, certain drugs.

Endogenous sources of ROS : Mitochondrial electron transport chain, Respiratory burst by phagocytosis, β oxidation of fat, auto oxidation of amino acids, ischemia reperfusion injury.

Free radical mediation of cell injury: One important mechanism of cell damage is injury induced by free radicals, particularly by activated oxygen species.

1. Lipid peroxidation of membranes
2. Mitochondrial damage
3. DNA damage
4. Protein damage

PHYSIOLOGICAL IMPORTANCE OF FREE RADICALS: [3]

Free radicals are produced continuously in the cells as accidental by products of metabolism, respiration and deliberately during phagocytosis. Free radicals are naturally produced in the body through the normal metabolism of amino acids and fats. In case of phagocytosis the bacteria to be phagocytosed are first coated with antibody (IgG) and compliment (C3), etc. This process is called opsonisation. The phagocytic cell then binds to this opsonised bacteria and phagocyte is activated this leads to increased movement and endocytosis. Release of enzymes and increased oxygen consumption (Respiratory burst) forms toxic substance like O_2^-, H_2O_2, HOCl, NO which will kill the bacteria. Subsequently endocytosed materials are digested.

ANTI-OXIDANTS : [4]

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents.

Classification of Anti-oxidants:

1. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic).

2. a) Endogenous anti-oxidants: [5] These are the physiological enzymes which are involved in cellular defense against uncontrolled oxidative process.

b) Exogenous anti-oxidants: Those which cannot be produced by human body, but may protect pro-oxidant forces when administered. Such as Vitamin-E, Vitamin-C, β – Carotene, lycopene, Selenium, Manganese, copper, Zinc.

3. According to action: [6]

a) Preventive anti-oxidants

b) Chain breaking anti-oxidants

c) Pharmacological anti-oxidants:

MEANING OF RASAYANA: [7]

The word Rasayana is composed of two words Ras + Ayan. The means by which one gets the excellence of Rasa (The nourishing fluid which is produced immediately after digestion) is known as Rasayana.

Rasayana is a specialized type of treatment influencing the fundamental aspect of body viz. Dhatus, Agni and Srotansi and ojus etc. Rasayana Chikitsa boosts the ojus and immune system. The adjective Ojaswiis used to describe those people who keep good health in all seasons and all stages of life. It is like obtaining a high rank in a physical or mental fitness. Ojus gives a bright look, sharp memory, high performance and every expected pleasure.

RASAYANA & ANTIOXIDANTS:[8]

Rasayana is one of the eight branches of Ayurveda. The world Rasayana made by worlds Rasa + Ayana. The world Rasa has multiple meanings. It means shrangaara, visha, veerya, guna, raga, drava etc. Ayana means the pathway. So literally Rasayana means the pathway to to get best quality of dhatus. It helps to maintain the intactness of body components also cures and prevents the diseases.

ACTION OF RASAYANA:

Generally action of rasayana can be explained in three levels.

1. Poshak stara: Some of the rasayana act as Rasa vardhaka. They mix with poshak rasa and does the nourishment of dhatus. Ex- Shatavari, Dugdha, Ghrita etc.

2. Agni stara: Some rasayana act on jatharagni and other agni's.They activate agni and increase metabolism and help dhatu's for nourishment. Ex- Pippali rasayana.

3. Srotas stara: Some rasayana act on srotas cleanses channels and help for the circulation of rasa. Thus helps for the nourishment of dhatus.Ex- Guggulu.

In modern view rasayana dravyas work as anti-oxidants.. Achara rasayana act as antioxidants, reduces the stress and thus prevent the release of free radicles

RAKTA DHATU IN AYURVEDA: [9]

Rakta is originated from rasa dhatu. Rakta is formed by raktagni through the prasadamsha of rasa dhatu.

SERUM: [10]

In blood, the **serum** is the component that is neither a blood cell (serum does not contain white or red blood cells) nor a clotting factor; it is the blood plasma with the fibrinogens removed. Serum includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms).The study of serum is serology and may also include proteomics. Serum is used in numerous diagnostic tests, as well as in blood typing

VITAMIN C (standard drug) : [11,12] Vitamin C, also known as ascorbic acid, is a water-soluble vitamin which is essential for normal functioning of the body.

$\underline{CCL_4}$ (Inducing drug) [13]

Synonyms: IUPAC Name -Tetra chloromethane

-1, 1, 1, 1-Tetrachloromethane

EXPERIMENTAL STUDY: [14]

ANTIOXIDANT ACTIVITY:

Aim: To evaluate the antioxidant effect of *Krishna Tila* (Sesamum indicum, Linn)

METHOD:

PARAMETERS TAKEN FOR ASSESMENT:

LIPID PEROXIDATION (TBARS METHOD) :

METHOD IN GENERAL:

- ✓ To take healthy human blood sample & separation of serum
- ✓ Addition of drugs to serum & to access the parameters

MATERIALS REQUIRED FOR LIPID PEROXIDATION:

The lipid peroxidation of the test sample is assessed by its absorption which react with the Thiobarbituric acid & thus produces the result. The protein in the serum undergoes lipid peroxidation.

