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IN VIVO EXPLORATION OF ANTIOXIDATIVE POTENTIAL OF BERBERINE IN RAT MODELS

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Abstract

Introduction: *Berberine, a naturally occurring compound found in several plants such as Berberis vulgaris (barberry), Berberis aristata (tree turmeric), and Phellodendron amurense (Amur cork tree), is a bioactive alkaloid with a long history of use in traditional Chinese and Ayurvedic medicine. Renowned for its broad spectrum of pharmacological properties, berberine is particularly celebrated for its potent anti-hyperglycemic, anti-inflammatory, and antimicrobial effects. It has been reported to have anti-oxidative potentials, scavenging free radicals and reducing oxidative stress, which may contribute to it therapeutic benefits in promoting health and well-being. It helps protect cells from damage, supporting various physiological processes and potentially mitigating the risk of chronic disease.*

Objective: *This study aims to investigate the antioxidative potential of berberine in edema-induced rat models, and understanding to its therapeutic applications.*

Material and Methods: *After study ERC approval (ERC/359/01/2023) Healthy male Sprague-Dawley rats (n=25) 7 weeks old were used in experiments performed inter-collaboratively at various affiliated Laboratories. All rats were divided into 5 groups containing 6 rats per group. Group I (Negative Control Group, Non-edema physiological rats), Group II (Positive Control Group, edema induced rats) III, IV and V (edema induced rats with Berberine extract treatment). Except for control of non-induced rats (Group I), edema was induced in rat's paws in groups II, III, IV and V by carrageenan. Following edema induction group III, IV and V were administered the crude powder extract of Berberine 1.9 g, 3.8 g and 5.7 g respectively by mixing in diet, rats were given a treatment of Berberine for the period of 24 days. Whole leaf aqueous extracts at various concentrations (100, 200, 400, and 600 mg/kg of body weight) were given to check the anti-inflammatory potential of Berberine. After treatment animals were sacrificed and samples were collected to analyze immunity and oxidative stress. Total Anti-oxidant capacity with stress biomarkers was analyzed. Oxidative stress parameters were measured spectrophotometrically by prescribed colorimetric assay. Data was subjected to ANOVA (two-way) using the statistical package SPSS-22.0.*

Results: *Results showed that treatments with Berberine showed highly significant effect on stress biomarkers and Total antioxidant capacity. Leaf extracts significantly reduced the formation of edema induced by carrageenan and formaldehyde and granuloma formation in a dose-dependent manner.*

Conclusion: *Berberine showed strong anti-oxidative potential with best pharmaceutical significance.*

Keywords: *Anti-oxidative potential, Berberine, Herbal plant, Phytochemicals, Rats model, Therapeutic insights*

INTRODUCTION

Due to antioxidant activity, berberine has significant prospects in the area of natural drugs and diagnostic equipment. Over the years simple biological research has changed its focus and targets natural compounds that possess medicinal attributes and these include tannins, alkaloids, flavonoids and phenolics

(1). Out of all these bioactive components, flavonoids, phenolic compounds, tannins and alkaloids are the most important. Herbs are very essential in relation to natural cure (2). It documents that throughout recorded history, traditional herbs have been used for treating fatal, easily communicable diseases including smallpox and other epidemic diseases. Berberine for instance has had historical testaments of beneficial effects on conditions resulting from bacteria, viruses, fungi as well as other microorganisms. It is also used in most drugs for humans and animals used administratively (3).

Oxidative stress threatens cellular functions and communication and stimulates the aging process due to the accumulation of oxidation byproducts. Furthermore, inflammation resulting from oxidative stress deepens tissue injury and thereby forms a cycle that threatens health at the molecular level (4). Qualitative analysis for phytochemicals presents in the leaves of berberine found out the presence of alkaloids, sterols, polysaccharides and anthraquinone. The main primary organic compounds are alkaloids which are well known for their medicinal as well as diagnostic importance; these have hypoglycemic property and are used to cure medicines for heart cancer (6). On the same note, glycosides are other compounds that are related in structure to hydrocarbons since they are organic compounds. Medicinal aloe thus has been widely used to cure human ailments due to its many years of use. Traditionally, bitter juice obtained from the aloe vera leaves which have medical uses as it purges the blood from infections has anti-bacterial activity. Another way through which berberine is said to be useful is through the preparation of juice which is said to increase appetite, cure fever and expel intestinal worms (7,8). Current literature has revealed that berberine has such properties like anti-inflammatory, anti-fungal, and anti-pyretic properties (9). Research in rats experiencing oxidative stress and cisplatin-induced nephrotoxicity established that methanolic leaves of berberine elevated antioxidant glutathione and the enzymes, SOD, catalase, glutathione reductase, GST, and GPx; concurrently, the study revealed a decrease in Nit and MDA levels (10).

