Research Article

Safety Profile for Evaluation of Berberine in Sprague- Dawley Rats: Effects on Renal, Liver and Myelopoietic Functions

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Abstract | The study was designed to investigate the effect of berberine toxicity on liver enzymes, renal function, serum uric acid and blood cell counts of Sprague-Dawley rats. For this purpose, twenty Sprague-Dawley rats were divided in two groups. Group A (n=10) was given distilled water and Group B (n=10) was supplemented berberine hydrochloride with dose of 75 mg/kg body weight/day. 24 weeks post-treatment, blood samples were collected by cardiac puncture from both groups and tested for Hb (%), WBC, RBC and platelet counts, SGPT, alkaline phosphatse, serum uric acid, blood urea and serum creatinine. All the numerical data were expressed as mean± SD. The values in two groups were analyzed with help of unpaired t-test. A p-value < 0.05 was considered significant. After 24 weeks of treatment with berberine hydrochloride, no significant differences were observed in Hb%, platelet count, liver enzymes and RFTs between two groups. There was a significant increase in RBC count and reduction in serum uric acid and WBC count in experimental group as compared to control group. There were no significant untoward effects of berberine on platelet count, LFTs and RFTs of Spargue-Dawley rats except neutropenia.

Received | July 08, 2017; **Accepted** | November 26, 2017; **Published** | -------- 00, 2017
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Citation | Anwar, A., S. Malik, Z. Usman and S. Chiragh. 2017. Safety profile for evaluation of berberine in sprague- dawley rats: effects on renal, liver and myelopoietic functions. *Annals of King Edward Medical University*, 23(4): xxx-xxx.
DOI | http://dx.doi.org/10.17582/journal.akemu/2017/23.....
Keywords | Berberine, SPRAGUE-Dawley rats, Hepato-toxicity

Introduction

Berberine, an isoquinolone derivative alkaloid, is present in roots, rhizome and stem bark of many plants including *Berbris equifolium*, *Berberis aristata*, *Berberis vulgaris* and *Hydratis canadensis*. It has been widely used in traditional Chinese medicines for hundreds of years[(1)]. Modern research has shown that berberine and its derivatives display several pharmacological effects through various mechanisms[(2)]. Its properties include anti-microbial and anti-protozoal effects[(3)], anti-diabetic effects[(4)], anti-lipidemic effects and hence beneficial effects on cardiovascular system[(5)]. In ayurvedic medicine, *Berberis aristata* and berberine is being used for prevention and treatment of cancer[(6)]. In a recently conducted research, berberine delayed and prevented development of 7, 12Dimethylbenz (α) anthracine induced mammary carcinogenesis in rats with improvement in histopathologic parameters[(7)].

Berberine is an over-the-counter (OTC) drug and is used for a wide variety of diseases in China. Despite its widespread use in ayurvedic and Chinese traditional medicine, limited studies are conducted on untoward effects and safety profile of the drug. The

present study was designed to evaluate the effects of berberine on various organ functions including liver functions, renal functions and myelopoietic system in Sprague-Dawley rats.

Materials and Methods

The study was carried out in the Department of Pharmacology, PGMI, Lahore during 2014. Healthy female Sprague-Dawley rats of 6 weeks age were purchased from University of Veterinary and Animal Sciences, Lahore, Pakistan. Animals were kept in animal house of PGMI for 1 week for acclimatization. They were fed on water and rat chow *ad libitum*and kept in iron cages at optimum room temperature according to criteria mentioned in guide for the care and use of laboratory animals[8]. After 1 week, rats were divided in two groups, A and B carrying 10 animals each. The body weight was recorded in grams. Berberine hydrochloride was purchased from Sigma Aldrich. For administration, 3 g of berberine was suspended in distilled water to make volume 80 ml,producing concentration of 75 mg berberine/2 ml[9]. Suspension was given to Group-B in a dose of 75 mg/kg daily by gavage for a period of 24 weeks as a morning dose whereas Group-A was given distilled water by similar route for same duration.

After 24 weeks, rats were anesthetized and blood samples collected through cardiac puncture. For CBC, blood was collected in EDTA vials. The vials were then put in roller mixer and attached to Sysmex KX-21 to get CBC reports. For alanineaminotranferase, alkaline phosphatase, urea and creatinine, clot activatedsamples were collected in vialsand centrifuged to separate serum. The serum was then put in Spectra fully automated machine to obtain results using commercially available kits.

Statistical analysis

All group data were evaluated statistically with SPSS version 20.All values were expressed as mean ± SD. Testing methods included unpaired-t test. The p-value < 0.05 was considered significant.

Results and Discussion

The levels of hemoglobin and blood cells counts in control and experimental groups of rats are shown in Table 1. It is evident that there were no statistically significant differences in mean hemoglobin, and mean platelets counts in two groups. However, significant difference was observed in mean WBC and RBC counts. There was decrease in WBC count but increase in RBC count in berberine treated group.

