Available online on 15.12.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Immunotherapeutic modification of *Escherichia coli* peritonitis and bacteremia by *Iris kashmiriana baker*

Javeed Iqbal Wagay[1*], Kirti Jain[2]

[*1]Research Scholar, Barkatullah University, Bhopal (MP), India 462026
[2] Department of Botany, Govt. Science and Commerce College, Benazeer, Barkatullah University, Bhopal(MP), India 462026

ABSTRACT

A larger number of medicinal plants and their purified constituents have been shown beneficial therapeutic potentials. We present here the protective effects of an Indian medicinal plant *Iris Kashmiriana* as compared to Ofloxacin in *E. coli* induced peritonitis. *Iris Kashmiriana* is one of an important member of family Iridaceae, locally known as Mazarmund in Kashmir. The plant has been widely used in traditional medicine and modern clinical preparations to treat cold, flu, malaria, toothache, cancer, bacterial, viral infections and bruise. Rats were pre-treated with 200 mg/kg and 400 mg/kg/bwt dose for 3 days and fourth day with *E. coli* (1×10^8 CFU/ml) strain and consecutively 3 days treatment. Mortality was monitored for 14 days. After the death of rats or completion of the experiment rats were sacrifice and kidney were used for our protocol. Colonies were count and statically analysis was done. Results showed dose dependent anti-microbial activity. Pretreatment of mice with *Iris Kashmiriana* improved bacterial clearance as well as improved phagocytic and intracellular bactericidal capacities of neutrophils. In the Ofloxacin treated mice although bacterial clearance was rapid, polymorph phagocytosis was depressed. Thus the results, obtained justify the traditional use of *Iris Kashmiriana*.

Keywords: *Iris kashmiriana, E. coli* induced peritonitis, Neutrophils, Ofloxacin.

Article Info: Received 06 Oct 2018; Review Completed 22 Nov 2018; Accepted 23 Nov 2018; Available online 15 Dec 2018

Cite this article as:

Wagay JI, Jain K, Immunotherapeutic modification of *Escherichia coli* peritonitis and bacteremia by *Iris kashmiriana baker*, Journal of Drug Delivery and Therapeutics. 2018; 8(6-s):1-4
DOI: http://dx.doi.org/10.22270/jddt.v8i6-s.2064

***Address for Correspondence:**

Javeed Iqbal Wagay, Department of Botany, Govt, Science and Commerce College, Benazeer, Barkatullah University, Bhopal, MP

INTRODUCTION

Interest in medicinal plants has burgeoned due to increased efficiency of new plant-derived drugs and the growing interest in natural products. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. The use of plants as medicines dates from the earliest years of man's growth [1, 2]. Medicinal plants serve as therapeutic alternatives, safer choices, or in some cases, as the only effective treatment. People in separate cultures and places are known to have used the same plants for similar medical problems. A larger number of these plants and their isolated constituents have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and immunomodulatory effects[1, 3-9]. Intra-abdominal sepsis continues to be a major cause of morbidity and mortality following trauma and abdominal surgery for bowel perforations[10]. Treatment of this condition has till now been focused on appropriate surgery, supplemented with antimicrobial agents and good nutritional support[11]. An important factor, which influences recovery from any infective process, is the status of the host's defense mechanism[10]. The approach of fortifying the cellular immune functions to increase resistance against infections has only recently been recognized[12]. Several substances, such as glucans, *C. parvum*, BCG and levamisol have been reported to increase resistance to infection by augmenting the immune response[13]. *Iris Kashmiriana* is one of an important member of the family Iridaceae, locally known as Mazarmund in Kashmir. The plant has been widely used in traditional medicine and modern clinical preparations to treat cold, flu, malaria, toothache, cancer, bacterial, viral infections and bruise. The phytochemical analyses of the different extracts of *Iris Kashmiriana* have revealed the presence of different compounds including flavonoids, isoflavonoids, glycosides and tannins. The medicinal importance of the plant prompted isolation of a variety of pharmacologically active compounds including quinones, triterpenoids, flavonoids, isoflavonoids and stilbene glycosides[14]. In view of the immunomodulation activities of *Iris Kashmiriana*, we undertook studies to initially evaluate the effect of *Iris Kashmiriana* upon survival of mice with *E.*

coli peritonitis in comparison with a standard antimicrobial agent, Ofloxacin. Further studies to investigate the mechanism of protective effects against *E. coli* sepsis were undertaken. These induced bacterial clearance studies and evaluation of polymorphonuclear functions.

