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In vivo assessment of shatter proof character of herbal preparations at odds with hydroquinone induced genotoxicity

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Abstract

Hydroquinone (QOH) is a crucial metabolite of benzene broadcating after benzene bioconversion mostly in liver, kidney, and bone marrow. Genotoxicity mention to inter linkage with, or vandalization to, DNA and/or other cellular constituents which synchronize the constancy of the genome. Chyawanprash, is a poly herbal preparation in which *Embilica offcinalis* is a major component is widely used as a health tonic. It is claimed to reduce aging and age-related ailments and are known immune modulatory and antioxidant properties. There by preventing mutagenesis and carcinogenesis. Aswagandhadi lehya is also an ayurvedic poly herbal preparation therapeutically used as Rejuvenating agent, aphrodisiac and used to treat disorders of Blood, Piles, and Psychosis. The aim of this study is to evaluate the conservetive role of herbal compounds against hydroquinone induced Genotoxicity using albino rats of wistar strain bone marrow cells. The data suggested that Hydroquinone induced oxidative damage was significantly attenuated by herbal formulations through up regulation of SOD, CAT, GSH levels and down regulation of MDA levels.

Keywords: Hydroquinone, biotransformation, Genotoxicity, Chyawanprash, mutagenesis, Carcinogenesis.

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Introduction

Hydroquinone

Hydroquinone is a white crystalline substance, but industrial use grades may be light grey or light tan. Hydroquinone is extensively used as a reducing agent, as a photographic developer, as an antioxidant for many oxidizable products, as a stabilizer or polymerizing inhibitor for certain materials that polymerize in the presence of free radicals, and as a chemical intermediate for the production of antioxidants, anti-ozonants, agrochemicals, and polymers. It is a skin-lightening agent and is used in cosmetics, hair dyes, and medical preparations.

The general population may be exposed to hydroquinone through consuming plant-derived foods that contain this chemical as a natural component, through smoking (active or passive), or through using cosmetics and skin-lightening creams. Amateur photographers who develop film manually may be exposed through skin contact and inhalation. Ingestion of large quantities may produce vomiting, convulsions, and coma. Repeated skin contact can lead to depigmentation, allergic contact dermatitis, and sensitization. Long-term occupational exposure to airborne hydroquinone can result in eye irritation, sensitivity to light, and visual disturbance. Hydroquinone is highly toxic for most organisms in the environment, though the toxicity varies considerably from species to species. However, the substance is readily degraded and does not persist in the environment (Douglas McGregor, 2007).

GENOTOXICITY

Genotoxicity is defined as a destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity and which are not necessarily associated with mutagenicity. Genotoxin is chemical or agent that can cause DNA or

chromosomal damage. Such damage in a germ cell has the potential to cause a heritable altered trait (germline mutation). DNA damage in a somatic cell may result in a somatic mutation, which may lead to malignant transformation (cancer) are mutagens; they can cause mutations. Genotoxins include, radiation (UV & Ionizing radiation), chemical genotoxins (David H. Phillips *et al*., 2009).

Chemical genotoxins are mainly three types, Group-I, Group-II, Group-III. Group-1 chemicals gives positive *invivo* and *invitro* genotoxicity tests, they are mutagenic in nature (eg: Cyclophosphamide, Hydroquinone). Group-II chemicals show negative *invivo* and *invitro* genotoxicity tests but they are genotoxic in rodents and non-mutagenic (eg: Ampicilline trihydrate). Group-III chemicals show negative *invivo* and *invitro* genotoxicity tests, but they induce mutations in Lympoma cells, positive Chromosomal aberration, and Micronucleus test (eg: Benzyl alcohol) (David Kirkland *et al.*, 2015).

Importance of Genotoxicity Studies

Genotoxicity studies is referred as various *in-vitro* and *in-vivo* tests designed to identify any substance or compounds which may induce damage to genetic material either directly or indirectly by various mechanisms (Mohamed *et al.*, 2017). Many in vitro and in vivo tests for genotoxicity have been developed that, with a range of endpoints, detect DNA damage or its biological consequences in prokaryotic or eukaryotic cells, These assays are used to evaluate the safety of environmental chemicals and consumer products and to explore the mechanism of action of known or suspected carcinogens, All the regulatory authorities require information on the genotoxic potential and safety profile of the new chemical entity.

