Phenylhydrazine Induced Haematotoxicity and Its Amelioration by Ethanolic Extract of *Momordica charantia*

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Abstract-It has been established that several plants serve as haemoprotective agents. The mechanism is that they bind to the oxidative radicals which is produced during the haemolysis of red blood cells and the binding restores the actual form of these cells. This way, the degenerative effect of plants as the blood is ameliorated by plants. In our study, phenyl hydrazine caused significant anaemia in Charles Foster rats which was recovered by ethanolic extract of *Momordica charantia.*

Keywords: *Momordica Charantia,* Haematological analysis, Anaemia

Introduction

Momordica charantia showed strong antioxidant activity in *in-vitro* experiments. This activity was correlated with its poly - phenolic constituents which play important roles in its anti -oxidative effects. Polyphenols act as reducing agents and as antioxidants by several mechanisms as by scavenging of free radicals , mediation and inhibition of enzymes and chelation of transition metals (Chang *et al*., 2011 ; Singh and Singh, 2019 ; Meena *et al*., 2019).

We have seen the effect of phenyl hydrazine on haematological parameters of Charles Foster rats and recovery of anaemia by ethanolic extract of *Momordica charantia*.

Material and Methods

Chemicals

Ethanol and phenylhydrazine were purchased from Sigma Aldrich, USA. All other chemicals used in the study were of analytical grade and available locally.

Plant Materials

Momordica charantia was purchased from local market of Lucknow city of Uttar Pradesh. These samples were authenticated by Botany division of CSIR-CDRI, Lucknow.(U.P.), India.

Preparation of Plant Extracts

The material was dried at room temperature and ground into powder. Dry powder 4 Kg was macerated in 95% ethanol and kept at room temperature for 48 hours. The resulting extract was filtered and filtrate the was concentrated in an oven at a temperature of 40^0 C for 24 hours. The extract was stored at 40^0 C.

Test Animals

The rats of both the sexes were obtained from National Laboratory, Animal Center, CSIR-Central Drug Research Institute, Lucknow and were allowed to acclimate for 7 days before the start of experiment. They were kept in a controlled environment at 24 ± 3^0C and 40-55 % relative humidity with a 12 hour dark and light cycle. The animals were fed a standard rodent pellet diet and water ad libitum. Animal studies were conducted as per regulations of Institutional Animal Ethics Committee.

Induction of Anaemia

Basal haematological values were determined by using MS-9 haematology

analyzer. Phenyl hydrazine is an established model for induction of anaemia (Biswas *etal.,* 2005 ; Chang *et al.,* 2011). In our study, phenyl hydrazine at a dose of 10 mg / kg body weight for seven consecutive days was used to induce anaemia. After 7 days, haematological values were determined and rats with a reduction of 35-45% in haemoglobin, total red blood cell count and haematocrit were considered anaemic.

Experimental Procedure

The rats were divided into four groups of 10 animals (5 males and 5 females) in each group. They were subjected to the following treatments.

Group I Control-Distilled Water (7 Days) (1% Gum Acacia)

Group II- 10 mg/ Kg B.W. PHZ (7 Days), then 500 mg/ Kg *M. charantia* extract

Group III- 10 mg/ Kg B.W. PHZ (7 Days), then 1,000 mg/ Kg *M. charantia* extract

Group IV- 10 mg/ Kg B.W. PHZ (7 Days), then 2,000 mg/ Kg *M. charantia* extract

At the end of 21 days, all the rats were anaesthetized with diethyl ether and sacrificed.

Body Weight

Body weight was recorded at days 0, 7, 14 and 21 of the experiment.

Food and Water Consumption

Monitoring of 24 hours food and water consumption of the animals in all the groups were done at days 0, 7, 14 and 21 of the experiment. This was done by giving a measured quantity of water and pellet diet followed by estimation of the amounts remaining at the end of 24 hours. Average food and water consumption per animal was calculated for each group.

Haematological Analysis

Blood samples were collected at days 0, 7, 14 and 21 of the experiment through tail vein in EDTA coated vials. The haematological parameters-haemoglobin, total red blood cell count and haematocrit were analyzed by using MS-9 fully automated haematology analyzer. At the end of 21 days, all the vital organs were collected by using standardized surgical procedure. The abdominal cavity of each animal was dissected and organs namely the heart, liver, lungs, spleen, kidney and brain were quickly removed, cleaned with normal saline, weighed and preserved in 10 % formalin.

Statistical Analysis

All the data were analyzed and results are expressed as Mean + Standard Deviation. Data were analyzed with one-way ANOVA. Statistically significant effects were further analyzed. The statistical significance was determined at p o.o5.

Results and Discussion

Induction of Anaemia

The rats which were given 10 mg / kg body weight for 7 days, significant decreases in haemoglobin (45 %), total red blood cell count (43 %) and haematocrit (44 %) were observed . This way, the anaemic conditions were recorded in all the rats.

Body Weight

After 7 days, there was decrease of 15-20 % in all phenyl hydrazine treated groups of rats. At the end of 21 days, the body weights were recovered and comparable to controls.

Food and Water Intakes

The animals treated with phenyl hydrazine at dose of 10 mg/ kg body weight for 7 days showed insignificant decreases in the consumption of food and water. After 7days of extract treatment , this was recovered and at the end of 21 days, insignificant increases were recorded.

In vivo Haemaprotective Activity

The haematological parameters in phenyl hydrazine treated animals were insignificantly decreased at the end of 7 days. After 7 days of treatment of different doses of extract , it recovered and was comparable to controls which after further treatment of extract for another seven days at different doses caused dose related increases of haematological parameters of all the animals.

Conclusion

In phenyl hydrazine induced anaemic rats, different doses of *Momordica charantia* extracts (500 mg, 1,000 mg and 2,000 mg) were evaluated. Rat is an established animal model for the induction of haemolytic anaemia (Mc Millan etal.,2005). Phenyl hydrazine generates ROS within both human and rat erythrocytes. The production of ROS was associated with extensive binding of oxidized and denatured haemoglobin to the membrane cytoskeleton. This way phenyl hydrazine induces haemolytic injury which is derived from oxidative alterations to red blood cell proteins rather than to membrane lipids .

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