MATERIALS AND METHODS

Plant material

The rhizome of Iris Kashmiriana was collected from district Bandipora of Jammu and Kashmir region. Herbarium of plant was prepared and submitted to Dr. Akhtar H. Malik, Curator, Centre of Biodiversity & Taxonomy, Department of Botany, University of Kashmir for authentication. Plant authentication voucher numbers obtained was 2625 for *Iris Kashmiriana*. Rhizome selected for the study was washed thoroughly under running tap water and then was rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the rhizome was shade dried without any contamination for about 3 to 4 weeks. Dried rhizome was grinded using electronic grinder. Powdered plant material was observed for their colour, odour and texture. Dried material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade. The test organisms *Escherichia coli* (*MTCC* 2075) were obtained from the stocks of the Pinnacle Biomedical Research Institute, Bharat scout guide bhawan, shyamla hills, Bhopal, (M.P.).

Extraction

Extraction was performed using continuous hot percolation soxhlation. Dried pulverized parts of *Iris Kashmiriana* were placed in thimble of soxhlet apparatus. Soxhlation was performed at 60°C using chloroform as non polar solvent at first. Exhausted plant material (mark) was dried and afterward extracted with ethyl acetate and methanol. Each solvent soxhlation was continued till no colour was observed in siphon tube. For confirmation of exhausted plant marc (i.e. completion of extraction) colourless solvent was collected from siphon tube and evaporated for residue. Absence of residual confirmed the completion of extraction. Obtained extracts were evaporated and using rotary vacuum evaporator (Buchi type) at 40°C. Dried extract was weighed and finally the percentage yields were calculated of the dried extracts.

In vivo study

Animals

All ethical and handling guidelines were followed as set by Indian Legislation and approved by Institutional Animal Ethics Committee. All animals were procured and housed in animal house maintained under standard hygienic conditions. All animals were given standard diet (Golden Feed, New Delhi) and water regularly. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal.

(Reg No. 1824/PO/ERe/S/15/CPCSEA). Protocol approval reference no. PBRI/IAEC/PN-17046.

Acute oral toxicity

The acute toxic class method set out in guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight[15].

Treatment

Animals were housed in a group of six in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (Golden feed, New Delhi) and water, *ad libitum.* The environment was also regulated at 25 ± 1 0C with 12/12 h (light/dark) cycle. Animals were further divided in five groups with six animals in each group.

Group I: **Normal control:** Normal saline were administrated by oral route at a dose of 5ml/kg body weight.
Group II: **Vehicle treated with *Escherichia coli* treated group:** *Escherichia coli* (1×10^8 CFU/ml) were dissolved in normal saline and were administrated by oral route at a dose of 10ml/kg body weight.
Group III: **Standard drug treated group:** Ofloxacin was dissolved in normal saline and was administrated by oral route at a dose of 5m g/kg body weight
Group IV: **200 mg/kg methanolic extract treated group:** Extract was dissolved in normal saline and was administrated by oral route at a dose of 200m g/kg body weight.
Group V: **400 mg/kg methanolic extract treated group:** Extract were dissolved in normal saline and was administrated by oral route at a dose of 400mg/kg body weight.

Preparation for bacterial inoculums

In brief, *E. coli* strain (MTCC 2075) were grown on nutrient broth medium (3 g of beef extract, 5 g of peptone and 5 g of NaCl, pH 7, sterilized by autoclaving at 120°C for 30 min.) from a single colony and incubated at 37°C for 16-18 h to obtain stationary growth phase cultures. The bacteria were then centrifuged (200 rpm) for 10 min at 4°C and the pellets were resuspended in PBS to an OD of 0.1 at 660 nm, with a spectrophotometer, corresponding to 10^8 CFU/ml[16].

