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Effect of methanolic extract of *Achyranthes aspera* on allergy induced by potassium dichromate

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Abstract

Potassium dichromate and other Cr (vi) forms are found as potent allergen in metal industries, cement industries where constant contact and inhalation or ingestion occurs which tend cause severe allergy. Several medicinal plants have potent anti allergic effects and drugs from natural products are free from side effects. The present study was aimed to evaluate the utility of *Achyranthes.aspera* in treating allergies caused due to heavy metals, particularly dichromate in albino mice. The allergy was induced in mice by giving oral administration of $K_2Cr_2O_7$ at a concentration of 200mg/kg body weight for the period of 10 days. The diagnosis of allergy was done by serum analysis of IgM and IgG antibodies, total Lymphocytic count and total WBC count. The methanolic extract of *A.aspera* (400mg/kg, 600mg/kg body wt) showed greater efficiency in reducing the effects of dichromate allergy induction.

Keywords: Potassium dichromate, *Achyranthes aspera*, Diagnosis of Allergy

INTRODUCTION

Achyranthes aspera L. (Amaranthaceae) is one of the plant used for medicinal purposes. It is an erect, annual herb, distributed in the hilly districts of India (Vetericchelvan et al.2003). The plant is used in indigenous system of medicine as emenagogue, antiarthritic, antifertility, laxative, ecobolic, abentifacient, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, antihypertensive, anti-coagulant, diuretic and anti-tumor(Ratra et al.,1970). Amongst the most chronic diseases elicited by allergens are allergic rhinitis and asthma. Asthma which is caused by air borne dust particles or plant pollens is a chronic inflammatory disorder of the airways leading to recurrent episodes of wheezing, breathlessness, chest toughness and coughing, due to leukotrienes secretion. It affects 100-150 million people across the world (W.H.O, Jan 2000).

Another common allergy is contact dermatitis. It is due to skin contact to a particular substance like nickel, chromium, latex causing skin rashes. Metals also may act as respiratory allergen in powder form of multivalent ions.

Food allergies are pretty common to cause undesirable reactions in the gut to a particular food allergen e.g., milk protein, peanut, groundnut, wheat etc.

Eczema is another commonly found allergy amongst the masses, which is accompanied by severe itching and sometimes secondary infections may also form.

The allergen that is being used is potassium dichromate. Potassium dichromate is also called as chromic acid, Dipotassium salt and potassium bichromate. It is a bright yellowish crystalline

substance, which is a strong potent allergen in metal and cement industries. It can act as contact allergen, respiratory allergen and also toxic to tissues and body. It is irritating to skin and mucous membrane, skin contact may cause skin irritation, rashes and burns, eye contact causes redness, swelling, tears, pairs and burns, even blindness. Inhalation may cause respiratory troubles, irritation of nose, throat, lungs, causing coughing, wheezing and/or shortness of breath.

MATERIALS AND METHOD

Collection of plant material

Whole plants of *Achyranthes aspera* were collected from Gulbarga University campus, Gulbarga during the months of October and November, 2006. After collection the plant specimens was authenticated by the Department of Botany, Gulbarga University as *Achyranthes aspera*.

Methods of extraction

Hot extraction method using soxhlet apparatus was used for successive solvent extraction of the harvested plant materials. The whole plants collected were shade dried to complete dryness and then the material was ground to fine powder in a mixer-grinder.

The powder was weighed and 2 packets of approximately 35g of plant material was extracted successively using solvent methanol (80°C) in a soxhlet. After complete extraction, the contents of each extraction were concentrated by distillation. The concentrated extracts were evaporated to dryness and weighed.

Experimental Animals

White albino mice weighing 15-20 g were brought from Animal House Facility, Biogen Biotechnologies Pvt. Ltd., Bangalore, kept and maintained in Animal house, at Department of Biotechnology, Gulbarga University, Gulbarga. Fed with balance diet as described

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by CFTRI, Mysore and water ad libitum. All the extracts were prepared in tween 80(1%), suspended in distilled water and administered orally to the animals with the help of intragastric catheter at desired doses. The control animals received an equivalent amount of vehicle only.

