Antileishmanial efficacy of *Boerhaavia diffusa* L. and *Ocimum sanctum* L. against experimental visceral leishmaniasis

Kaur S*, Bhardwaj K & Sachdeva H

Parasitology Laboratory, Department of Zoology, Panjab University, Chandigarh-160 014, India

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The chemotherapy of visceral leishmaniasis (VL) has several limitations including resistance and toxicity of the existing drugs. Down regulation of immune system further aggravates the problems. To combat this situation we evaluated the leishmanicidal efficacy of *Boerhaavia diffusa* and *Ocimum sanctum* through oral route in *L. donovani* infection in BALB/c mice. Results have demonstrated maximum clearance of the parasites from infected animals treated with combination of *B. diffusa* and *O. sanctum* (@ 100 and 400 mg/kg body wt., respectively 5 days) as depicted through Leishman Donovan Units in liver. Up-regulation of cell-mediated immunity was also observed in animals of this group as heightened delayed type hypersensitivity responses and increased IgG2a levels were observed. Moreover, increased levels of SGOT, SGPT, serum urea, blood urea nitrogen and serum creatinine were brought down to normal levels. Since VL is associated immunosuppression, the above treatment is a good option as it helps in the up-regulation of Th1 responses and reduction in parasite load in *L. donovani* infected mice. These findings suggest a new option for antileishmanial chemotherapy at lower cost and nil toxicity.

**Keywords**: Black fever, Dumdum fever, Folk medicine, Herbal, Holy basil, Indian medicine, Kala-azar Punarnava, Red spiderling, Santhi, Tarvine, Thulasi

Visceral leishmaniasis (VL) is one of the most important infectious diseases caused by the parasite *Leishmania donovani* and is fatal if left untreated. The currently available drugs for the treatment of VL have several limitations\(^1\). The intensity of VL is associated with immunological dysfunctions of T cells, natural killer cells and macrophages and as a result the treatment of VL is compromised. Therefore, an antileishmanial drug that can effectively and quickly reverse the immunosuppression of the infected host, besides killing the parasite, is desirable\(^2\). So, there is a need to evaluate the drugs which are totally safe, and have ability to modulate humoral immune responses by antibody production; and release of the mediators of hypersensitivity reactions. Medicinal plants play significant roles in the protecting human beings from various pathogenic microorganisms and diseases\(^3\). Many plants and their products have been exploited for modulation of immune system in a number of ayurvedic formulations either alone or in groups\(^4,5\).

Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases. The presence of alkaloids, carbohydrates, tannins, phenolic compounds and saponins, glycosides in herbal plants have been reported to have immunomodulatory activities\(^6\). They are helpful in the development of a Th1 phenotype, activation of macrophages and CD4 and T lymphocytes for the better clearance of parasites\(^7\).

*Boerhaavia diffusa* L. (Nyctaginaceae), commonly called Red spiderling or Tarvine, locally, Punarnava, is an important medicinal plant used in traditional medicines in many parts of the world\(^8\). *B. diffusa* contains large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins and glycoproteins apart from boeravinone, caffeoyltartaric acid, hypoxanthine-9-L-arabinofuranoside, liirodendrin, potassium nitrate, punarnavine, punarnavoside and ursolic acid\(^9,10\).

The root, leaves, aerial parts or the whole plant of *B. diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine. *B. diffusa* is used for the treatment of many diseases, such as abdominal pain, anaemia, anthelmintic,
Antibacterial, anticarcinogenic, anti-inflammatory, antineutodatal, antihypertotoxy, blood impurities, enlargement of spleen, jaundice, and also act as an immunomodulator. Intraperitoneal administration of *B. diffusa* extract @ 40 mg/kg body wt. in BALB/c mice and enhanced the total WBC count, bone marrow cellularity, circulating antibody titer and the number of plaque forming cells (PFC) in the spleen. It also showed enhanced proliferation of splenocytes, thymocytes and bone marrow cells. It did significantly reduce the lipopolysaccharide (LPS) induced elevated levels of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 in mice demonstrating the immunomodulatory activity of *B. diffusa*.

