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EVALUATION OF ANTIOXIDANT ACTIVITY OF HERBAL FORMULATION (TIAM) ON CHROMIUM (VI) INDUCED OXIDATIVE STRESS IN ALBINO RATS

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ABSTRACT

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging. *Emblica officinalis* (Amla) Belong to the Family of Euphorbiaceae, it is found natively in India. So in the present study we have determined the antioxidant activity or potential of the plant by inducing the free radicals in the animal model with the help of chromium (VI). Rats were divided randomly into five groups of six animals each and treated for four weeks i.e. 28 days as follows: Group I Served as normal control group received normal saline at a dose of 10ml/kg, Group II Served as Toxic Control group and was administered chromium 30mg/kg (30% V/V, 1.0ml/100kg) orally, Group III Served as a standard group and was administered LIV-52 in a dose of 56mg/kg orally, Group IV Served as a treatment control group and was administered herbal formulation (TIAM) in a dose of 250mg/kg orally and Group V Served as a treatment control group and was administered herbal formulation (TIAM) in a dose of 500mg/kg, Group III to V was given the herbal formulation 1 hr prior to the administration of the chromium (VI). In animals fed with both doses of 'TIAM' significant protection was observed against the chromium induced oxidative stress. The 'TIAM' inhibited the chromium induced increase in MDA levels and restore the intracellular antioxidant. Like GSH and catalase levels to that control. The 'TIAM' also protected the animals significantly from the hepatotoxicity induced by chromium is revealed by the decreased AST and ALT activity compared to the chromium (VI) treated animals. In the present investigation we concluded that the 'TIAM' possess a potent antioxidant activity.

KEYWORDS

Antioxidant, Chromium and *Emblica officinalis*.

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INTRODUCTON

Organisms are exposed over their life span to the effects of exogenous oxidizing agents from environmental pollutants, life style and to endogenous ones produced by metabolism. Chemical entities that act as oxidizing agents contain reactive oxygen species (ROS), namely superoxide anion ($O_2^{\bullet-}$), hydroxyl ($HO^{\bullet}$), and

peroxyl ($ROO^{\bullet}$) radicals, or reactive nitrogen species (RNS), which include agents like peroxynitrite anion ($ONOO^{-}$) and nitric oxide ($NO^{\bullet}$) radical, among other; in addition, there are non-free radical species such as hydrogen peroxide (HOOH), nitric oxide (NO) and hypochlorous acid (HClO) which also behave like oxidizing agents[1].

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging[2,3].

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule[4]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[5]. Herbal plants considered as good antioxidant since ancient times.

Emblica officinalis (Amla) Belong to the Family of Euphorbiaceae**,** it is found natively in India. Indian gooseberry has been used as valuable ingredient of various medicines in India and abroad. The active ingredient that has significant pharmacological action in Amla is designated by Indian scientists as 'phyllemblin'. The other ingredients present are gallic acid, tannins, pectin, and ascorbic acid (Vitamin C). It is a tonic, has a haematinic and lipolytic function. It is one of the strongest rejuvenatives in Indian pharmacopoeia. It contains 30 times the amount of Vitamin C found in oranges. It is aperient, carminative, and diuretic, aphrodisiac, laxative, astringent and refrigerant. It is the richest known source of vitamin 'C'. It is useful in anaemia, jaundice, dyspepsia, haemorrhage disorders, diabetes, asthma and bronchitis. It cures insomnia and is healthy for hair. It is considered as one of the most rejuvenating drugs, imparting a long healthy life and weight gain. It also acts as an antacid and anti-tumorgenic agent.

In recent years, the chemical importance of the herbal drugs has received considerable attention as many synthetic antioxidants have been shown to have one or the other side effects. There has been an upsurge of interest in the therapeutic potential; of the medicinal plants as antioxidants in reducing free radicals induced tissue injury. Numerous plant products have been shown to have the antioxidant activity and the antioxidant vitamins, flavonoids and polyphenolic compounds of the plant origin have been extensively reported as scavengers of free radicals and inhibitors of the lipid peroxidation. So in the present study we have determined the antioxidant activity or potential of the plant by inducing the free radicals in the animal model with the help of chromium (VI).

The present study was undertaken to determine the anti-oxidant or free radical scavenger property of "TIAM" (judicious blend of green Tea with Amla) and the evaluations of the various biochemical parameters involved there in.