Systemic infection by E. coli

To produce infection, the rats were induced by the intra-peritoneal with suitable inoculums in a volume of 0.2 to 0.25 ml. After infection, the rats were observed twice daily and animals exhibiting profound inanition or an inability to reach food and water were sacrificed. The experimental design involved administration of each of the three test agents by daily oral dosing for a period of 14 days. dosing regimens were started on days -7,-6, -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, 6, 7 and relative to the day of challenge (day 0) with 1×10^8 CFU of *E. coli*/ml. Before and after the challenged day animals were treated with 200 mg/kg body weight and

400 mg/kg body weight *Iris Kashmiriana* extract and 5 mg/kg body weight Ofloxacin respectively. Survival was monitored for all experimental groups till 14 day. These conditions were in accordance with those of previously described method [17], with slight modification. The pathological status of the rat was determined by visual examination of internal organs after their death or sacrifice at the completion of the experiment. All surviving rats were killed by cervical dislocation on 15 day determination of the numbers of CFU of *E. coli* per gram from the kidney[18, 19]. This determination was made by aseptically removing and weighing both kidneys, homogenizing kidneys in w/v ml of saline with a high speed Homogenizer (Remi RQ-124A) and Kidney burden was determined by culturing of homogenates in physiological saline followed by plating 0.1 ml aliquots onto Nutrient agar plates. The plates were incubated at 37°C and the number of colonies was enumerated after 48 h of growth[20]. All animal care procedures were supervised and approved by the Institutional Animals Ethics Committee (IAEC) of PBRI, Bhopal.

Bacterial clearance

E. coli was determined in the different groups by sampling retro blood at various intervals after 16 hrs and up to 14 days (at which time maximum mortality will be recorded). The sample was serially diluted and plated on nutrient agar media. Incubate it at 24 hours at 37°C temperature. After incubation colony count was determine by colony counter method.

Neutrophiles function test

The rats were pre-treated orally with vehicle or extracts for 14 days. At the end of treatment day 14, blood samples were collected from the retro-orbital plexus into heparinized vials and analyzed for differential leukocyte count (DLC). After the initial counts, blood samples were incubated with 80 mg nylon fibres/ml for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC, respectively to give neutrophil index of blood samples. The percent neutrophil adhesion was calculated as follows:

$$\text{Neutrophil adhesion \%} = NIu - NIt \times 100 / NIu$$

Where, NIu is the neutrophil index of untreated blood samples and

NIt is the neutrophil index of treated blood samples.

Sample harvesting

After the monitoring of mortality rats were sacrificed after infection, at this time rats were anesthetized by inhalation of diethyl ether and peritoneal cavity was washed with 5 ml of sterile PBS saline by using an 18-gauge needle and peritoneal lavage fluid was collected in sterile tubes for determination of CFU[21].

Determination of CFU

Fifty microliters of peritoneal lavage fluid from each Rat were placed on ice and serially diluted with sterile saline. Twelve microliters of each dilution were spread on sterile nutrient agar plates and incubated overnight at 37°C after which the number of colonies was counted

RESULTS AND DISCUSSIONS

In previous paper phytochemical analysis, quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoids content (TFC) result showed that methanolic extract of *Iris Kashmiriana* has highest methanolic extractive percentage compare to other extracts. In the acute toxicity study, no signs of toxicity were found up to the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 200 mg/kg and 400 mg/kg have been fixed as ED50 for present study Table 1.

