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Haematopoietic Activity of Mandoor Parpati on Cyclophosphamide induced Anaemia in Wistar strain albino Rats

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ABSTRACT

Ayurvedic formulations are single to multi component mixtures containing plant and animal derived products and minerals.Iron Deficiency Anaemia is a worldwide problem with the highest prevalence in developing countries. The present study aims to evaluate haematopoietic activity of mandoor parpati in wistar strain albino rats. Test animals were divided into four groups containing six Wistar strain albino rats either sex in each group. Group I kept as normal control (without drug treatment) for 28 days . Anaemia was produced by cyclophosphamide (3 mg/kg body weight) given i.p. for 7 days to group II,III and IV. After the 7th day, cyclophosphamide was withdrawn from groups II and III. Group III treated with only Mandoor Paripati of once a day continuously upto next 21 days. Group IV treated with cyclophosphamide (3 mg/ kg body weight i.p.) and Mandoor Paripati (45mg/kg) of once a day continuously upto next 21 days. The blood sample was collected on the 29th day and evaluated for haematological parameters. Comparison of group II and IVshowed significant (^{a} P <0.05) increase in haematological parameters. However, change in values after 29 th showed that the haematological count was approximately restored to normal even after discontinuing of cyclophosphamide treatment. This further possess good haematopoietic activity of Mandoor Parpati. Mandoor Parpati may reduce the risk of aplastic and iron deficiency anaemia.

Keywords: *AyurvedaMandoor, Parpati, Panduroga, Iron deficiency Anaemia, Cyclophosphamide ,Wistar strain albino rats.*

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INTRODUCTION

Ayurveda is the science of life deals maintain of healthy life as well as prevention and cure of diseases[1].Ayurvedic formulations are single to multi component mixtures containing plant and animal derived products and minerals.[2] Iron Deficiency Anaemia (which is described under the disease Panduroga in Ayurveda) is a worldwide problem with the highest prevalence in developing countries. According to WHO, the prevalence of Anaemia in pregnancy in South East Asia is around 56%. In India incidence of anaemia in pregnancy has been noted as high as 40-80%. 15%- 22% of maternal mortality has been estimated due to Anaemia during pregnancy.[3] There is need to evaluate the potential of Ayurvedic drugs to counteract toxicity of modern therapy. Ayurvedic formulation, Mandoor Parpati is used in the treatment of anaemia, hepatomegaly, spleenomegaly, loss of appetite, sprue syndrome.[4] The louha bhasma ,mandoora bhasma and navayaslauha , the components of ayurvedic formulations are reported as cytoprotective, hematinic and possessing haemoglobin regeneration ability. [5,6,7] The present study was conducted for evaluate haematopoietic activity of mandoor parpati in wistar strain albino rats .

MATERIAL AND METHODS

Preparation of Drug

Mandoor Parpati were prepared by following standard guidelines as prescribed in siddhayoga samgraha [4] Raw material of Mercury (Parad), Sulphur (Gandhak) & Iron slag (Mandoor) was procured from local market of Puri (Odisha) India.

Preparation of Mandoor Parpati was carried out as per the reference of Siddhayoga Sangraha [4]. Purified mercury (Shuddha Parada) :100gm, purified Sulphur (Shuddha Gandhaka): 200gm and incineration iron slag (mandoor bhasma): 100gm were taken for thepreparation of Kajjali. In a Khalvayantra, Purified mercury(ShuddhaParada) :100gm, purified Sulphur(Shuddha Gandhaka): 200gm were taken and triturated. It was continued until the powder became black,smooth, and lusterless.100g Mandoor bhasma was add and triturated well until became homogenous mixture.Iron frying pan filled with sand was placed on fire and heated. 25gm of prepared Kajjali was taken in a Darvi(Iron laddle) smeared with Cow ghee(Goghrut). This Darvi was placed over the heated sand and mild fire was given to melt the Kajjali till the mixture turned into semisolid (Pankwat) form. In the mean time, cow dung was spread on an even surface and over it an intact Kadalipatra smeared with Cow ghee (Goghrut) was placed. Kajjali was heated till the mixture turned into semisolid (Pankvat) form. The melted Kajjali was immediately poured on the smooth surface of Kadalipatra (Musa paradisiacal) and covered with another Cow ghee (Goghrut) smeared Kadalipatra. These leaves were then immediately compressed gently by using a steel plate. Thus, this obtained melted Kajjali which was solidified and flat in shape, was collected as Mandoor Parpati.It was collected and cleaned with a cloth. These Parpaties were then powdered and stored in a glass bottle.

Animals

Wistar strain albino rats of either sex, weighing 200 ± 20 g, were used as per the guidelines of the Institutional Animal Ethics Committee (IAEC). The animals were obtained from the animal house attached to the pharmacology laboratory, Columbia Institute of Pharmacy Raipur (C.G.). The animals were maintained under ideal husbandry conditions in terms of standard conditions of temperature (22 ± 3^0C), relative humidity (50-60%) and exposed to 12 h light and dark cycles. All animals were exposed to the same environmental conditions and were maintained on standard diet and drinking water ad libitum. The experimental protocol was approved by the IAEC/1321/PO/ReBi/S/10/CPCSEA Dated 22/10/2014 as per guideline of committee for the purpose of control and supervision of experiments on animals in India.