MATERIAL AND METHODS

Chemicals

Thiobarbituric acid (TBA, Research-Lab Fine Chem. Industries Mumbai, India) nitro blue tertazolium chloride (NBT, Himedia Laboratories Pvt. Ltd, Mumbai, India). 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB Alfa Aesar, A Johnson Mathey company). Bovine serum albumin (Spectrochem Pvt. Ltd, Mumbai, India) carboxy methyl cellulose (Research Lab, Mumbai, India) Ethyl alchol (absolute ethanol) All the chemical used were of analytical grade and purchased from standard manufactures.

Animals

Male albino wistar rats each weighing 180-220 were obtained from K. M. College of Pharmacy, Madurai. Rodent laboratory chow was access and water *ad libitum*, and rats were maintained on a 12 hour light/dark cycle in a temperature regulated room (20-25^{o}C) during the experimental procedures. The animals were cared for according to the guiding principles in the care and use of animals. The experiments were approved by the institutional animal ethics committee.

Chromium Ingestion and Herbal Formulation of TIAM (judicious blend of green tea with Amla) Administration[6,7]

Rats were divided randomly into five groups of six animals each and treated for four weeks i.e. 28 days as follows: Group I Served as normal control group received normal saline at a dose of 10ml/kg, Group II Served as Toxic Control group and was administered chromium 30 mg/kg (30% V/V, 1.0ml/100kg) orally, Group III Served as a standard group and was administered LIV-52 in a dose of 56mg/kg orally, Group IV Served as a treatment control group and was administered herbal formulation (TIAM) in a dose of 250mg/kg orally and Group V Served as a treatment control group and was administered herbal formulation (TIAM) in a dose of 500mg/kg, Group III to V was given the herbal formulation 1 hr prior to the administration of the chromium (VI).

Biochemical Analysis

Dissection and Homogenization

On the 29th day all animal were killed by decapitation. Blood was collected and serum was separated for estimation of Alanine aminotransferase (ALT) and Aspartate aminotranferase (AST). The liver was rapidly excised rinsed in ice-cold saline and a 10% W/V homogenate was prepared using (0.15MKCl) potassium chloride. Centrifuged at 800rpm for 10 min at 4^{o}C. The supernatant obtained was used for the estimation of Catalase, and lipid per oxidation. Further the homogenate was centrifuged at 1000rpm for 20 min at 4^{o}C and the supernatant was used for estimation of SOD and glutathione.

Lipid per Oxidation Assay (LPO)

Malondialdehyde (MDA), a secondary product of lipid per oxidation reacts with thiobarbituric acid at PH 3.5. The red pigment produced was extracted in n-butanol-pyridine mixture and estimated by measuring the absorbance at 532nm.

Superoxide dismutase activity (SOD)

Superoxide dismutase activity was assayed according to the method of kono[130](8). Where in the reduction of nitro blue tetrazolium chloride (NBT) was inhibited by superoxide dismutase and measured at 560nm spectrophotometrically. Briefly the reaction was initiated by addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of liver homogenate. The results were expressed as units per milligram of protein with one unit of enzyme defined as the amount of SOD required to inhibit the rate of reaction by 50%.

Catalase activity (CAT)

Catalase activity was assessed by the method of luck[131](9). Where the breakdown of H_2O_2 was measured at 240nm. Briefly the assay mixture consisted of 3ml of H_2O_2 phosphate buffer (0.0125M; H_2O_2) and 0.05ml of supernatant of liver homogenate and the change in the absorbance was measured at 240nm. The enzyme activity was calculated using the mill molar extension coefficient of H_2O_2 (0.07). The results were expressed as micromole of H_2O_2 decomposed per min per milligram of protein.

Estimation of Reduced Glutathione

Reduced glutathione (GSH) in the liver was assayed according to the method of Ellman[132](10). Sample (0.75ml) of homogenate was precipitated with 0.75ml of 4% sulphosalicyclic acid and centrifuged at 1200g for 15 min at 4^{o}C. The assay mixture contained 0.5ml of supernatant and 4.5ml of 0.01M, DTNB. (5-5'-dithiobis (2-nitro benzoic acid)) in 0.1M, phosphate buffer (PH 8.0). The yellow colour developed was read immediately at 412nm. The results were expressed as micromole of GSH per milligram of proteins.

Determination of AST (Aspartate aminotransferase)

AST (aspartate aminotransferase) from the liver in the blood serum was assayed according to the method of Reitman'S, Frankel S.