Table 1: Acute oral toxicity of methanolic extract

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg Bodyweight	0/3
2.	50 mg/kg Bodyweight	0/3
3.	300 mg/kg Bodyweight	0/3
4.	2000 mg/kg Bodyweight	0/3

In-vivo antimicrobial activity was tested by inducing peritonitis through *E. coli*. The activity of the extract was calculated by measuring the CFU/ml of the microorganisms. The experiment was done in groups of 5 containing control, vehicle, standard (5mg/kg), extract (200 mg/kg) and extract (400 mg/kg). The results obtained indicated a dose dependent antimicrobial activity of the extracts; the extract given at a concentration of 400 mg/kg had better activity than the one administered at 200 mg/kg Table 2& Fig. 1.

Table 2: *In-Vivo* antimicrobial activity in *E. coli* induced peritonitis

S. No.	Groups	CFU/ml
1.	**Normal Control**	105.50±5.089
2.	**Vehicle control**	277.67±6.532
3.	**Standard (5 mg/kg BW)**	80.00±6.753
4.	**Extract (200 mg/kg BW)**	201.50±7.369
5.	**Extract (400 mg/kg BW)**	128.33±13.155

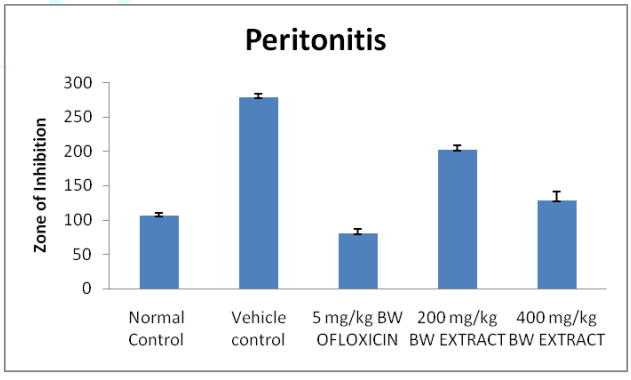

Figure 1: *E. coli* induced peritonitis

Neutrophil adhesion test is an indication of the marginalization of phagocytic cells in the blood vessels, i.e. an indication of immunostimulation. Increase in % neutrophil adhesion is attributed to marginalization of phagocytic cells which reflects improvement in defensive response under normal circumstances Table 3.

Table 3: Neutrophiles function test

S. No.	Groups	Neutrophiles %		Neutrophiles Adhesion
		Untreated	Treated	
1.	Normal Control	25.60±1.416	23.91±0.774	17.84±5.752
2.	Vehicle Control	24.34±0.934	23.91±0.866	6.01±0.974
3.	Standard (5 mg/kg BW)	27.04±3.996	24.38±0.861	30.25±8.113
4.	Extract (200 mg/kg BW)	24.53±0.903	23.81±0.824	27.97±4.597
5.	Extract (400 mg/kg BW)	26.60±1.157	21.25±1.061	29.65±2.014

CONCLUSION

The present study demonstrates clearly that pretreatment with *Iris Kashmiriana* protect mice against mortality due to induced *E. coli* sepsis and this is comparable to Ofloxacin. This is associated with a rapid clearance of bacteremia, mediated probably through a stimulation of phagocytic and bacterial function of polymorphs. It appears therefore that activation of neutrophils by *Iris Kashmiriana* leads to a rapid bacterial clearance thus affording protection against *E. coli* induced peritonitis and morality. Extrapolation of animal data to a clinical setting is not always appropriate. However, the results obtained with *Iris Kashmiriana* in this study coupled with its relative lack of toxicity and oral efficacy suggests that it may prove to be useful therapeutic modality in patients with abdominal sepsis. The observed potential can be attributed to the presence of phenolic compounds and flavonoids present in plant. Further detailed study is required to pinpoint the exact mechanism and active principle involved in it.