*Ocimum sanctum* L. commonly known as Holy Basil or sacred thulasi, belongs to family Lamiaceae, a medicinal herb used in the indigenous system of medicine. It has been adored in ancient ayurvedic texts for its extraordinary medicinal properties. The leaves of *O. sanctum* contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. Fresh leaves and stem of *O. sanctum* extract contains some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugenol. Apart from the two flavonoids, orientin and vicenin, apigenin-7-O-glucuronide, luteolin, luteolin-7-O-glucuronide, molludustin, orientin and Ursolic acid have also been isolated from the leaf extract of *O. sanctum*. Further, it contains a number of sesquiterpenes and monoterpenes viz., bornyl acetate, campesterol, cholesterol, stigmasteryl. The roots, leaves and seeds of *O. sanctum* possess several medicinal properties such as antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic, analgesic, antiinflammatory, antiallergic, antihypertensive, cardioprotective, antihypercholesterolaemic, hepatoprotective, antidiabetic, antiasthmatic, antioxidant, anticancer, chemopreventive, radioprotective and immunomodulatory.

*Ocimum sanctum* orally at the doses of 50, 100 and 200 mg/kg/day for 14 days to healthy albino Swiss mice demonstrated a stimulatory effect on delayed type hypersensitivity (DTH) and improved humoral immunity. Foot volume was enhanced after *O. sanctum* treatment suggesting cell mediated immune response enhancement. *O. sanctum* seeds have been proved to modulate both humoral and cell mediated responsiveness in both non-stressed and stressed experimental animals and mechanism behind these immunomodulatory properties could be due to their activity on GABAergic pathways.

In the present study, we explored whether *B. diffusa* and *O. sanctum* can reverse the immunosuppression caused during *L. donovani* infection in BALB/c mice.

**Materials and Methods**

**Parasite**—*Leishmania donovani* promastigotes of strain MHOM/IN/80/Dd8, originally obtained from the London School of Tropical Hygiene and Medicine, London, was used for the present study and maintained in vitro at 22±1°C in modified Novy, McNeal and Nicolle’s (NNN) medium by serial subcultures after every 48-72 h.

**Animals**—Inbred BALB/c mice of either sex, 4-6 wk old, weighing 20-25 g were procured from the CSIR-Institute of Microbial Technology, Chandigarh, India and prior approval for their use was obtained from the Institutional Animal Ethics Committee, Panjab University, Chandigarh. The animals were kept under standard conditions of temperature (25±2°C), fed with standard pellet diet and water ad libitum.

**Drugs**—Drugs, *Boerhaavia diffusa* and *Ocimum sanctum* (as whole plant extracts in the form of capsules) were purchased from the Himalaya Drugs company, Bangalore, India. These were dissolved in distilled water to get the required concentration as per body weight of mice.

**Experimental design**—Inbred BALB/c mice were infected with 10⁷ promastigotes of *L. donovani* and left for one month. A month after infection, the animals were administered with different doses of drugs orally for 15 days daily. For experimental purpose, the mice were grouped as follows: Group I, Normal without infection; Group II, Infected with *L. donovani* promastigotes; Group III, infected + *B. diffusa* (100 mg/kg body wt.); Group IV, Infected + *O. sanctum* (400 mg/kg body wt.); Group V, Infected + *B. diffusa* (50 mg/kg) + *O. sanctum* (200 mg/kg body wt.); and Group VI, Infected + *B. diffusa* (100 mg/kg, + *O. sanctum* (400 mg/kg body wt.). The animals were sacrificed on 0, 7 and 15 post infection/treatment days (p.i.d./p.t.d.). The protective efficacy of the drugs was tested by the assessment of hepatic parasite burden and the generation of humoral and cellular immune responses. The effect of drugs was also tested on the haematological
and biochemical parameters to see the toxicity, if any, caused by the drugs.

Assessment of infection—Mice from each group were sacrificed after 0, 7 and 15 post infection and post treatment days. Parasite load was assessed as Leishman Donovan Units by the method of Bradley and Kirkley. Delayed type hypersensitivity (DTH) responses—DTH was determined as an index of cell mediated immune responses. All groups of mice were challenged in the right foot pad with a subcutaneous injection of leishmanin. The control paw received equal volume of PBS only. After 48 h, the thickness of the right and left foot pad was measured using a pair of vernier callipers. The percentage increase in the thickness of the right foot pad as compared to the left foot pad was calculated.

