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Research Article

In-Vivo Anti-Oxidant of "ATH-2K13" in Normal and CCl_4 Induced Rats

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ABSTRACT
To evaluate the in-vivo antioxidant potential of poly herbal formulation ATH-2K13 against CCl4 induced toxicity in rats. All the animals are divided in to 5 groups and in each group consist of six animals each. All the animals were treated with the poly herbal formulation ATH-2K13 for 7 days. The toxicity was induced to the animals with the intraperitoneal injection of carbon chloride (ccl_4). The treatment of animals with the poly-herbal formulation may produce its effectives when compared with the control. The two doses of the poly herbal formulation were 100mg/kg, 200mg/kg. The present study reveals that the present drug shows significant in-vivo anti-oxidant activity.

Keywords: ATH-2K13, In-vivo antioxidant activity, CCl_4 (carbon tetra chloride), SOD (Superoxide dismutase), catalase, LPO.

INTRODUCTION

Free radical induced oxidative damage has long been thought to be the most important consequence of the aging process[1]. Such conditions are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer, and cardiovascular diseases[2-3]. Studies show that these radicals also affect the equilibrium between pro-oxidants and antioxidants in biological systems, leading to modifications in genomes, proteins, carbohydrates, lipids and lipid peroxidation[4] thus inactivating antioxidant defence. Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential[5].Natural antioxidants are in high demand for application as neutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference. The present work undertaken to prove the pharmacological action of the ATH-2K13.The poly herbal formulation of ATH-2K13 consists of three herbals called dried rhizomes of Curcuma longa linn, Embilica officinalis,Honey.

MATERIAL AND METHOD

Plant Material: Procurement and identification of plant material: The dried rhizomes of Curcuma longa, fruits of Embilica officinalis and honey were procured at Erragadda rythu bazar. The dried rhizomes and fruits were brought to the laboratory, cleaned the rhizomes and washed fruits thoroughly in running tap water to clean the adhering sand particles and then rinsed in distilled water, shade dried, coarsely powdered and stored in air tight containers for further use.

Preparation of crude methanol extracts of dried rhizome of Curcuma longa linn. and Embilica officinalis: After washing the rhizomes dried and fruits, powdered and submitted to successive extraction by soxlet apparatus with 100%methanol at 72degrees temperature for 18hrs. All the extract was filtered through membrane filter and then the extract dried in room temperature. By using Tween 80 the poly herbal formulation is prepared in the form of suspension. The two doses of ATH-2K13 100mg/kg, 200mg/kg were prepared by adding methanolic extracts of dried fruits of amla and rhizomes of turmeric in 1:1ratio and honey is used as vehicle to test the in vivo anti-oxidant activity.

Chemicals used: The various chemicals used for the investigation of invio-antioxidant activity were CCl4 (Qualigens Ltd. India), Liv-52 (Himalaya, India), hydroxylamine hydrochloride (Hi- media, India), nitro-blue tetrazoleum (NBT) (Hi-media, India), hydrogen peroxide, EDTA, and Ellman's reagent (Sigma, India)[6].

Animals: Healthy adult albino rats of Wister strain of either sex between the age of 2-3 months and weighing 200-300 grams were used for the present study. The animals were housed individually in polypropylene cages, maintained under standard conditions (12 hours light and12 hours dark cycle, 23±5°C and 40-60% humidity). They were fed with standard rat pellet diet (National Institute for Nutrition, Hyderabad) and provided water ad libitum. All the animals are collected from central animal house SICRA LABS PVT.LTD, KUKATPALLY, HYDERABAD and all experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC reg. no. (769/2011/CPCSEA).

Phytochemical Screening: A preliminary phytochemical screening of methanolic extracts was carried by using standard procedures.The phytoconstituents present in the

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Table-1 Phytochemicals constituents of curcuma longa

Phyto constituents	Curcuma longa
Alkaloids	+
Carbohydrates	+
Glycosides	+
Tannins	+
Proteins and amino acids	+
Saponins	+
Steroids	+
Flavonoids	+
phenols	+

Table-2. Phytochemicals constituents of Embilica officinalis

Phyto constituents	Embilica officinalis
Alkaloids	+
Carbohydrates	+
Glycosides	+
Tannins	+
Proteins and amino acids	+
Saponins	-
Flavonoids	+
phenols	+

herbals present in the current formulation were shown in table1, 2.

Acute Oral Toxicity Studies: Acute oral toxicity studies[7] of the extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted and received from Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India.

Albino wistar rats (n=30) were fasted for 16 to 18hrs and were divided into four groups of 6 animals each and the treatment protocol was described below. Before the drug administration, blood samples were collected by spinning the tail vein for the estimation of glucose levels.

Treatment protocol: All the rats were randomized into five groups comprising of six animals in each group as given below.