Induction of Allergy by $K_2Cr_2O_7$ (Potassium dichromate)

The mice were divided into 3 groups. Group 1 mice received vehicle only and were used as negative controls. Group 2 is considered as allergen induced group, these mice were induced with allergen $K_2Cr_2O_7$ and were used as positive controls. The 3rd groups of mice were induced allergy by treating with $K_2Cr_2O_7$ and were treated with crude plants extract of *A.aspera*. Animals of Group 2 and Group 3 received potassium dichromate at a concentration of 20mg/kg body weight dissolved in distilled water for 10 days continuously. The mice were given allergen by oral route i.e. gavage once a day for the given period. On the 10th day, animals from Group 2 were sacrificed for serum analysis of IgM and IgG to confirm allergy induction and type of allergy induced in the mice. Analysis was done at Pooja Diagnostic Laboratory, Gulbarga. Minimum 10 animals were maintained in each group.

Diagnosis of Allergy

Total WBC count

Total WBC count is done to enumerate the total number of leucocytes in unit volume (1mm³) of blood. It can be used as an indicator for the presence of any pathological condition in the person, either pathogenic infection, blood cancers or hypersensitivity conditions.

Differential cell count

White blood cells, or leukocytes, are classified into two main groups: Granulocytes and non-granulocytes or Agranulocytes.

The granulocytes, which include neutrophils, eosinophils, and basophils, have granules in their cell cytoplasm. Neutrophils, eosinophils, and basophils also have a multilobed nucleus. The non-granulocyte white blood cells, lymphocytes and monocytes, do not have granules and have non-globular nuclei. The leukocytes were detected by using giemsa staining technique.

Serum Antibody Detection

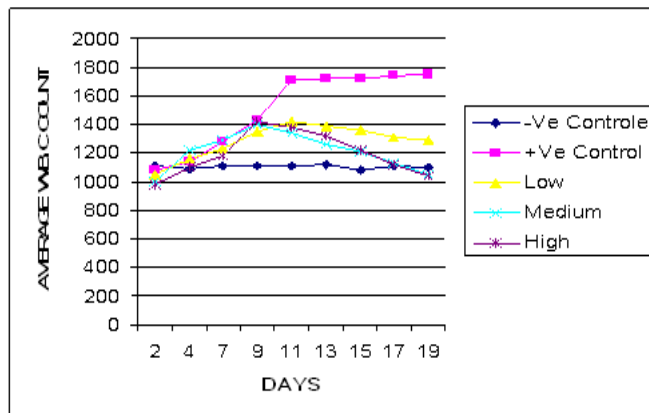
ELISA (Enzyme linked immuno sorbent assay) is a useful immunological technique for rapid and accurate detection of specific Ab and Ag in biological samples and both qualitative and quantitative analysis can be done by this technique. ELISA may be employed in several way, viz., direct, indirect, sandwich etc. depending on the type of analysis to be done and the biological component to be assayed. ELISA has been employed for several applications including clinical diagnosis of pathogens, detection of several diseases and pathological conditions etc.

Serum IgG and IgM antibodies were quantitatively estimated by sandwich ELISA (Enzyme linked immuno sorbent assay). The blood serum was isolated and its was given to pooja diagnostic laboratory, Gulbarga for the analysis of IgG and IgM antibodies present in the serum. The normal range of mice IgM IS 60-250mg/dL and that for mice IgG is 90-450/mg/dL.

