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Research Article

Beneficial Effect of Qurs-E-Damavi, A Traditional Unani Formulation in Cyclophosphamide Induced Haematological Perturbations in Rats

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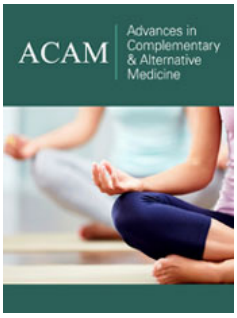

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Abstract

Qurs-e-Damavi (DM) is a polyherbal Unani formulation. It contains Rewand Chini (*Rheum emodi*), Zanjabeel (*Zingiber officinale*), Samagh-e-Arabi (*Acacia arabica*) and Hira Kasees (sulftes of Iron). It is used in conditions like anaemia. The aim of the current study was to validate the use of DM and its hydroethanolic extract (DME) in cyclophosphamide induced haemotoxicity in rats for the assessment of erythropoietic activity. DM was prepared as per classical methodology. Extract (DME) was obtained from crude formulation (DM) using extraction with ethanol and water (1:1; v/v). Haemotoxicity was induced by intraperitoneal administration of cyclophosphamide 3mg/kg bw in rats for seven consecutive days. Drug treatment was started from day-8 and continued till day-22. Blood samples were analysed on day-7 and day-22 using haematology analyser. Treatment with DM at 25 and 50mg/kg bw significantly reversed haemotoxicity induced by cyclophosphamide and haematological parameters of treated groups were comparable to vehicle control except a significant decrease ($p<0.01$) in WBC count at DM 25mg/kg bw group. DME 10mg/kg treatment normalized Hb and PLT count, however, RBC, WBC and HCT values were still significantly lower ($p<0.05$) compared to vehicle control. DME 20mg/kg treatment restored all hematological parameters except a significant decrease ($p<0.001$) in WBC count was persisted on day-22. Treatment with DM at 25 and 50mg/kg bw restored the haematological parameters in rats induced by cyclophosphamide. DME effectively restored haematological parameters only at the dose of 20mg/kg bw. Observed effect may be exerted by synergistic effect of the phytoconstituents of DM ingredients such as *Rheum emodi, Zingiber officinale, Acacia arabica* and iron. Present findings validate the indication of this traditional Unani formulation in the management of iron deficiency conditions like anaemia.

Keywords: Unani; Erythropoiesis; Polyherbal formulation; Haemopoiesis; Cyclophosphamide; Myelosuppression

Abbreviations: DME: Hydroalcoholic Extract; CP: Cyclophosphamide; TED: Therapeutic Equivalent Dose; Hb: Haemoglobin, RBC: Red Blood Cell; WBC: White Blood Cell; HCT: Haematocrit; PLT: Platelet

Introduction

Qurs-e-Damavi (DM) is a polyherbal Unani formulation. It contains Rewand Chini (*Rheum emodi*), Zanjabeel (*Zingiber officinale*), Samagh-e-Arabi (*Acacia arabica*) and Hira Kasees (sulfates of Iron). Though this traditional Unani formulation is being used clinically since long time (based on traditional knowledge), scientific data is lacking to support definite use of this Unani formulation. Therefore, the present study is designed to evaluate the effect of Qurs-e-Damavi and its 50% hydroalcoholic extract (DME) on haematopoiesis.

Materials and Methods

Effect of Qurs-e-Damavi and its 50% hydroalcoholic extract (DME) was evaluated in cyclophosphamide (CP) induced haematological perturbations in rats.

Preparation of the formulation

Qurs-e-Damavi (DM) was prepared in the GMP certified Pharmacy Section of National Research Institute of Unani Medicine for Skin Disorders, Hyderabad as per the composition which includes Rewand Chini (*Rheum emodi*), Zanjabeel (*Zingiber officinale*), Samagh-e-Arabi (*Acacia arabica*) and Hira Kasees (sulphates of Iron).

50% hydroethanolic extract of DM was prepared by Drug Standardisation Research Unit of the Institute. Briefly, DM was soaked in 1:1 mixture of water and ethanol (v/v) for 24 hrs

with intermittent shaking, followed by filtration. The supernatant was discarded, and filtrate was evaporated to obtain dry extract (DME) which was weighed to calculate the yield and stored in desiccators till further use.