Group I : normal rats received 100mg of ATH-2K13
Group II : normal rats received 200mg/kg ATH-2K13
Group III: normal rats received 500mg/kg ATH-2K13
Group IV: normal rats received 750mg/kg ATH-2K13
Group V : normal rats received 1000mg/kg ATH-2K13

ATH-2K13of various doses were administered orally using an intra-gastric tube and monitored for 48hrs.After the administration of single dose of ATH-2K13 no mortality rate is observed. The results are shown in table-3.

Experimental

Group-I animals - Normal control, treated with vehicle (gum acacia 10% solution).

Group-II animals - Toxic control, treated with CCl4 in a single dose of 1.5 ml/kg, i.p., to produce acute toxicity.

Group III animals- Standard group, treated with Liv-52 in a dose of 56 mg/kg, p.o.

Group-IV animals-Test group 1treated with daily doses of 100mg/kg ATH-2K13

Group-V animals-Test group 2 treated with daily doses of 200 mg/kg, p.o., ATH-2K13

From the animal of Groups III–V were given single dose of CCl4, 1.5 ml/kg, i.p., 6 h after the last treatment. After 7 days of drug treatment on the 8th day the rats were sacrificed by carotid bleeding and liver was rapidly excised, rinsed in ice-cold saline. The 10%w/v homogenate was prepared by using 0.15M KCI, centrifuged at 800 g for 10 min at 4°C. The supernatant fluid which is obtained after centrifugation was used for the estimation of LPO, SOD, and Catalase.

Biochemical estimation: Estimation of LPO[8]: Malondialdehye, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of peroxidation reaction. Malondialdehye reacts with thiobarbituric acid (TBA) to produce red colour species which is measured at 540 nm. To the 1 ml of supernatant, 0.5 ml of 30% trichloroacetic acid (TCA) was added followed by, 0.5 mL of 0.8% TBA. The tubes were kept in a shaking water bath for 15 min at 100 °C. After 15 min of incubation the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800 g for 15 min. The amount of MDA was assessed by measuring the absorbance of supernatant at 540 nm at room temperature against an appropriate blank. The percentage inhibition of Lipid peroxidation was calculated using the equation:

$$\% \text{ inhibition} = A_0 - A_1 / A_0 \times 100$$

Since A_0 = absorbance of the control without extract

A_1 =absorbance of the sample extract.

Estimation of Catalase: To estimate the catalase activity by the method[9] the reaction mixture consisted of 1.95 ml phosphate buffer (0.1 M, pH 7.4), 1.0 ml hydrogen peroxide (H_2O_2) (0.019 M), and 0.05 ml of supernatant in a final volume of 3 ml. Changes in absorbance were recorded at 240 nm. The percentage inhibition of free radicals by CAT was calculated using the equation

$$\% \text{ inhibition} = A_0 - A_1 / A_0 \times 100$$

Where, A_0 is the absorbance of the control without extract and A_1 is the absorbance of the sample extract.

Estimation of superoxide dismutase (SOD): The Estimation of superoxide dismutase was done by auto oxidation of hydroxylamine at pH 10.2, which was done by the reduction of NBT, and the nitrite produced in the presence of EDTA was detected colorimetrically[10]. One enzymatic unit of SOD is the amount in the form of proteins present in 100il of 10% liver homogenate required to inhibit the reduction of 24 mm NBT by 50% and is expressed as units per milligram of protein.

STATISTICAL ANALYSIS

All the results were subjected to one-way ANOVA. $P<0.05$ was considered significant. The post hoc analysis was carried out by Dunnet's multiple comparison test. The statistical analysis was done by using prism graph pad software.