Several pathophysiological mechanisms that are underlying the neuroprotective effects of berberine extract were studied in Wistar rats which had ischemia/reperfusion partial sciatic nerve injury. This attenuation of the treatment checked oxidative stress and neuronal apoptosis through the inhibition of oxidative nitrosamine stress in addition to the diminishing of pro-inflammatory cytokines (11). This ability of berberine leaf extract was established by such indicators as nitric oxide, hydrogen peroxide, and cytochrome-C in concentrations; caspase 3-9 activation as well as DNA fragmentation (12). Oxidative stress in general is therefore controlled by the endogenous antioxidant system in which GSH-Px, SOD and Catalase have been noted to play significant roles are shown in Fig. 1.

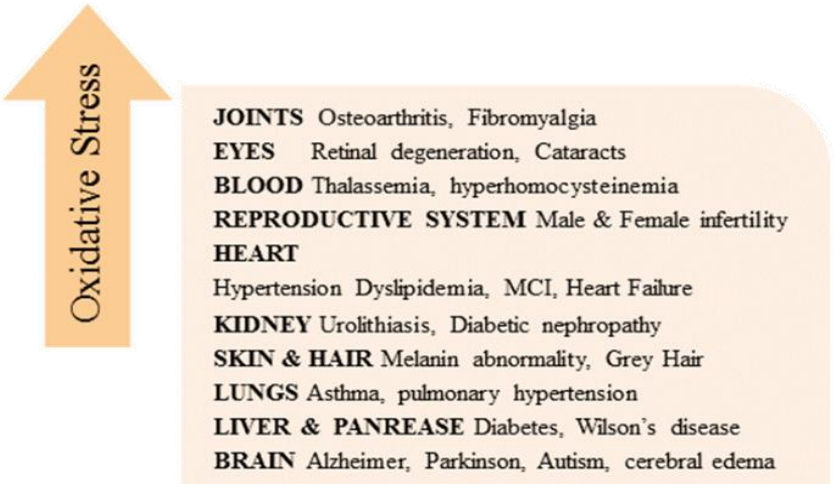

Fig. 1. Effects of oxygen stress (13, 14)

MATERIALS AND METHODS

After study ERC approval (ERC/359/01/2023) the experiments on mouse handling and analytical proceedings were done inter-collaboratively at Kohat University of Science and Technology, University of Kotli AJK, COMSATS University Islamabad, University of Veterinary and animal sciences Lahore. Fresh leaves of Berberine plant about 0.7 Kg were obtained from the local areas and the botanical gardens in Mirpur University of Sciences and Technology and Government College University Faisalabad which were

identified and authenticated from botanist of concerned Institutes. Leaves were shade dried and were grinded to make powder. Experimental scheme is shown in Fig. 2.

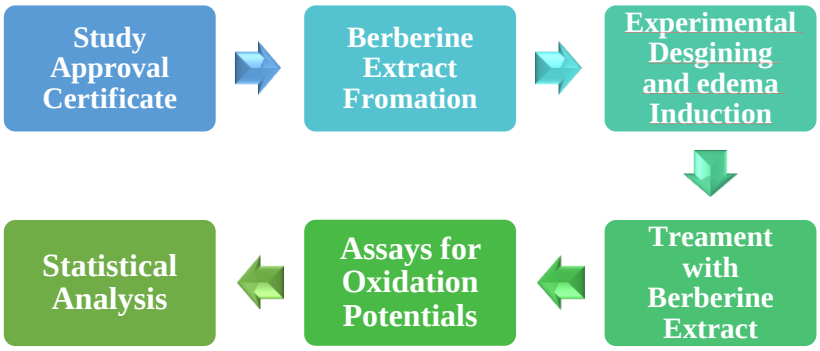

Fig. 2. Scheme of Experiments

A total of 24 male Sprague-Dawley rats of 7-week-old, reared in animal nursery, were selected for the experiment. Animal's weight was 190-250grams. All rats were kept for 7 days at standard conditions (Temperature was 26oC ± 3.5oC) and humidity (30-50%) with the light maintained at 10h (light-dark cycle). Chow maintained diet (CMD) was given to animals and water ad libitum. Their daily consumption of diet was measured at fixed intervals. All procedures and protocols used during animal handling were approved from respective head of departments from various labs at different institutes.