Experimental animals

Sprague Dawley rats (220±30g; Male) were used for the present study. Animals were procured from EDARA research foundation, Hyderabad. Rats were group housed in polysulfone cages in the temperature-controlled room maintained at the temperature of 22°C ± 3°C and relative humidity of 30-70%, with a 12:12 h light/dark illumination cycle. Study was approved by Institutional Animals Ethics Committee vide protocol no. 1034/GO/Re/S/07/CPCSEA. National guidelines of laboratory animal care (CPCSEA) were followed throughout the experiment [1]. Rats were maintained on standard diet (SDS diet, England) and water ad libitum, unless mentioned otherwise. Corn cob bedding was used for housing the animals. Only male rats were used to avoid the influence of the estrus cycle on drug metabolism and/or efficacy. Rats were acclimatized to the laboratory conditions for one week before using them for experiment.

Dose selection and study design

Therapeutic dose of DM for adult human is reported as 250mg per day. Accordingly, as per body surface area conversion method [2], Therapeutic Equivalent Dose (TED) for rat is about 25mg/kg bw per day. Therefore, present study was performed at two dose levels of DM i.e., 25 and 50mg/kg bw/day. The percentage yield of hydroethanolic extract was found to be 40.46% (w/w). Accordingly, equivalent doses of extract (DME) for rats are 10 and 20mg/kg bw/day.

Cyclophosphamide induced haemotoxicity

Hematopoietic activity was evaluated cyclophosphamide induced haemotoxicity model in rats [3]. Haemotoxicity was induced by intraperitoneal administration of cyclophosphamide (3mg/kg bw) for 7 consecutive days. Rats were divided into six groups (6 male animals in each group) and treated as Table 1.

Table 1: Treatment for rats.

S.No:	Group (n=6)	Treatment Schedule
Group I	Vehicle Control	0.9% saline i.p. from Day 1 to Day 7 followed by 0.3% CMC, p.o. from Day 8 to Day 22.
Group II	Cyclophosphamide Control	Cyclophosphamide 3mg/kg bw, i.p. from Day 1 to Day 7 and 0.3% CMC, p.o. from Day 8 to Day 22.
Group III	DM 25 +Cyclophosphamide	Cyclophosphamide 3mg/kg bw, i.p. from Day 1 to Day 7mg/kg bw and DM 25mg/kg bw, p.o. from Day 8 to Day 22.
Group IV	DM 50 + Cyclophosphamide	Cyclophosphamide 3mg/kg bw, i.p. from Day 1 to Day 7mg/kg bw and DM 50mg/kg bw, p.o. from Day 8 to Day 22.
Group V	DME 10 +Cyclophosphamide	Cyclophosphamide 3mg/kg bw, i.p. from Day 1 to Day 7mg/kg bw and DME 10mg/kg bw, p.o. from Day 8 to Day 22.
Group VI	DME 20 + Cyclophosphamide	Cyclophosphamide 3mg/kg bw, i.p. from Day 1 to Day 7mg/kg bw and DME 20mg/kg bw, p.o. from Day 8 to Day 22.

DM and DME were suspended in 0.3% CMC every day using mortar pestle. The test drugs were orally administered as an aqueous suspension at the maximum volume of 2mL/100gm bw. The control rats were administered with vehicle (i.e., 0.3% CMC) only. Test drugs or vehicle were administered via stainless steel gavage, by calculating the individual dose based on the body weight of each rat.

On day 7, blood samples were collected from the retro-orbital plexus under isoflurane anesthesia and evaluated for various blood parameters using automatic haematology analyser. Hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT) and platelet (PLT) counts were analyzed. Further, blood will be collected on the 22nd day and evaluated for the same hematological parameters.

Statistical analyses

Data from the experiments was expressed as mean ± standard error of mean (SEM). The mean difference between the control and treatment groups was analysed by one-way Analysis of Variance using Graph Pad prism (version 5) Graph Pad Software, Inc., CA, USA. p value<0.05 was considered as statistically significant.