REFERENCES

1. Dattner AM. From medical herbalism to phytotherapy in dermatology: back to the future, Dermatologic Therapy, 2003; 16:106-113.
2. Fong HH. Integration of herbal medicine into modern medical practices: issues and prospects, Integrative Cancer Therapies, 2002; 1:287-293.
3. Huffman MA. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants, Proceedings of the Nutrition Society, 2003; 62:371-381.
4. Miller KL, Liebowitz RS, Newby LK. Complementary and alternative medicine in cardiovascular disease: a review of biologically based approaches, American Heart Journal, 2004; 147:401-411.
5. Thatte UM, Rege NN, Phatak SD, Dahanukar SA. The flip side of Ayurveda, Journal of Postgraduate Medicine, 1993; 39:179-82, 182a–b.
6. Thatte UM, Kulkarni MR, Dahanukar SA. Immunotherapeutic modification of Escherichia coli peritonitis and bacteremia by *Tinospora cordifolia*, Journal of Postgraduate Medicine, 1992; 38:13-15.
7. Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine, Phytotherapy Research, 1999; 13:275-291.
8. Parab S, Kulkarni R, Thatte U. Heavy metals in dherbalT medicines, Indian Journal of Gastroenterology, 2003; 22:111-12.
9. Salem ML, Hossain MS. Protective effect of black seed oil from Nigella sativa against murine cytomegalovirus infection, International Journal of Immunopharmacology, 2000;22(9):729-740.
10. Alexander JW. The role of host defense mechanism in surgical infections, Surgical Clinics of North America, 1980; 60:107-108
11. Condon RE, Malangoni MA. Peritonitis and intra- abdominal abscesses. In: 'Principles of Surgery'. SI Schwartz, GT Shires, FC Spencer, EH Storer, editors. 4th edition. Singapore: McGraw Hill; 1984, pp 1391-1491
12. Hadden JW. Immunomodulators in the immunotherapy of cancer and other diseases, Trends in Pharmacology Sciences, 1982; 3:191-194
13. Drews J. Immunomodulation. In: Recent advances in infection, Vol. 2. DS Reeves, AM Geddes, editors. New York: Churchill Livingstone; 1982, pp 89-100.
14. Koka JA, Wani AH, Bhat MY, Wani TA, Parveen S. Antimycotic activity of ethanolic and aqueous leaf extracts of Ajuga bracteosa Wall. ex Benth. (Lamiale: Lamiaceae) and Iris kashmiriana Baker (Asparagales: Iridaceae) against some vegetable rot fungi, Brazilian Journal of Biological Sciences, 2018; 5(9):75-84.
15. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.
16. Teixeira-da-Cunha MG, Gomes RN, Roehrs N, Bozza FA, Prescott SM, Stafforini D et al. Bacterial clearance is improved in septic mice by platelet-activating factor-acetylhydrolase (PAF-AH) administration, PloS one, 2013; 8(9):e74567.
17. Ikeda F, Wakai Y, Matsumoto SS, Maki K, Watabe E, Tawara S et al. Efficacy of FK463, a new lipopeptide antifungal agent, in mouse models of disseminated candidiasis and aspergillosis, Antimicrobial Agents and Chemotherapy, 2000; 44:614-618.
18. MacCallum DM, Odds FC. Need for early antifungal treatment confirmed in experimental disseminated candida albicans infection, Antimicrobial Agents and Chemotherapy, 2004; 48(12):4911- 4914.
19. Rex JH, Nelson PW, Paetznick VL, Lozano-Chiu M, Espinel-Ingroff A, Anaissie EJ. Optimizing the correlation between results of testing in vitro and therapeutic outcome in vivo for fluconazole by testing critical isolates in a murine model of invasive candidiasis, Antimicrobial Agents and Chemotherapy, 1998; 42(1): 129-134.
20. Arthington-Skaggs BA, Warnock DW, Morrison CJ. Quantitation of candida albicansergosterol content improves the correlation between in vitro antifungal susceptibility test results and in vivo outcome after fluconazole treatment in a murine model of invasive candidiasis, Antimicrobial Agents and Chemotherapy, 2000; 44(8):2081-2085.
21. Wilkinson PC. Neutrophil Adhesion Test. In Vanejr, Ferraria, S.H., (eds). Handbook of Experimental Pharmacology, Vol. 1, Springer Verlag, Berlin, 1978:109.