Assay of parasite-specific IgG1 and IgG2a isotypes by ELISA—The serum specific immunoglobulin G (IgG) isotype antibody responses were measured by conventional enzyme-linked immunosorbant assay (ELISA). Dynatech 96-well ELISA plates were coated with crude antigen of L. donovani at a concentration of 200ng/well. Sera from mice of all the groups were then added at two fold serial dilutions, followed by washes and addition of isotype specific HRP-conjugated secondary antibodies (rabbit anti-mouse IgG1 or IgG2a) after which the substrate and chromogen were added and absorbance was read on by ELISA plate reader at 450 nm.

Evaluation of haematological parameters—The blood of mice was collected and Hb estimation was done by Sahli's haemometer method. The estimation of TLC was done using a haemocytometer. Blood was diluted in diluting fluid in the ratio of 1:20 in glass pipette. The contents were mixed for at least 1 min. About 10 μl of this mixture was placed on Neubauer's counting chamber and leukocytes were counted in the four corner areas containing 16 squares each. TLC per liter was calculated by the following formula:

\[
\text{No of leukocytes counted} \times \text{dilution factor} \times \text{Area counted} \times \text{depth of fluid} \times 106.
\]

Liver function tests—Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), bilirubin and alkaline phosphatase were estimated in serum samples of all the groups of mice by using commercially available kits (Kinetik Koncepts, Gurgaon, India).

Kidney function tests—Urea, blood urea nitrogen (BUN) and creatinine were estimated in serum samples of all the groups of mice by using commercially available kits (Reckon Diagnostics Pvt. Ltd, Baroda, India; Coral Clinical Systems, Goa, India).

Statistical analysis—The student's t-test was performed to calculate P value by employing Graph pad software.

Results

Parasite Load—The results have demonstrated that administration of B. diffusa alone @ 100 mg/kg body wt. (Group III) and O. sanctum alone @ 400 mg/kg body wt. (Group IV) 55.2 % and 67.22 % reduction in the hepatic parasite load, respectively in L. donovani infected BALB/c mice. Percentage of hepatic parasite load further decreased to 76.7% in low dose combination in Group V (infected and B. diffusa 50 mg/kg body wt. and O. sanctum 200 mg/kg body wt.). However, a maximum decrease in hepatic parasite load (79.1%) was observed in Group VI that were treated with high dose combination (infected and B. diffusa 100 mg/kg body wt. and O. sanctum 400 mg/kg body wt.) (Fig. 1).

Delayed type hypersensitivity responses (DTH)—The DTH responses were determined as an index of cell mediated immune responses. Progressive infection is associated with poor delayed-type hypersensitivity (DTH), whereas containment is associated with a strong cell-mediated DTH response. In the current study profound development of DTH response was found in the groups III and IV which were treated with B. diffusa (100 mg/kg) and O. sanctum (400 mg/kg) alone and in low dose combination with O. sanctum (200 mg/kg body wt.) and B. diffusa (50 mg/kg body wt.). The progressive infection in these two combinations was associated with a strong delayed-type hypersensitivity (DTH) response. The infection was contained in group V and VI which were treated with high dose combination (infected and B. diffusa 100 mg/kg body wt. and O. sanctum 400 mg/kg body wt.). The DTH response was found to be strong in group VI which were treated with high dose combination (infected and B. diffusa 100 mg/kg body wt. and O. sanctum 400 mg/kg body wt.). Therefore, the combination therapy is more effective than the monotherapy in containing the infection.

![Fig. 1](image_url)—Parasite load in different groups of BALB/c mice. [Group II, Infected with L. donovani promastigotes; Group III, infected + B. diffusa (100 mg/kg body wt.); Group IV, Infected + O. sanctum (400 mg/kg body wt.); Group V, Infected + B. diffusa (50 mg/kg) + O. sanctum (200 mg/kg body wt.); and Group VI, Infected + B. diffusa (100 mg/kg) + O. sanctum (400 mg/kg body wt.). Group I is normal, hence not given here.]
combination group V (infected and *B. diffusa* 50 mg/kg and *O. sanctum* 200 mg/kg). However, highest DTH induction was noticed in high dose combination group VI (infected and *B. diffusa* 100 mg/kg and *O. sanctum* 400 mg/kg). In contrast, the infected mice failed to mount DTH responses, which coincide with the progression of the disease (Fig. 2).