Chyawanprash

Avaleha or Lehya is a semi-solid preparation of drugs, prepared with addition of jaggery, sugar or sugar-candy and boiled with prescribed drug juice or decoction. They are also known as Modaka, Guda, Khanda, Rasayana, Leha etc. Chyawanprash, is a poly herbal preparation in which *Embilica offcinalis* is a major component is widely used as a health tonic. It is claimed to reduce aging and age-related ailments (Ojha, 1988). Preparation of chyavanaprash involves making of decoction from 35 herbs, of which many are known immunomodulatory and antioxidants and made into a past with brown sugar and taste with species. chyawanaprash, were shown to be potent free radical scavenging agents (Jeena and Kuttan, 1995), thereby preventing carcinogenesis and mutagenesis (Jeena et al., 1997).

Medical Uses of Chywanprash

Chyawanprash is helpful in clearing the accumulated excreta by promoting digestion and excretion. It relieves nausea & vomiting and corrects hyperacidity, dyspepsia & flatulence, gastritis, peptic ulcer and intestinal cramping, hepatoprotective, strengthens liver, streamlines the metabolism of fats & Protiens (Jeena KJ *et al*, 2000). Used to treat Bronchial asthma, common cold and respiratory infections, Brain tonic, Cardiotonic, anti-oxidant, anti-mutagenic, anti-carcinogen (Milind Parle, *et al*., 2006)

Aswagandhadi Lehya

Aswagandhadi lehya is a classical ayurvedic poly herbal formulation, mainly it contains Aswagandha (*Withania somnifera*), *Hemidesmus indicus, Smilax china, Cuminum cyminum, Elettara cardamonum, vitis vinifera.* It is therapeutically used as the Raktavikara (Disorders of Blood), Krsatva (Cahexia), Arsa (Piles), Unmada (Psychosis), Balya Rasyana (Rejuvenating agent), Vajikara (Aphrodisiac).

Medical Uses of Aswagandhadi Lehya

Adaptogenic, Aphrodisiac, Anabolic effect, Anti-arthritic, Anti-depressant, Anti-inflammatory, Anodyne, Antioxidant, Anti-stressor, Immunomodulatory, Rasayana (Rejuvenating) (Gupta mansi *et al*., 2011)

AIM & OBJECTIVE

Aim

The aim of the present study is to evaluate the protection role of herbal preparations against hydroquinone induced genotoxicity.

Objective

To evaluate *in-vivo* antioxidant studies

- Super oxide dismutase (SOD)
- Reduced glutathione (GSH)
- Catalase
- Lipid peroxidation

MATERIAL AND METHODS

Procurement of Test Compounds

The test compounds Aswagandhadi lehya and chyawanprash were procured from local Ayurvedic market, Ongole. Andhra Pradesh, India.

Animal Husbandry

Healthy albino rats of Wistar strain of either sex body weighing about 150-220 g were used for the study. The rats were purchased from Sri Venkateswara Agencies, Bangalore. The animals were caged individually and kept in air conditioned room, at the temperature of 22±24 with 50%±10% relative humidity with 12 hours light and dark cycle. Throughout the study the animals

were maintained at normal laboratory conditions. Animals were maintained at standard rat pellet diet and drinking water *ad libitum*. After acclimatization animals were selected randomly and were divided into 4 groups (n=6).

Instruments

UV-Visible spectrophotometer (analytical systems, model no: AUV 2060), electronic balance (Shimadzu, Model no: DS-852 J), homogenizer (Ever shine, Model no: 607), auto analyser (Mispa excel, Version: 14e), Cooling centrifuge (Remi, Model.no: C-24 BL), electronic microscope, incubator, p^H meter.