EXPERIMENTAL SCHEME

Five Groups were made with each individual group containing 5 rats. Study Experimental scheme is given below:

Group I: (Non-edema/Normal Rats) Negative Control group (kept at CMD only)
Group II: (Edema Rats) Positive Control group (No treatment given, kept at CMD only)
Group III: (Edema Rats) Treatment group 1 (1.9 g/kg Berberine in CMD)
Group IV: (Edema Rats) Treatment group 2 (3.8 g/kg Berberine in CMD)
Group V: (Edema Rats) Treatment group 3 (5.7 g/kg Berberine in CMD)

Edema was induced in rats' paws groups II, III, IV and V by the injection of carrageenan 1% (w/v) solution in normal saline, 0.1 ml of this solution was injected into the sub-plantar region of the rat's hind paw by using a 25–30-gauge needle for the injection to minimize tissue damage. Rats were kept under observation for few hours after giving injection. Upon confirmation of Edema in rats, designated groups were given treatment of Berberine leaves crude extract powder for 24 days.

The total anti-oxidant capacity in the serum sample was measured using the novel automated colorimetric method along with comparative analysis of various oxidants and ROS (samrtchem®wet chemistry analyzer M-6589). Total oxidant status was assessed via automated colorimetric assay along with superoxide dismutase SOD and paraoxonase activity assay.

Data was subjected to one way ANOVA and SPSS-28 was used (significant p-value < 0.01). In experiments involving five rat groups with different treatments, one-way ANOVA is highly effective for assessing differences in outcomes, such as paw edema. This method allows for simultaneous comparison of means across all five groups, ensuring a comprehensive evaluation without increasing the risk of Type I error.

RESULTS

The TOS levels were significantly reduced in treatment groups compared to the positive control, indicating the antioxidative effect of berberine. One way ANOVA was used because of its robust against deviations from normality and facilitates differential statistics specific groups differ from each other as shown in Table I. Overall, it offers a reliable and efficient approach to evaluate the efficacy of various treatments in the study.

Table I. Accumulative mean ± standard error of oxidative parameters in five groups (n=25)

Groups	Group I	Group II	Group III	Group IV	Group V	Statistics
Parameters	NC	PC	B1	B2	B3	*P*-Value
TOS(µmol/l)	18.42±0.551	71.45±11.351	21.21±2.452	18.25±2.552	15.97±2.922	0.01
TAC(mmol/l)	2.45±0.013	0.606±0.043	0.974±0.084	2.188±0.544	1.929±0.154	0.00
Paraoxonase (KU/min/ml)	425.25±116.515	41.65±16.965	179.86±47.96	249.84±74.97	239.79±69.27	0.01

Results are means ± SE. NC = Negative Control, PC = Positive Control, B1 = Berberine 1.9g, B2 = Berberine 3.8g, B3 = Berberine 5.7g TOS = Total oxygen status, TAC= Total Anti-oxidant capacity. Statistically Significant p-value (p≤ 0.01). Means values with similar numbers (superscript) were statistically found Non-significant (p-value >0.05) to each other

One-way analysis of variance was applied to compare mean concentration of serum TOS from different groups. Positive control (diabetic rats) group showed highly significant difference ($p \leq 0.01$) with all other groups. The TOS level significantly decreased in both treatment groups as compared to positive control (diabetic rats). The overall mean of Total oxidant status is presented in Fig. 3 and Table I.