*Parasite-specific IgG1 and IgG2a isotype levels*—To evaluate the humoral immune responses induced by the drugs, the serum specific levels of antileishmanial antigen specific IgG isotypes, IgG1 and IgG2a from all the groups of mice were assessed. *L. donovani* infected BALB/c mice produced IgG1 antibodies than IgG2a. However, following treatment with *B. diffusa* (Gr. III) and *O. sanctum* (Gr. IV) alone and in low/high dose combination (Gr. V/VI) in infected mice, higher levels of IgG2a over IgG1 were found. The effective stimulation of IgG2a isotype is associated with the presence of antileishmanial Th1 cells after drug treatment, and it suggests the role of protective immunity stimulated by the drugs (Figs 3 A and B).

*Haematological parameters*—In our study here, *L. donovani* infection in mice led to a transient decline in the concentration of Hb. However, infected mice after treatment with *B. diffusa* and *O. sanctum* alone and in low/high dose combination showed reversal of Hb concentration to normal levels. As regard to the total leucocyte count, all infected mice treated with both drugs alone and in low/high dose combination showed significant normal levels as compared to the infected mice (Table 1).

![Fig. 2.](image) Delayed type hypersensitivity responses in different groups of BALB/c mice. [Group I, Normal; Group II, Infected; Group III, infected + *B. diffusa* (100 mg/kg body wt.); Group IV, Infected + *O. sanctum* (400 mg/kg body wt.); Group V, Infected + *B. diffusa* (50 mg/kg) + *O. sanctum* (200 mg/kg body wt.); and Group VI, Infected + *B. diffusa* (100 mg/kg.) + *O. sanctum* (400 mg/kg body wt.).] *P* value: Normal vs. Infected. $^aP <0.0001$

![Fig. 3.](image) (A) IgG1; and (B) IgG2a levels in different groups of BALB/c mice. [Group I, Normal; Group II, Infected; Group III, infected + *B. diffusa* (100 mg/kg body wt.); Group IV, Infected + *O. sanctum* (400 mg/kg body wt.); Group V, Infected + *B. diffusa* (50 mg/kg) + *O. sanctum* (200 mg/kg body wt.); and Group VI, Infected + *B. diffusa* (100 mg/kg.) + *O. sanctum* (400 mg/kg body wt.).] *P* value: Normal vs. Infected. $^aP <0.0001$

### Table 1. Haematological analysis in various groups of BALB/c mice

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Hb Concentration (gm/dl)$^a$</th>
<th>Total leucocyte count (per mm$^3$)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 p.t.d.</td>
<td>7 p.t.d.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>10.30±0.37</td>
<td>10.63±0.52</td>
</tr>
<tr>
<td></td>
<td>7.41±0.40$^5$</td>
<td>8.08±0.56$^5$</td>
</tr>
<tr>
<td>Infected + <em>B. diffusa</em> (100 mg/kg body wt.)</td>
<td>10.92±0.56$^5$</td>
<td>11.10±0.35$^5$</td>
</tr>
<tr>
<td>Infected + <em>O. sanctum</em> (400 mg/kg body wt.)</td>
<td>10.54±0.29$^5$</td>
<td>12.33±0.45$^5$</td>
</tr>
<tr>
<td>Infected + <em>B. diffusa</em> (50 mg/kg)+ <em>O. sanctum</em> (200 mg/kg body wt.)</td>
<td>9.82±0.55$^3$</td>
<td>11.31±0.37$^5$</td>
</tr>
<tr>
<td>Infected + <em>B. diffusa</em> (100 mg/kg)+ <em>O. sanctum</em> (400 mg/kg body wt.)</td>
<td>11.13±0.42$^5$</td>
<td>11.82±0.59$^5$</td>
</tr>
</tbody>
</table>

$^a$Concentration of haemoglobin in various groups of BALB/c mice; $^b$Total leucocyte count in various groups of BALB/c mice. p.t.d. = post treatment days. *P* value: $^aP <0.0001$
### Table 2—Liver function tests in various groups of BALB/c mice