1. Drug *: Krishna Tila Taila*

2. Healthy human blood sample with the separation of serum
3. Equipments & glasswares

CHEMICALS & REAGENTS:

- 20% Trichloro acetic acid (TCA)
- 0.05N sulphuric acid
- 2 molar sodium sulphate
- Thibarbituric acid reagen
- N- Butyl alcohol

METHODS:

LIPID PEROXIDATION (TBARS):

Principle:

The proteins in serum are precipitated by trichloroacetic acid (TCA) and the mixture is heated with thiobarbituric acid in 2 M. sodium sulphate, in a boiling water bath for 30 mins. The resulting chromogen

Control Group	CCl_4 Treated	Vit- C Treated	Test Drug 1%	Test Drug 2%	Test Drug 5%
0.647	1.134	0.455	1.025	0.775	0.608
0.583	1.108	0.467	1.044	1.064	0.821
0.756	1.211	0.666	0.878	0.583	1.000
0.654	1.121	0.487	0.673	0.778	0.666
0.596	1.243	0.608	1.006	1.019	0.794

is extracted with n-Butyl alcohol and absorbance of organic phase is determined at 530 nm wavelength. The values are expressed in term of absorption of each sample which is calculated by the Beer- Lamberts Law.

Method of assessing Lipid peroxidation:

1. Preparation of Chemical Reagents
2. Separation of serum from blood
3. preparation of Test Sample
4. observance of color change in different samples
5. Estimation of serum lipid peroxide

1) Method of Preparation of Chemical Reagents:

- 20 % Trichloroacetic acid: 20 gms of TCA was dissolved in 100 ml of distilled water and kept in a bottle.
- 0.05 N sulphuric acid: 1.23 ml of conc. H_2SO_4 was diluted with 250 ml of distilled water & kept in bottle.
- 2M sodium sulphate : 28.4 gms of unhydrous sodium sulphate was dissolved in 90 ml of distilled water by heating & stirring. Then volume was made up to 100 ml with distilled water.
- Thiobarbituric acid reagent: 0.67 gms (670 mg) of TBA was dissolved in 2M sodium sulphate solution by heating & the volume was made upto 100 ml, kept in brown coloured bottle.

2) Preparation of Serum:

The blood from the healthy person is taken & is been kept in centrifugation chamber for the separation of the serum. This is used for the assessment.

3) Method of preparation of Test Sample:

The fixed oil from the sesamum seeds is been extracted through the soxhlet apparatus & the solvent used is petroleum ether. Hence the oil obtained is taken as the test sample.

4) **Method of Observation of absorption :**

- 1 ml of T.B.A. reagent was added to each dilutions (Test tube).
- Kept in boiling water bath for 30 min for coupling of lipid peroxide with TBA-reagent.
- Pink color starts appearing in this stage.
- Then cooled in cold water
- Resulting chromogen extracted with 4 ml of n-Butyl alcohol by vigorous shaking.
- Then centrifuged at 3000 rpm for 10 mins for the separation of organic phase
- Absorbance is Measured at 530 nm wavelength
- The individual samples absorption are calculated with Beer- Lamberts law formula.

This formula is applied to calculate the molar concentrations of each sample separately.

5) Estimation of serum lipid per oxidation:

Procedure: Aliquots of 4ml of homogenate were taken in 2 different 25ml conical flasks. All the test tubes are added with 6ml of potassium sulphate buffer (pH, 7.4) , 8mlof 0.15ml potassium chloride. In test group three different conical flasks added three concentrations of drug like 1%, 2% & 5% . In control group distilled water is added, in standard group vit-c is addd, in inducing group ccl_4 is added & in test group test sample is added. Totally 6 test tubes are incubated at 37°c in incubator.

Lipid peroxidation is assessed on 1 st day(after 45 min), 2 nd day and 4 th day.On each day 0.5ml of reaction mixture from the homogenate which is kept for incubation is taken in a test tube and added with 4ml of 10% Trichloroacetic acid(TCA). Contents centrifuged at 4000 rpm for 10 min. 2ml of clear supernatant is taken in a graduated tube. 2 ml of 0.67% Thiobarbituric acid (TBA) is added and heated in boiling water bath for 15 min. Then tubes are cooled, pH is adjusted to 12-12.5.Colour which is developed stabilized. Absorbance is measured at 540 nm in an UV spectrometer & calculated by using Beer- Lamberts Law.