Chemicals

Hydroquinone, Aswagandha, Chyawanprash, Hanks' balanced salt solution, Cold fixative, Giemsa stain, Hypotonic solution, citric acid.

Preparation of Test Compounds

Stock solutions of both Aswagandha lehya and chyawanprash were prepared in distilled water of concentrations.

Dose Selection

The doses of Chyawanprash for the present study were selected based on the earlier work done on anti-oxidant (Jeena *et al*., 1995), anti-tumour activity (Jeena *et al*., 1997). Acute toxicity of Aswagandha lehyam was performed according to OECD-423 guide lines. The herbal formulation was found to be safe at 5000 mg/kg. Pilot studies were carried out with doses of 1000 mg/kg and 1200 mg/kg and the dose which was more effective was (1200 mg/kg) for the present study.

Experimental Protocol

Table 1:-Treatment schedule for assessing Protective role of Herbal formulation against hydroquinone induced genotoxicity

S.NO	GROUP (n=6)	TREATMENT	PURPOSE
1.	Normal	Vehicle (Water)	To study the normal parameters
2.	Control (Hydroquinone (HQ)- 500 mg/kg; *p.o*)	Hydroquinone (HQ) (500 mg/kg; *p.o*)	To assess the normal parameters in disease control
4.	Test -1 (Hydroquinone (HQ) 500 mg/kg; *p.o* + Chyavanprash (CH)2.5 g/kg; *p.o*)	Hydroquinone (HQ) 500mg/kg; *p.o.* + Chyavanprash (CH) 2.5 g/kg; *p.o.*)	To study the effect of Test-1
5.	Test- 2 (Hydroquinone (HQ) 500mg/kg; *p.o.* + Aswagandadi lehya (AL) 1.2 g/kg; *p.o.*)	Hydroquinone (HQ) (500mg/kg; *p.o.*+ Aswagandadi lehya (AL)1.2 g/kg; *p.o.)*	To study the effect of Test-2

Estimation of Tissue Antioxidant Parameter

At the end of the treatment schedule the animals were sacrificed by cervical dislocation, liver and kidney was isolated then it was immediately processed for antioxidant parameters.

Procedure

Excised liver and kidney was cross chopped with surgical scalps into fine slices and were chilled in the cold 0.25 M sucrose, quickly blotted with filter paper the tissue were minced and homogenized in ice cold 10mM tris HCL buffer (to p^H 7.4) at a concentration of 10% (w/v) with 25 stokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition was designed to disrupt as far as possible the ventricular structure of cells so rpm release soluble protein and leave only membrane and non-vascular matter in sedimentation form. It was then centrifuged at 5000 rpm at 20^oc temperature and clear supernatant was separated and used to estimate superoxide dismutase (SOD), reduced glutathione (GSH), Catalase (CAT) and lipid peroxidation (LPO).

Measurement of Superoxide dismutase (SOD)

SOD was estimated by the method of Misra and Fridovich.

Measurement of Catalase (CAT)

Catalase activity was measured by the method of Claiborn, *et,al*., 1979.

Measurement of reduced glutathione (GSH)

Glutathione was measured according to the method of Ellman, *et al*., 1959

Measurement of lipid peroxidation (LPO)

Thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation, was measured as described by (Ohkawa, *et al*., 1979).

STATISTICAL ANALYSIS

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Bonferroni multiple comparison test. The significance level was set at P<0.001 for all tests.

RESULTS

Antioxidant levels in Liver

Effect of Herbal preparations on *In vivo* antioxidant SOD levels of Liver homogenates

Disease control group showed prominent (p<0.001) reduction in the SOD levels when compared with of normal control group. Increased SOD levels were found to significant (p<0.001) in test groups treated with Chyavanprash (2.5g/kg, *p.o*) and Aswagandhadi lehya (1.2 g/kg. *p.o*) when compared with that of disease control group. When compared with the Aswagandhadi lehya treated groups, Chyavanprash treated groups showed increase in the liver SOD levels.