RESULTS AND DISCUSSION

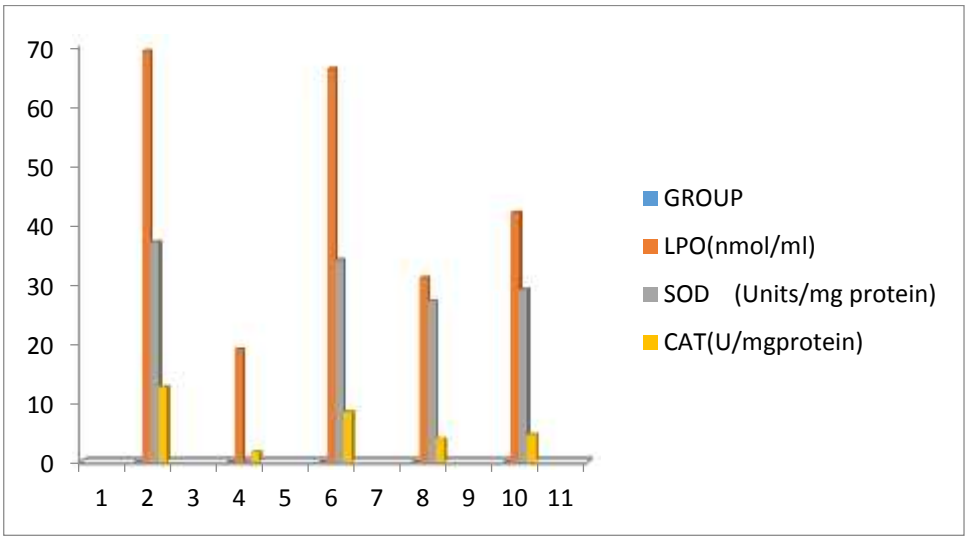
Table-3.Acute Oral Toxicity Studies

	NO OF RATS/NO.OF MORATLITY			
DOSE	6Hh	12hr	24hr	48hr
100mg/kg	6/0	6/0	6/0	6/0
200mg/kg	6/0	6/0	6/0	6/0
500mg/kg	6/0	6/0	6/0	6/0
750mg/kg	6/0	6/0	6/0	6/0
1000mg/kg	6/0	6/0	6/0	6/0

Table-4: Table For Anti Oxidant Activity

GROUP	LPO(nmol/ml)	SOD (Units/mg protein)	CAT(U/mgprotein)
Normal	69±0.021	37±0.13	12.7±0.017
Control(ccl_4)	19±.11	19±0.14	1.71±0.091
Standard Liv 52	66±.09**	34±0.10***	8.46±0.007***
ATH-2K13(100mg/kg)	31±.011	27±0.11	3.9±0.008
ATH-2K13(200mg/kg)	42±.013*	29±0.19**	4.6±.008**

Values are in Mean±SEM. Where p<0.05, **p<0.01, ***p<0.0001*



Anti-oxidants are acting as fighting free radicals and they are protecting us from various clinical manifestations which are caused due to the endocrinal disorders and neurological disorders. They play vital role in the metabolism. Antioxidant exerts their activity by scavenging of oxygen free radicals or they produce some defence mechanisms. Here in the present study after the preliminary phytochemical screening of herbs the in-vivo antioxidant activity screening was done by using carbon chloride as toxic agent which was used here as control. It is a seven days study. Two test doses that are 100mg/kg, &200mg/kg of ATH-2K13formulation was used. Under the screening of in-vivo antioxidant activity we have studied three parameters called SOD, CAT, and LPO. The standard drug use in this screening Liv-52 in a dose of 56 mg/kg, p.o.The changes caused due to the induction of ccl_4 was liver damage and hepatotoxicity was produced. In CC14 induced toxicity the test drug shows significant anti-oxidant activity. We propose that the additive and synergistic antioxidant activity of phytochemicals such as flavonoids present in the ATH-2K13 formulation showed some significant activities and the anti-oxidant enzymes were almost back to their normal levels. Thus our investigation showed that the enzymatic antioxidants so called SOD, LPO, and CAT were improved significantly when compared with the control group to the drug treated group. The poly herbal formulation due to the presence of active phytochemicals involved in the removal of oxygen free radicals and showed good antioxidant property.

REFERNCES

1. Harman D. Role of free radicals in aging and disease. Annuals of the New York Academy of Sciences 1992; 673: 598– 620.
2. Prior RL, Cao G. Analysis of botanicals and dietary supplements for antioxidant capacity: a review The Journal of AOAC International 2000; 83: 950–956.
3. Yamaguchi F, Saito M, Ariga T, Yoshimura Y, Nakazawa H. Free radical scavenging activity of garcinol from Garcinia indica fruit rind. J Agri Food Chem 2000; 48: 2320–2325.
4. Romero FJ, Morell FB, Romero MJ, Jareno EJ, Romero B, Marin N. Lipid peroxidation products and antioxidants in human disease. Environ Health Persp 1998; 106: 1229– 1234.
5. Craig WJ. Health-promoting properties of common herbs. Am J Clin Nutr 1970; 70: 491–499.

6. Pawan Kaushik, Dhirender Kaushik* and Sukhbir Lal Khokra, In vivo antioxidant activity of plant abutilon indicum, J Pharm Educ Res Vol. 2, Issue No. 1, June 2011.
7. OECD/OCDE, OECD Guidelines for the testing of chemicals, revised draft guidelines 423: Acute Oral toxicity- Acute toxic class method, revised document, CPCSEA, Ministry of Social Justice and Empowerment, Govt. of India; 2000.
8. Venkateshwarlu E1*, Raghuram Reddy.A, P Goverdhan1, Swapna Rani K, Jayapal Reddy G In Vitro and In Vivo Antioxidant activity of Methanolic Extract of Solena amplexicaulis (Whole Plant) IJPBS |Volume 1| Issue 4 |OCT-DEC |2011|522-533.
9. Clairbone A. Catalase activity. In: Greenwald RA, editor. CRC Handbook of methods for Oxygen Radical Research. Boca Raton, FL: CRC Press; 1985. pp. 283–4.
10. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys 1978; 186:189–95.