Test group treated with : *Krishna tila taila*
Control group treated with: Distilled water
Induced group treated with : CCl_4
Standard drug treated with : Vit- C

OBSERVATIONS:

OBSERVATION – ANTIOXIDANT EFFCT OF Sesamum indicum:
OBSERVATION OF EXPERIMENTAL STUDY:
The molar concentrations of the samples are : Table no 10 **LIPID PROFILE OF THE SAMPLES:**

1. CONTROL GROUP: Table no 11

Control group	LPO in µM concentrations
A	0.647
B	0.583
C	0.756
D	0.654
E	0.596

2. INDUCED GROUP : Table no 12

CCl_4 treated	LPO in µM concentrations
A	1.134
B	1.108
C	1.211
D	1.121
E	1.243

3. STANDARD GROUP: Table no 1

Vit-C treated	LPO in µM concentrations
A	0.455
B	0.467

C	0.666
D	0.487
E	0.608

4. 1% CONCENTRATION : Table no 14

1% concentration treated	LPO in µM concentrations
A	1.000
B	1.044
C	0.878
D	0.673
E	1.006

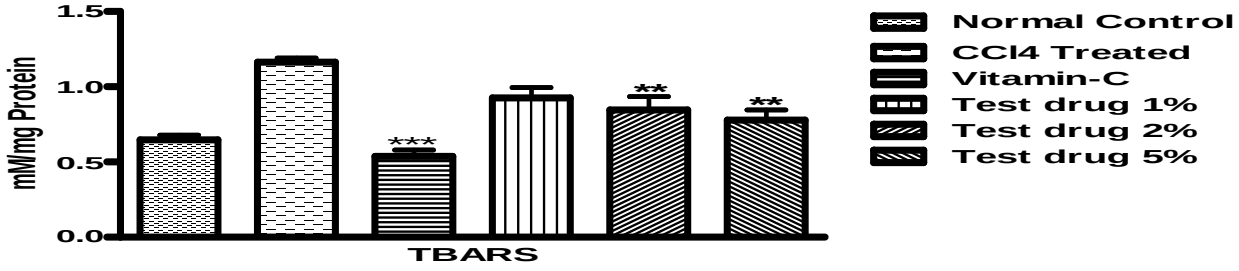
2% concentration treated	LPO in µM concentrations
A	0.775
B	1.064
C	0.583
D	0.788
E	1.019

5. 2% CONCENTRATION: Table no 15

6. 5% CONCENTRATION : Table no 16

5% concentration treated	LPO in µM concentrations
A	0.608
B	0.821
C	1.000
D	0.666
E	0.794

GRAPHICAL REPRESENTATION OF RESULTS:



DISCUSSION ON FREE RADICALS: Free radicals are molecules or fractions of molecule having unpaired electron in

their outer most orbit .Free radicals produced continuously in cells during metabolism, respiration and deliberately during phagocytosis. These cytotoxic free radicals play an important role in immune system dysfunction which is responsible for many disorders like arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer and cardiovascular disease.

DISCUSSION ON ANTIOXIDANT PROPERTY:

Anti-oxidants are molecules that are useful in preventing early oxidation of body cells. Oxidation of body cells can lead to generation of free radicals that are solely responsible for aging as well as degenerative process happening in the body these antioxidants are involved in inhibiting the production of free radicals there by making the person feel younger and healthier both mentally and physically. Antioxidants are the agents which stimulates immunity of an individual either in healthy or with immune deficiency. These produce effect either by cellular or humoral or by both.

DISSCUSION ON ACTIVITY:

The result is been withdrawn on the basis of the color change of the samples to pink when TCA is added. This helped to estimate the overall lipid per oxidation in the samples. And the same is been calculated through absorption factor which is calculated by Beer- Lamberts law.

PROBABLE MODE OF ACTION:

The Rasayana (particularly those with madhur vipaka that are advocated as adapt gens in Ayurveda) primarily activate immune cells, leading to secretion of cytokines, which in turn act on multiple target organs to produce the myriad effects ascribed to these treatments.

It has been found that the nervous, endocrine and immune systems are all interrelated. Immune products like various cytokines have been found to stimulate the hypothalamus-pituitary-adrenal axis and corticotrophin release factor (CRF), which ultimately enhances the production of adrenal corticotrophic hormone (ACTH) resulting into increased secretion of glucocorticoids which have an overall suppressive effect on the immune system. Stress also acts on the same axis and brings about changes in the immune status of the body. These Rasayana drugs probably reduce stress levels by affecting antioxidant levels. So these Rasayana drugs act as potent antioxidants.

CONCLUSIONS:

1. Madhura, tikta, kashaya –rasa, madhura-vipaka , guru, snigdha- gunas, these qualities in *Krishna tila* acts as rasayana.
2. Presence of flavonides & tannins in Krishna tila beeja dominates antioxidant property.
3. Study has shown significant antioxidant activity on molar concentrations of 2% & 5%.
4. Easily available *Krishna tila* can be used safely as substitute over modern antioxidant drugs.

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