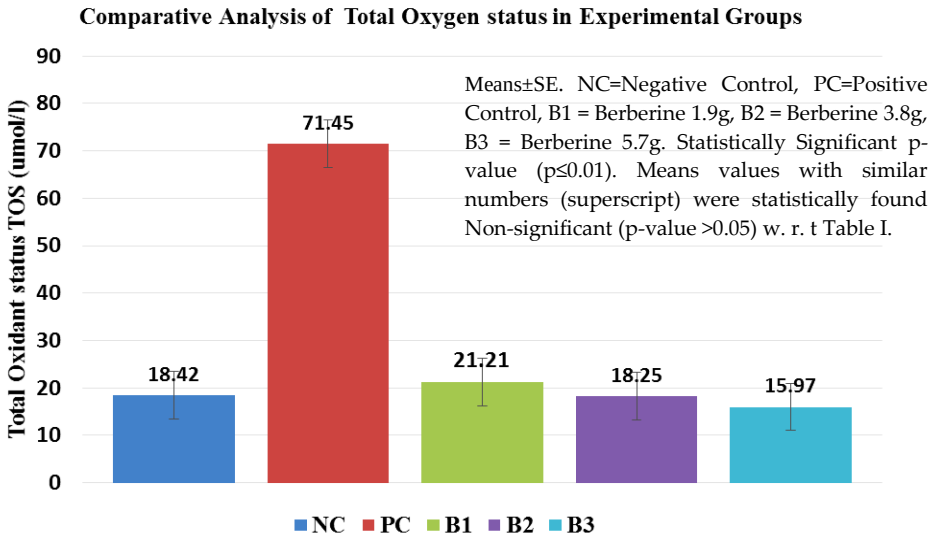

Fig. 3. Mean total oxidant status TOS levels of study groups

One-way analysis of variance was applied to compare mean concentration of serum TAC from different groups. There was no significant difference ($p \leq 0.01$) among all groups. However, the TAC level increased in high dosage group (1.929±0.154) as compared to positive control (0.606±0.043). The overall mean of Total antioxidant capacity is presented in Fig. 4 and Table I.

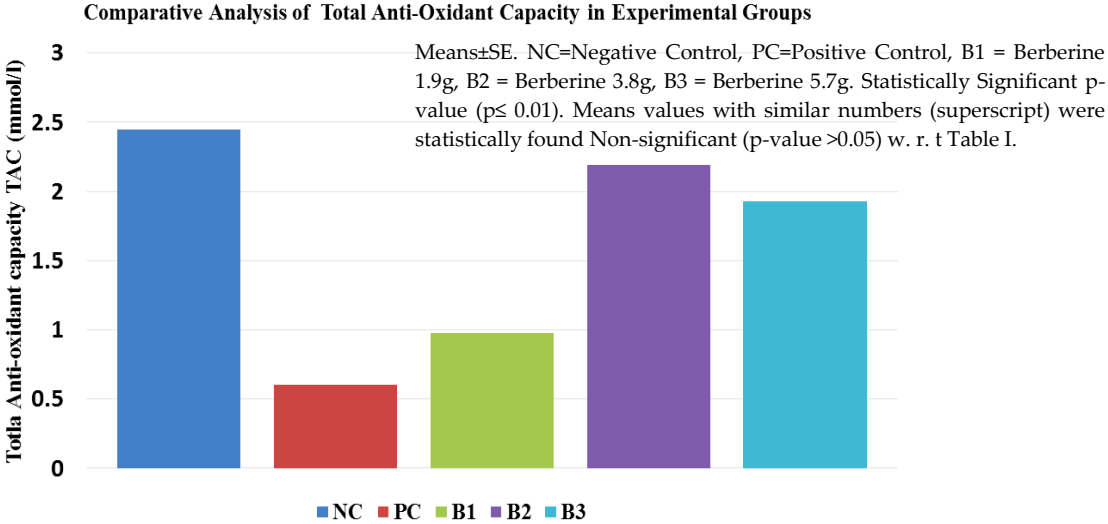

Fig. 4. Accumulative means values of TAC in experimental groups

One-way analysis of variance was applied to compare mean concentration of serum PON-1 from different groups. Positive control (diabetic rats) group showed highly significant difference ($p \leq 0.01$) with all groups and value of PON-1 increased in both treatment groups. The overall mean of Paraoxonase (PON-1) is presented in Fig. 5 and Table I.