Principle: SGOT (AST) catalyses the following reaction

α - Ketoglutarate + L-aspartate $\longrightarrow$ L-glutamate + oxaloacetate

Oxaloacetate so formed is coupled with 2, 4 Dinitrophenyl hydrazine (2, 4 DNPH) to give the corresponding hydrazine which gives brown colour in alkaline medium and this is measured colorimetrically.

Determination of ALT (Alanine aminotransferase)

ALT (alanine aminotransferase) from the liver in the blood serum was assayed according to the method of Reitman'S, Frankel.

Principle: ALT catalyses the following reaction

α - Ketoglutarate + L- alanine ⟶ L- glutamate + pyruvate

Pyruvate so formed coupled with 2, 4 Dinitrophenylhydrazine (2, 4 DNPH) to give the corresponding hydrazine which gives brown colour in alkaline medium and this can be measured colorimetrically.

Statistical Analysis

The results are expressed as mean ± SEM. Data was evaluated using one way ANOVA followed by Newman Keul's multiple range left. Probability values less than ($P<0.01$) were considered significant.

RESULTS AND DISCUSSION

Effect of 'TIAM' On Body weight, Food and Water Consumption

The effect of 'TIAM' herbal formulation on body weight changes during the chromium induced oxidative stress is shown in Table No.1. Chromium feeding resulted in significant decrease in the body weight with the duration of treatment; however, in animals fed with two doses of 'TIAM' and chromium; there was no significant change as compared to the control group. Administration of chromium did not cause any significant change in the food and water intake.

Effect of 'TIAM' on Body weight Ratio

Table No.2 shows organ to body weight ratio in the chromium and 'TIAM' treated animals. Administration of chromium caused a significant increase in the heart, liver, spleen and kidney to body weight ratio in all the animals. However, the lung to body weight ratio was not altered in the chromium treated animals. Pretreatment with 'TIAM' herbal formulation in both doses (250mg/kg and 500mg/kg) maintained the organ to body weight ratio comparable to that control values.

Effect of 'TIAM' on SOD and Catalase Levels

Administration of chromium caused a significant increase ($p<0.01$) in the liver tissue catalase levels but did not affect SOD levels (Table No.3). The 'TIAM' in a dose of 250mg/kg and 500mg/kg body weight was able to restore the catalase levels to that of control values.

Effect of 'TIAM' on Reduced GSH and MDA (Lipid Peroxidation)

Liver tissue GSH levels were significantly decreased following the chromium treatment, whereas significant increase in plasma MDA levels was observed (Table No.3). Administration of 'TIAM' in 250mg/kg and 500mg/kg body weight, dose reverted the GSH and MDA levels to that of control values.

Effect of 'TIAM' on AST and ALT Levels

AST and ALT levels were increased ($p<0.01$) in all the animals treated with chromium (Table No.3). Administration of 250mg/kg and 500mg/kg body weight dose of 'TIAM' significantly inhibited the chromium induced increase in enzyme levels and restored to that of control values.

DISCUSSION

One of the most important early events in cell degeneration leading to necrosis, is the Lipid peroxidative damage that occurs mainly in the cell membrane. In addition, lipid peroxidation represents one of the most reaction resulting from free radicals attack on biological structures Cr(VI) and Cr(V) are both able to yield ROS[8]. The majority of oxidative stress studies in rat have used TBARS as a tissue damage indicator[9].

In addition there was no study relating 'TIAM' with chromium intoxication. Therefore in this study was undertaken to evaluate for the antioxidant activity against the chromium (VI) induced oxidative stress in male albino rats. The results of the present study demonstrate that the 'TIAM' at a concentration of 250mg/kg and 500mg/kg body weight protected the animals significantly from the chromium induced oxidative damage.

Oral feeding of chromium resulted in a significant decrease in body weight and increase in organ to body weight ratio. Chromium (IV) Compounds are well known oxidizing agents capable of directly inducing tissue damage and possess carcinogenic, mutagenic and teratogenic potency[10]. Chromium (VI) compounds are easily taken up by cells and are subsequently reduced to Cr (III) species. This reduction generates free radicals, which play major role in the adverse biological effect of these compounds[11]. Administration of chromium significantly increases the lipid peroxidation as

evident by the increase in MDA levels. To cope with the oxidative stress, there was a significant decrease in reduced glutathione (GSH) and catalase level in the liver tissue. No significant change in the SOD activity was observed in the Chromium-treated animals and our results fall in confirmation with earlier studies. Besides activating the oxidative stress, Chromium also caused a marked increase in AST and ALT levels suggesting that the Chromium treatment also causes hepatic damage. Many workers have also demonstrated the hepato-toxic effect of Chromium (VI), which is mainly due to lipid peroxidation.