Result

Rats treated with cyclophosphamide 3mg/kg for seven days (group II-VI) showed marked decrease (statistically significant in most of cases; refer to (Table 2) in haematological parameters such as RBC, Hb, WBC, HCT and PLT compared to vehicle control. Haematological perturbations persisted on day-22 in cyclophosphamide control rats (group-II) and there was a significant reduction in RBC ($p<0.01$), Hb ($p<0.05$), WBC ($p<0.05$), and HCT ($p<0.05$) compared to vehicle control (Table 3). Treatment with DM at 25 and 50mg/kg bw significantly normalised these haematological parameters and all values were comparable to vehicle control except a significant decrease ($p<0.01$) in WBC count at DM 25mg/kg bw (4000±184.4 vs. 5840±201.5 of control). DME 10mg/kg treatment normalized Hb and PLT count, however,

RBC, WBC and HCT values were still significantly lower ($p<0.05$) compared to vehicle control. DME 20 mg/kg treatment normalized all hematological parameters except a significant decrease ($p<0.001$) in WBC count was persisted on day-22 (3700±312.0 *vs.* 5840±201.5 of control).

Table 2: Effect of Damavi (DM) and its hydroethanolic extract (DME) on haematological parameters in cyclophosphamide-treated albino rats (after 7 days).

Parameter	Treatment Group					
	I	II	III	IV	V	VI
	Vehicle Control	CP Control 3mg/kg	CP 3mg/kg + DM 25mg/kg	CP 3mg/kg + DM 50mg/kg	CP 3mg/kg + DME 10mg/kg	CP 3mg/kg + DME 20mg/kg
Mean RBCs (10^6/µl)	8.58±0.317	7.40±0.368*	7.50±0.116*	7.43±0.239*	7.53±0.117	7.43±0.199*
Mean Hb (gm/dl)	16.22±0.244	14.47±0.643*	14.67±0.163*	14.52±0.405*	14.78±0.101	14.57±0.301*
Mean WBCs (10^3/µl)	6800±820.60	3650±312.80***	2850±254.00***	3083±135.20***	2700±203.30***	2633±187.40***
Mean HCT (%)	42.50±0.224	40.17±0.749	39.83±0.601	38.67±1.022**	39.50±0.428	38.83±0.980*
Mean Platelets (lakhs/mm^3)	6.05±0.241	3.52±0.287***	3.27±0.216***	3.67±0.549***	3.82±0.248***	3.92±0.323**
Values are shown as Mean ± SEM (n=6); One-way ANOVA; *=p<0.05, **=p<0.01, ***=p<0.001 vs. vehicle control.						

Table 3: Effect of Damavi (DM) and its hydroethanolic extract (DME) on haematological parameters in cyclophosphamide-treated albino rats (after 22 days).

Parameter	Treatment Group					
	I	II	III	IV	V	VI
	Vehicle Control	CP Control 3mg/kg	CP 3mg/kg + DM 25mg/kg	CP 3mg/kg + DM 50mg/kg	CP 3mg/kg + DME 10mg/kg	CP 3mg/kg + DME 20mg/kg
Mean RBCs (10^6/µl)	8.72±0.271	7.75±0.180**	8.03±0.0803	7.98±0.220	7.95±0.092*	8.12±0.147
Mean Hb (gm/dl)	16.20±0.268	14.38±0.612*	15.20±0.058	14.98±0.422	15.08±0.150	15.38±0.244
Mean WBCs (10^3/µl)	5840±201.5	4383±192.2*	4000±184.4**	4600±323.5	4317±409.4*	3700±312.0***
Mean HCT (%)	43.00±0.447	39.00±1.342*	40.00±0.258	39.67±0.667	39.33±0.333*	41.33±0.882
Mean Platelets (lakhs/mm^3)	4.56±0.431	3.88±0.343	3.63±0.186	4.60±0.262	5.32±0.214	4.32±0.361
Values are shown as Mean ± SEM (n=6); One-way ANOVA; *=p<0.05, **=p<0.01, ***=p<0.001 vs. vehicle control.						