Acute toxicity study:

Toxicological study revealed that Wistar strain albino rats tolerated considerably high dose of Mandoor Parpati (2 g/kg body weight i.p.) without any toxic manifestations.

Studies on haematopoietic activity:

Test animals were divided into four groups containing six Wistar strain albino rats either sex in each group. Group I kept as normal control (without drug treatment) for 28 days . Anaemia was produced by cyclophosphamide (3 mg/kg body weight) given i.p. for 7 days to group II,III and IV. On day 7, blood samples were collected from all four groups from the retro-orbital plexus vein of the rat's eyes in vials containing EDTA as the anticoagulant and evaluated for blood parameters. Haematological parameters were evaluated in anaemic animal model. After the 7^{th} day, cyclophosphamide was withdrawn from groups II and III. Group III treated with only Mandoor Paripati (45mg/kg) of once a day continuously upto next 21 days. Group IV treated with cyclophosphamide (3 mg/ kg body weight i.p.) and Mandoor Paripati (45mg/kg) of once a day continuously upto next 21 days. The blood sample was collected on the 29^{th} day and evaluated for haematological parameters.

Statistical analysis

Statistical analysis was performed as mean of variance ± SEM (n = 6) followed by ANOVA test using Graph Pad Prism and for multiple comparison test among the groups, Bonferroni test was performed. A probability level of $P < 0.05$ was accepted statistically.

RESULT

Haematopoietic activity was evaluated by using cyclophosphamide-induced anaemia in animal model.

Comparison of group I (without drug treatment) with II, III and IV exhibited significant ($P < 0.05$) (Table 1) decrease in haematological parameters after 7 days of induced with cyclophosphamide.

In Comparison of group II, the group III and IV showed significant (a $P < 0.05$) (Table 2) increase in haematological parameters. However, change in values in group III after 29th showed that the haematological count was approximately restored to normal even after discontinuing of cyclophosphamide treatment. This further possess good haematopoietic activity of Mandoor Parpati [8-11].

Table 1: Hematopoietic Activity (After 7 days)

Observation	Group I Normal control	Group II Cyclophosphamide (3mg/kg) Control	Group III MandoorParipati (45mg /kg)	Group IV Cyclophosphamide (3mg/kg) + MandoorParipati(45mg/kg)
WBC (10^3/µl)	9.16±0.24	2.82±0.63*	2.76±0.56*	2.70±0.49*
Hb (g/dl)	13.21±0.63	6.31±0.72*	6.37±0.64*	6.41±0.34*
RBC (10^6/µl)	7.52±0.47	2.69±0.28*	2.75±0.41*	2.64±0.36*
Neutrophil (%)	28.65±0.21	14.26±0.43*	14.34±0.18*	14.40±0.38*
Lymphocyte(%)	41.53±0.09	20.35±0.58*	20.50±0.17*	20.42±0.39*
Monocyte (%)	3.24±0.43	1.62±0.12*	1.54±0.28*	1.68±0.20*
Eosinophils(%)	2.36 ± 0.50	2.39 ± 0.12*	2.46 ± 0.31*	2.42 ± 0.23*

Table 2: Hematopoietic Activity (on 29th days)

Observation	Group I Normal control	Group II Cyclophosphamide (3mg/kg) Control	Group III MandoorParipati (45mg /kg)	Group IV Cyclophosphamide (3mg/kg) + MandoorParipati (45mg/kg)
WBC (10^3/µl)	9.82±0.12	4.12±0.65*	10.14±0.87[a]	10.29±0.24[a]
Hb (g/dl)	14.24±0.71	8.53±0.42*	14.82±0.54[a]	14.37 ±0.32[a]
RBC (10^6/µl)	8.16±0.58	3.46±0.36*	8.53±0.61[a]	8.17±0.81[a]
Neutrophil(%)	29.25±0.35	19.18±0.76*	29.17±0.38[a]	29.34±0.57[a]
Lymphocyte(%)	41.68±0.41	26.47±0.38*	42.01±0.16[a]	41.68±0.21[a]
Monocyte (%)	3.82±0.59	2.03±0.43*	3.75±0.41[a]	3.52±0.36[a]
Eosinophils(%)	2.38 ± 0.46	2.43 ± 0.31*	2.93 ± 054*	2.56 ± 0.14*

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 compared to normal control group; [a]P<0.05 compared to cyclophosphamide control group

DISCUSSION

The study aimed to evaluate the effect Mandoor Parpati on the aplastic and iron deficiency anaemia induced by Cyclophosphamide Wistar strain albino rat. Cyclophosphamide has a bone marrow suppressive effect and induces aplastic anaemia and iron deficiency anaemia [12]. Mandura Bhasma as haematinic on $HgCl_2$ induced anaemia in rats, and reported significant haematinic and cytoprotective activity [13] Cyclophosphamide treatment at the dose of 3 mg/kg, body weight and i.p. resulted in the significant lowering of haematological parameters on the 7th day. All the haematological indices as aplastic anaemia and iron deficiency anaemia were restored to almost normal counts after continuous administration of the drug via repairing of bone marrow (Erythropoiesis) and iron recovering mechanism in RBCs [14].

CONCLUSION

It is concluded that the haematopoietic activity of Mandoor Parpati were found to be statistically significant biological activity without inducing any apparent acute toxicity on dose dependent study and it may reduce the risk of aplastic and iron deficiency anaemia.

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