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>SGOT Concentration (U/L)*</th>
<th>SGPT Concentration (U/L)*</th>
<th>ALP Concentration (KA)*</th>
<th>BILIRUBIN Concentration (mg/dl)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 p.t.d.</td>
<td>7 p.t.d.</td>
<td>15 p.t.d.</td>
<td>0 p.t.d.</td>
</tr>
<tr>
<td>Normal</td>
<td>34.96±0.34</td>
<td>36.66±0.75</td>
<td>38.71±0.74</td>
<td>28.49±0.35</td>
</tr>
<tr>
<td>Infected</td>
<td>44.12±1.60</td>
<td>46.63±1.62</td>
<td>51.24±1.32</td>
<td>43.55±0.83</td>
</tr>
<tr>
<td>Infected+B. diffusa (100 mg/kg body wt.)</td>
<td>33.53±0.87</td>
<td>36.73±0.57</td>
<td>38.58±1.12</td>
<td>33.87±0.87</td>
</tr>
<tr>
<td>Infected+O. sanctum (400 mg/kg body wt.)</td>
<td>31.95±0.85</td>
<td>34.82±0.94</td>
<td>37.14±0.73</td>
<td>31.98±0.85</td>
</tr>
<tr>
<td>Infected+B. diffusa (50 mg/kg)+O. sanctum (200 mg/kg body wt.)</td>
<td>22.26±0.79</td>
<td>28.94±0.35</td>
<td>34.82±1.43</td>
<td>30.21±0.43</td>
</tr>
<tr>
<td>Infected+B. diffusa (100 mg/kg)+O. sanctum (400 mg/kg body wt.)</td>
<td>22.91±0.78</td>
<td>29.10±0.47</td>
<td>37.27±1.52</td>
<td>29.76±0.93</td>
</tr>
</tbody>
</table>

*Concentration of SGOT; †Concentration of SGPT; ‡Concentration of ALP; ††Concentration of BILIRUBIN in various groups of BALB/c mice. p.t.d. = post treatment days. P value: *P < 0.0001

### Table 3—Kidney function tests in various groups of BALB/c mice.

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Serum creatinine (mg/dl)a</th>
<th>Blood urea concentration (mg/dl)b</th>
<th>Blood urea nitrogen concentration (mg/dl)c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal range 0.8-1.35</td>
<td>Normal range 10-45</td>
<td>Normal range 5-21</td>
</tr>
<tr>
<td></td>
<td>0 p.t.d.</td>
<td>7 p.t.d.</td>
<td>15 p.t.d.</td>
</tr>
<tr>
<td>Normal</td>
<td>0.73±0.16</td>
<td>1.03±0.12</td>
<td>1.21±0.02</td>
</tr>
<tr>
<td>Infected</td>
<td>1.43±0.12</td>
<td>1.39±0.17</td>
<td>1.66±0.35</td>
</tr>
<tr>
<td>Infected+B. diffusa (100 mg/kg body wt.)</td>
<td>0.76±0.12</td>
<td>1.26±0.17</td>
<td>1.34±0.04</td>
</tr>
<tr>
<td>Infected+O. sanctum (400 mg/kg body wt.)</td>
<td>0.90±0.08</td>
<td>1.26±0.28</td>
<td>1.32±0.40</td>
</tr>
<tr>
<td>Infected+B. diffusa (50 mg/kg)+O. sanctum (200 mg/kg body wt.)</td>
<td>0.66±0.16</td>
<td>1.23±0.12</td>
<td>1.35±0.26</td>
</tr>
<tr>
<td>Infected+B. diffusa (100 mg/kg)+O. sanctum (400 mg/kg body wt.)</td>
<td>0.69±0.28</td>
<td>1.28±0.25</td>
<td>1.30±0.30</td>
</tr>
</tbody>
</table>

*aConcentration of serum creatinine; †serum urea; ‡serum BUN in various groups of BALB/c mice. p.t.d. = post treatment days. P value: *P < 0.0001
Liver function tests—The infected groups exhibited liver dysfunction with increased levels of SGOT, SGPT and serum bilirubin. However, administration of whole plant extract alone (Groups III & IV) and in low dose combination (Group V) it attenuated and resulted in normal liver function tests. Group VI that had high dose combination of B. diffusa and O. sanctum did not reveal any disturbance in liver function tests, thus, suggesting the safety of these drugs at higher doses. It was also found that alkaline phosphatase remained in normal range in all the groups of animals (Table 2).

Kidney function tests—Urea, BUN and creatinine in serum samples of mice from all the groups were assessed as markers of kidney function tests. In L. donovani infected BALB/c mice, all these levels were found exaggerated. However, treatment with B. diffusa and O. sanctum alone and in low/high dose combination afforded normal kidney function (Table 3).