Effect of Herbal preparations on *In vivo* antioxidant Catalase levels of Liver homogenates

Disease control group showed prominent (p<0.001) reduction in the Catalase levels when compared with of normal control group. The test groups administered with Chyavanprash (2.5 g/kg, *p.o*) and Aswagandhadi lehya (1.2 g/kg. *p.o*) are significantly increased in catalase levels in liver when compared with that of disease control group (p<0.001). When compared with the Aswagandhadi lehya treated groups, Chyavanprash treated groups showed increase in the liver Catalase levels.

Effect of Herbal preparations on *In vivo* antioxidant GSH levels of Liver homogenates

Significant (P<0.001) reduction in levels of GSH were seen in disease control group when compared to that of normal control group. There was a significant (P<0.001) elevation of reduced glutathione levels was observed in Chyavanprash (2.5 g/kg, *p.o*) and Aswagandhadi lehya (1.2 g/kg) treated groups when compared with disease control group. Chyavanprash treated groups showed increased levels of reduced glutathione when compared to Aswagandhadi lehya treated groups.

Effect of Herbal preparations on *In vivo* Lipid peroxidation levels of Liver homogenates

There was a notable (p<.001) rise in the malondialdehyde levels in the control groups when compared to the normal group depicting the lipid peroxidation. Treatment with Chyavanprash (2.5 g/kg) and Aswagandhadi lehya (1.2 g/kg) treated groups showed significant (p<0.001) decreased levels when compared with disease control groups.

Antioxidant levels in Kidney

Effect of Herbal preparations on *In vivo* antioxidant SOD levels of Kidney homogenates

There was a significant (p<0.001) reduction in the SOD levels in disease control group when compared with the normal group. Increased SOD levels were found to significant (p<0.001) in test groups treated with Chyavanprash (2.5g/kg, *p.o*) and Aswagandhadi lehya (1.2 g/kg. *p.o*) when compared with that of disease control group. Chyavanprash treated groups showed increased levels of SOD levels when compared with the Aswagandhadi lehya treated groups

Effect of Herbal preparations on *In vivo* antioxidant Catalase levels of Kidney homogenates

Disease control group showed prominent (p<0.001) reduction in the Catalase levels when compared with of normal control group. The test groups administered with Chyavanprash (2.5 g/kg, *p.o*) and Aswagandhadi lehya (1.2 g/kg. *p.o*) are significantly increased in catalase levels in kidney when compared with that of disease control group (p<0.001). When compared with the Aswagandhadi lehya treated groups, Chyavanprash treated groups showed increase in the liver Catalase levels.

Effect of Herbal preparations on *In vivo* antioxidant GSH levels of Kidney homogenates

Significant (P<0.001) reduction in levels of GSH were seen in disease control group when compared to that of normal control group. There was a significant (P<0.001) elevation of reduced glutathione levels was observed in Chyavanprash (2.5 g/kg, *p.o*) and Aswagandhadi lehya (1.2 g/kg) treated groups when compared with disease control group. Chyavanprash treated groups showed increased levels of reduced glutathione when compared to Aswagandhadi lehya treated groups.

Effect of Herbal preparations on *In vivo* Lipid peroxidation levels of kidney homogenates

There was a notable (p<.001) rise in the malondialdehyde levels in the control groups when compared to the normal group depicting the lipid peroxidation. Treatment with Chyavanprash (2.5 g/kg) and Aswagandhadi lehya (1.2 g/kg) treated groups

showed significant ($p<0.001$) decreased levels when compared with disease control groups.

DISCUSSION

SOD, CAT and GSH take part in maintaining homeostasis in the tissues. These antioxidants are involved in the defense system against free radical mediated tissue or cellular damage Increased activity of SOD and CAT indicates increased removal of superoxide radicals thereby reducing cell damage caused by free radicals.

CONCLUSION

We evaluated the protective role of herbal formulations against hydroquinone induced genotoxicity which was assessed by estimating oxidative stress markers such as MDA, GSH and SOD levels. Hydroquinone induced oxidative damage was significantly attenuated by herbal formulations through up regulation of GSH and SOD levels and down regulation of MDA levels.

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