Table 1: *Effect of berberine on hemoglobin and blood cell count of female Sprague Dawley rats.*

Parameter	Group n=10	Mean±SD	p- value
Hemoglobin (g/dl)	Control Treated	13.59±1.10 13.58±1.33	0.980
WBC (/mm^3)	Control Treated	8475±1089.66 5820±1522.28	0.002
RBC (10^6/mm^3)	Control Treated	4.83±0.37 6.45±0.24	0.0001
Platelet (/mm^3)	Control Treated	408000±59029.18 365000±106796.80	0.150

Table 2: *Effect of berberine on renal function, liver enzymes and uric acid level of female Sprague Dawley rats.*

Parameter	Group n=10	Mean±SD	p- value
Blood urea (mg/dl)	Control Treated	34.40±4.30 33.00±4.69	0.220
Serum Creatinine (mg/dl)	Control Treated	0.50±0.24 0.55±0.14	0.300
Alkaline Phosphatase (U/l)	Control Treated	206.20±93.51 244.40±43.23	0.155
SGPT (U/l)	Control Treated	29.10±6.21 30.40±5.42	0.299
Serum Uric acid (mg/dl)	Control Treated	4.20±0.69 2.73±0.57	0.001

Results shown in Table 2 indicate that the comparison of liver enzymes, renal function and serum uric acid levels of the rats in two groups. No statistically significant differences were seen in mean blood urea, serum creatinine, alkaline phosphatase and SGPT levels. However, there was a significant decrease in serum uric acid level in berberine treated group.

Berberine is a bitter tasting yellow plant alkaloid used for long time in Chinese and ayurvedic medicine. Several clinical trials have been conducted using berberine for various conditions in animal as well as humans. Before the present study, no information describing the toxic effects of berberine compounds on liver, kidneys and blood cells of laboratory animals was found in the literature. However, regarding developmental anomalies in rats and mice, one study has been published[10]. Present study was designed with a

view to observe any toxic effects of berberine on various organ systems in experimental rats.

No adverse clinical signs were observed during the treatment or the trial period. Table 1 compares the blood cell counts of control and berberine treated groups. No significant differences were found in hemoglobin level and platelet counts in the two groups. The compound has rather previously been tested for effects on platelet count and platelet functions in patients of primary and secondary thrombocytopenia showing improvement in both[11].

The mean WBC count in control group was 8475±1089 and in experimental group, it was 5820±1522 per mm^3. Although this relative neutropenia observed had no apparent clinical effects but the difference is statistically significant (p=0.002). This may be attributed to a non-specific response of berberine. Leucopenia has been observed with use of some herbs like Echinacea (published as a case report) and was considered to be a reversible allergic response[12]. This may be a possible explanation in case of berberine but it requires further investigation. Significantly high RBC count was observed in berberine treated rats. This effect on RBC count with concomitant decrease in WBC count suggests stimulation of one progenitor cell type in bone marrow with simultaneous suppression of other. Additional data as red cell volume and differential white cell count may have been helpful in providing an explanation.

Table 2 shows the comparison of hepatic enzymes and renal function tests of the two groups. The mean blood urea levels were 34.4 ± 4.3 and 33.0 ± 4.69 mg/dl in control and treatment groups respectively. The mean serum creatinine levels were 0.50 ± 0.24 and 0.55 ± 0.14 mg/dl respectively. These differences were not significant thus ruling out any possible nephro-toxicity of berberine in normal Spargue-Dawley rats. In one study, the drug showed nephro-protective role in streptozotocin induced diabetic rats[13]. Moreover, berberine wasfound to have protecting effect on hypertensive renal impairment model rats, fed with high fat-salt-fructose diet associated with elevated antioxidant capability in body and kidney tissues[14].

Regarding hepatic enzymes, the mean SGPT was 29.1 ± 6.21 and 30.4 ± 5.4 U/l in control and treated groups respectively. Similarly, alkaline phosphatase was 206.2 ± 93.5 and 244.4 ± 43.2 U/L. These differences were not significant either, ruling out any possible hepato-toxicity. In a previously reported study, berberineshowed hepato-protective activity against CCl_4 induced hepato-toxicity. The activity was both preventive and curative [15]. In another study, it was concluded that berberine not only reduced elevated levels of ALT and AST, but also showed improvement in histo-pathological changes of necrosis and fibroblast proliferation in livers of rodents in which liver toxicity was induced[16].

An inhibitory effect on serum uric acid levels was observed in berberine treated group. Chang et al found an inhibitory effect of 12 phenolics including berberine on xanthine oxidase activity in experimental mice. This xanthine oxidase inhibition may be a possible explanation for reduced uric acid levels in the current study[17].

The above-mentioned results of the present study establish safety of berberine use in normal Sprague Dawley rats. Rather, a beneficial effect on serum uric acid level was observed for a possible therapeutic usage for hyperuricemia in human subjects. Berberine may also be helpful in elevated uric acid levels associated with cancers. However, further investigation in human volunteers is required to see the safety for long term human use.

Conclusions

The results of the present study showed that berberine at the dose of 75 mg/kg body weight/day for 24 weeks in Sprague- Dawley rats possesses no clinical and biochemical evidence of effects on liver and renal functions. However, a relative neutropenia was observed which provides guidance for further studies to determine the mechanisms for berberine effects.

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