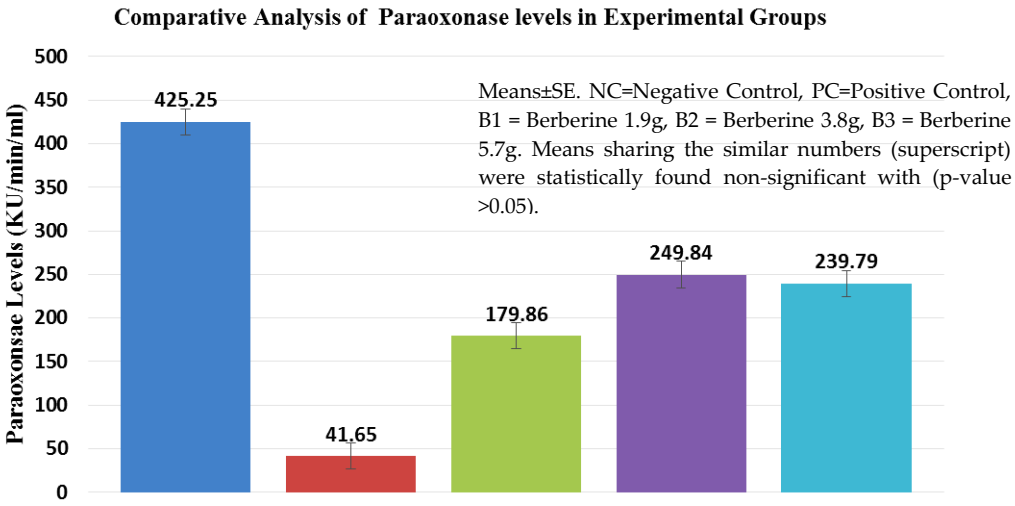

Fig. 5. Accumulative means values of PON-1 in experimental groups

DISCUSSION

Strong antioxidant qualities shown by Berberine, which scavenge free radicals and lower oxidative stress, may be part of the plant's healing advantages in enhancing general health and wellbeing. Its high antioxidant concentration aids in cellular damage prevention, supporting a number of physiological functions and perhaps lowering the risk of chronic illnesses (15). The significant reduction in oxidative stress biomarkers suggests that berberine's antioxidative properties contribute to its therapeutic potential. These findings align with previous studies demonstrating berberine's ability to modulate oxidative stress and inflammation. Highly reactive molecules known as free radicals have the ability to harm lipids, DNA, and proteins found in cells, impairing their ability to function and possibly accelerating the onset of a number of diseases, including cancer, cardiovascular disease, neurodegenerative diseases, and conditions associated with aging (16).

Researchers have confirmed that the chemical component of berberine, acemannan, possesses anti-inflammatory and anti-oxidative qualities. Studies conducted in vivo have also proven Berberine's anti-tumor properties (17).

Many authors have not only established the fact that the chemical part of berberine, acemannan, has good anti-inflammatory and anti-oxidative properties (18). Clinical investigations have shown that Berberine can decrease oxidative stress from the human body and it has been proved that it has anti-tumor effects in animal tests (19). The study design showed a significant difference between the groups after checking the mean concentration of TAC. Particularly, TAC levels in the diabetic group were found to decrease, however, efficiently came to normality in rats receiving berberine treatment, which supports the antioxidant influence (20). The plasma enzyme with exceptional antioxidative properties named Paraoxonase-1 (PON-1) is involved with the breakdown of lipid peroxidase. It is confirmed that it becomes lesser active in diabetic and other pathological states (21). Indeed, in the present work the treatment group revealed a relatively higher concentration of PON-1 compared to that in the PC group suggesting the effectiveness of berberine in modulating oxidant stress. Also, another in vivo study supported these observations by assessing the levels of PON-1 in two groups of rats; the healthy one and the diabetic one. Upregulation of RALDH2 following post-therapy characterized also by augmentation of the RANECS PON-1 was observed in the rat receiving berberine for three weeks, thus supporting the antioxidative impact of berberine in the amelioration of the oxidative stress in diabetic conditions (22). Results from additional research demonstrated that berberine's enhanced inflammatory response and neovascularization promote wound healing. Infusion of berberine leaves has immunomodulatory and growth-promoting effects in chicks. The infusion of berberine improved growth performance and antibody titer, according to the results. The efficacy of guava and medicinal aloe (also known as true aloe) extracts against food pathogens was reported in a different investigation. The findings showed that certain food-rotting pathogens can be inhibited by the antibacterial chemicals found in these extracts (23). An additional investigation

demonstrated the ethanolic extract of berberine leaves' antiangiogenic capacity against the volume of breast tumors and its ability to prevent the growth of new tumors. By suppressing the production of proangiogenic genes and vascular endothelial factor, it prevents the growth of malignancies (24, 25).

Oxidative stress is the result of the production of reactive oxygen species (ROS) over the local antioxidant capacity. It is the destruction of body cells caused by ROS. The body contains natural molecules that act as a counteractant to the ROS and prevent the body from being destroyed by ROS. The body experiences oxidative stress when the number of antioxidants decreases. Oxidative stress causes the macromolecules in cells, such as DNA, proteins, carbohydrates, and lipids, to break down. The use of herbal plants during therapy had a highly significant impact on stress biomarkers and total antioxidant capacity, according to the results. In a dose-dependent manner, leaf extracts dramatically decreased the formation of granuloma and edema brought on by formaldehyde and carrageenan. Berberine demonstrated the greatest medicinal significance and potent anti-oxidative potential.

CONCLUSION

Berberine demonstrated strong antioxidative potential, indicating its pharmaceutical significance for managing oxidative stress-related conditions. The pharmaceutical research for Berberine based on nano-formulations with various drugs is of great prospects in the field of medical diagnostics in order to enhance the efficacy of these compounds with lower doses and less post-treatment complications.

Conflict of Interest:

None

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