These adverse effects of Chromium (VI) could be significantly curtailed by pretreating the animals with the 'TIAM' (herbal formulation).

In animals fed with both doses of 'TIAM' significant protection was observed against the chromium induced oxidative stress. The 'TIAM' inhibited the chromium induced increase in MDA levels and restore the intracellular antioxidant. Like GSH and catalase levels to that control. The 'TIAM' also protected the animals significantly from the hepatotoxicity induced by chromium is revealed by the decreased AST and ALT activity compared to the chromium (VI) treated animals.

Table No.1: Effect of Body Weight of Normal and Experimental Animals in each Group

S.No	Groups	Initial body weight	Final body weight
1	Group-I	206 ±4.26	210.4 ±3.26
2	Group-II	198.80 ±3.95	160.30 ±2.16 a*
3	Group-III	210.5 ±4.06	225.20 ±5.16 b*
4	Group-IV	215.66 ±5.10	228.40 ±3.70 b*
5	Group-V	195.20± 4.14	220.4 ±2.16 b*

Values are expressaed as mean ± SEM, No. of animals in each group (n) = 6, (a*) values were significantly different from Normal control (G_1) at ($P < 0.01$), (b**) values were significantly different from toxic group (G_2) at ($P < 0.01$).

Table No.2: Organ to Body Weight Ratio in the Chromium and TIAM Treated Animals

S.No	Organ	Group-I	Group-II	Group-III	Group-IV	Group-V
1	Heart	3.44±0.05	4.56±0.26a*	3.52±0.14b*	3.26±0.15b*	2.85±0.14b*
2	Liver	0.05±0.04	0.08±0.02 a*	0.028±0.02 b*	0.030±0.03 b*	0.028±0.01 b*
3	Lung	5.10±0.60	5.50±0.60	5.38±0.22	5.45±0.45	5.10±0.30
4	spleen	1.5±0.40	2.68±0.28a*	1.63±0.24b*	1.86±0.16b*	1.65±0.20b*
5	kidney	7.06±0.67	9.30±0.42a*	7.26±0.62b*	7.30±0.42b*	6.90±0.58b*

Values are expressaed as mean ± SEM, No. of animals in each group (n) = 6, (a*) values were significantly different from Normal control (G_1) at ($P < 0.01$), (b**) values were significantly different from toxic group (G_2) at ($P < 0.01$).

Table No.3: Effect of "TIAM" Herbal Formulation on Chromium Induced Free Radicals in Rats

S.No	Groups	SOD U/L	Catalase min/mg of protein	Reduced GSH mg/dl	Lipid Peroxidation nmoles/ml	AST U/L	ALT U/L
1	Group-I	30.66±1.65	275.4±4.21	112.15±2.79	166.95±2.84	184.78±3.01	88.95±2.68
2	Group-II	28.41±0.93	187.41±3.25*a	60.16±1.30*a	258.28±2.34 *a	331.86±2.66*a	221.90±3.13*a
3	Group-III	27.25±1.58	238.75±4.07*b	93.3±1.95*b	216.8±4.19*b	234.25±3.90*b	127.42±3.33*b
4	Group-IV	32.95±0.84	202.03±2.31*b	72.62±3.23*b	198.69±2.38*b	284.66±3.21*b	181.41±2.30*b
5	Group-V	31.79±1.31	217.45±2.11*b	84.21±3.05*b	207.44± 4.49*b	255.51±3.73*b	152.72±2.75*b

Values are expressaed as mean ± SEM, No. of animals in each group (n) = 6, (a*) values were significantly different from Normal control (G_1) at ($P < 0.01$), (b**) values were significantly different from toxic group (G_2) at ($P < 0.01$).

CONCLUSION

In the present investigation we concluded that the 'TIAM' possess a potent antioxidant activity. 'TIAM' composition such as Black tea and Amla are claimed to be a rich source of many antioxidant substances, like catechin, emblicanin A and B compounds. The main antioxidant components of 'TIAM' are epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, ascorbic acid, carotenoids, ellagic acid and tannins. Further investigations are in progress to develop the formulation as a neutraceuticals.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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