Discussion

Present study was carried out to determine erythropoietic activity of traditional Unani formulation DM and its hydroethanolic extract DME. Cyclophosphamide induced model in rats was used to assess the effect of DM and DME on various haematological parameters. Cyclophosphamide induced toxicity model has been routinely used to evaluate the beneficial effect of herbal formulation in blood dyscrasia [3-5]. Cyclophosphamide is an antineoplastic drug that causes myelosuppression due to its metabolites which limits its clinical use [6]. The severity of myelosuppression increases with higher doses of cyclophosphamide. Cyclophosphamide induces myelosuppression by oxidative stress. Traditional medicines have been reported to reverse such changes [7,8].

In the present study, administration of cyclophosphamide at 3mg/kg for seven days (group II-VI) resulted in significant reduction of the RBC, Hb, HCT, WBC and PLT counts on day-7 as compared to vehicle control animals. Treatment with DM at 25 and 50mg/kg from day 8 to day 22 significantly normalised these haematological parameters. Oral administration of DME at the dose of 20mg/kg from day-8 to day-22 restored hematological alterations induced by cyclophosphamide except a significant decrease in WBC count. However, DME 10mg/kg did not effectively reversed haematological parameters induced by cyclophosphamide.

Observed beneficial effect of DM may be exerted by the presence of iron i.e., Hira Kasees (Sulphates of Iron) and atleast partly due to other constituents of DM such as ginger. It is reported that Rainbow trout (*Oncorhyncus mykiss*) fed with powdered ginger rhizome showed significant immune-stimulatory effect, increasing WBC, haematocrit, RBC count compared with the control group [9]. A recent study reported a significant increase in RBC, WBC, as well as the level of haematocrit and haemoglobin in fish (*Cyprinus carpio*; common carp) fed with ginger (*Zingiber officinale*) supplemented diet [10].

R. emodi another ingredient of DM has traditionally been used as diuretic, liver stimulant, purgative/cathartic, stomachic, anticholesterolemic, antitumour, antiseptic, immunomodulatory, as tonic and in menstrual disorders like dysmenorrhoea and menorrhagia (Zargar Hina). Several anthraquinone derivatives including emodin, aloe-emodin, physcion, chrysophanol, rhein, emodin glycoside and chrysophanol glycoside occur as the main chemical constituents [11,12]. *R. emodi* is reported as antioxidant and bioactivity-guided isolation of roots of *R. emodi* revealed eugenol, gallic acid, quercetin, rutin, epicatechin, desoxyrhapontigenin, rhapontigenin and mesopsin as major phenolic compounds responsible for the antioxidant activity [13]. Water soluble fraction of alcoholic extract of *R. emodi* is reported to have nephroprotective effect on all the proximal tubule segments possibly through antioxidant action of the tannins present in the fraction [14]. *R. emodi* also contains various micro and macro elements such as K, Ca, Fe, Mn, Na, Zn, Co, Li and Cu [15]. Aqueous extract of *R. emodi* was found to be safe up to 4000mg/kg/day in rats in a repeated dose 90-day oral toxicity study in rats [16]. Reported pharmacological profile of *R. emodi* including antioxidant potential clearly support the observed beneficial effect of DM in present study. A. arabica, another ingredient of DM is reported as a potent free radical scavenger and hepatoprotective and the polyphenol rich fraction is responsible for free radical scavenging activity [17,18]. Taken together, cumulative synergistic effect of individual ingredients is responsible for observed beneficial effect of this compound Unani formulation in cyclophosphamide induced haemotoxicity in rats. Findings of the study support that DM is a potentially effective therapy to overcome conditions like anaemia and leukocytopenia. Test formulation may be particularly useful as adjuvant therapy to overcome or curtail cyclophosphamide-induced damage during cancer chemotherapy. Further studies are warranted to explore the underlying mechanism DM and DME against cyclophosphamide induced toxicity.

Conclusion

Treatment with DM at 25 and 50mg/kg bw restored the haematological parameters in rats induced by cyclophosphamide. DME effectively restored haematological perturbations only at 20mg/kg bw. Observed effect may be exerted by the presence of iron and other constituents of DM such as flavonoids, terpenoids, and steroids. Present findings validate the indication of this traditional Unani formulation in the management of iron deficiency anaemia and DM could be a potential formulation for erythropoietic activity.

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