RESULTS AND CONCLUSION

Effect on total WBC count

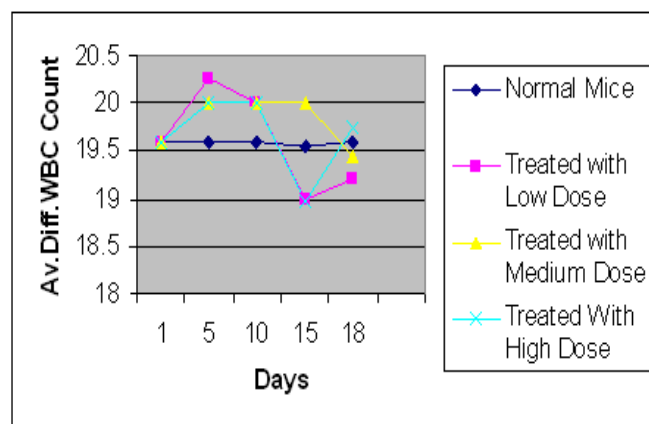
Increases in the WBC's were observed during allergy induction in mice. On treating allergy induced mice, the methanolic extract (400mg/kgbd.wt) of *A.aspera* showed decrease in the WBC level. (Graph1)



Graph 1.

Effect on differential WBC count

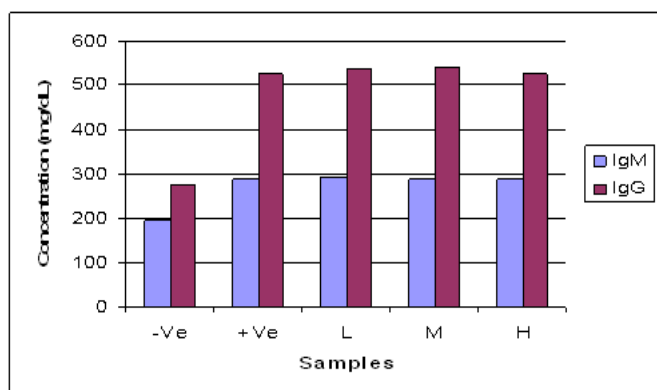
Differential WBC counting revealed the percentage of WBC in the blood of mice at various stages of induction and treatment .increase in the eosinophils, monocytes and lymphocytes were observed during allergy induction in mice. On treatment of allergy induced mice with methanolic plant extract of *A.aspera*, number of the differential cells was reduced. (Graph 2)



Graph 2. Effect of Methanolic Extract on Differential WBC count

Effect on serum antibodies

Immunoglobulin titres of IgM and IgG in the serum were quantitatively estimated by sandwich ELISA. The normal range of mice IgM is 60-250mg/dL and that for mice IgG is 90-450mg/dL. Serum titres were significantly raised during the course of allergy induction as indicated by the serum Ab titre values. When the mice were treated with methanolic plant extract, there was a significant reduction the serum Ab (Graph 3).



Graph 3. Effect of Petroleum ether Extract on serum IgM and IgG titre

DISCUSSION

Allergy induction by dichromate may be classified as type II hypersensitivity due to the increase in cells associated i.e. neutrophils, lymphocytes, eosinophils and macrophages and increase in IgM and IgG titre after allergy induction (Abdul Ghaffar et al, 2002, and Cohen.S.R., Davis D.M, Zelokoff et al. and Kramkowski P E, 1974, Clinical manifestation of chromic acid toxicity)

An increase in total W.B.C count was observed on allergy induction due to $K_2Cr_2O_7$ treatment. This count was reduced at the end of the treatment with various extracts like petroleum ether and methanol of which methanolic extract showed the greater effecting lowering the W.B.C count to near normal. A lowering in the differential W.B.C cells involved in Type II allergy was typically greater by the methanolic extract. Moreover the higher IgM titre seen on allergy induction was reduced to greater extent by the methanolic extract treatment.

The somatic changes like reduction in the body weight due to allergy was regained greater weight increase in case of methanolic extract treated mice. Hence, the methanolic extracts as a potent efficiency in reducing the effects of dichromate induced allergy. The above changes due to plant extract treatment may be due to the bioactive compounds like alkaloids present in it.

The active principles involved in the beneficial effects of the extracts in reducing the effects of potassium dichromate induced allergy *Achyranthes aspera* can be further tested against other forms and types of allergies for its treatment

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