Discussion

In an endeavor to find new orally active and affordable antileishmanial drug, we evaluated the potential of B. diffusa and O. sanctum (as whole plant extracts) against experimental visceral leishmaniasis. Our study performed in mouse model clearly showed that these whole plant extracts are highly active against L. donovani infected BALB/c mice. Infected animals treated with B. diffusa (50 mg/kg body wt.) and O. sanctum (200 mg/kg body wt.) (Gr. V), and in combination showed decreased percentage of parasites in liver. The maximum decrease was achieved by high dose combination of B. diffusa (100 mg/kg) and O. sanctum (400 mg/kg) (Gr. VI) followed by low dose combination (Gr. V).

A major factor of immune mechanism is the development of cell mediated immune response like delayed type hypersensitivity, which is responsible for protection, and is also contributes to healing in visceral leishmaniasis. We further assessed the delayed type hypersensitivity (DTH) responses in the experimental groups of mice to see whether B. diffusa and O. sanctum alone and in combination were able to generate immune response in infected mice. From the results, it was evident that B. diffusa and O. sanctum alone and in combination treated infected mice displayed significant heightened DTH responses with maximum responses in high dose combination group (Gr. VI). On the other hand, DTH response was impeded in the infected mice (Gr. II).

As the outcomes of visceral leishmaniasis infection may also be arbitrated by the extent of immune system modulation, it is also highly important to characterize the changes in the immunoglobulin levels before and after the drug treatment. Outcomes from the present study showed that the antileishmanial IgG1, a surrogate marker of the Th2 cell differentiation, was elevated progressively in L. donovani infected BALB/c mice. In contrast, B. diffusa and O. sanctum alone and in combination treated L. donovani infected mice showed significant elevation in IgG2a, a surrogate marker for Th1 type of immune response. As a measure of cell mediated immunity, elevation of IgG2a was consistent with the development of the effective immune response. Thus, a significant increase in IgG2a isotype levels in B. diffusa and O. sanctum treated infected mice (Gr. III-VI) as compared to only infected mice (Gr. II) suggests the immunomodulatory role played by the drugs in the induction of protective immunity in murine model of visceral leishmaniasis.

Our results are in accordance with earlier reports where alkaloidal fraction of B. diffusa was studied for its effect on cellular and humoral functions in mice. It was found that oral administration of the alkaloid fraction (25–100 mg/kg) significantly increased the antibody titre and elevated the levels of cytokines such as TNF-α, IL-2 and IL-6 in mice. The comparative evaluation of both alcoholic and aqueous extracts of O. sanctum at various dose levels in albino Swiss mice showed that aqueous extract of O. sanctum @ 200 mg/kg body wt. showed maximum increase in the antibody production.

Regarding haematological parameters, there was no significant difference in the haematological composition of the blood parameters between the normal control group and B. diffusa and O. sanctum alone and in combination treated infected group. Moreover, in infected mice treated with B. diffusa and O. sanctum, the haemoglobin concentration was significantly higher as compared to infected group. The total leucocyte count was found to be significantly higher in infected group but after treatment with B. diffusa and O. sanctum the total leucocyte count was decreased in and attained normal levels. Thus, this study demonstrated that B. diffusa and O. sanctum were helpful in the restoration of haematological parameters within the normal range. Supportive evidence for the confirmation of the our above mentioned results was obtained by Jeba et al.
who showed that aqueous extract of *O. sanctum* orally @ 100 and 200 mg/kg/day in wistar albino rats for 45 days enhanced the production of red blood cells and haemoglobin\(^\text{39}\). On observing liver and kidney function tests, it was found that *L. donovani* infection in BALB/c mice also resulted in liver and kidney dysfunction as depicted by the increased levels of SGOT, SGPT, serum bilirubin, serum urea, BUN and creatinine. However, infected mice treated with *B. diffusa* and *O. sanctum* alone and in combination showed protective activity of these drugs by demonstrating significant reduction in the levels of SGOT, SGPT, serum bilirubin, serum urea, BUN and creatinine after drug treatment. Eugenol, flavonoid and ursolic acid components, present in *O. sanctum* leaves, have free radical scavenging and anti-lipoperoxidative effects. Therefore, the hepatoprotective effect of *O. sanctum* may be due to the antioxidant properties of its constituents. The membrane stabilizing property of *O. sanctum* is also responsible for its hepatoprotective action\(^\text{40}\). Chattopadhyay et al. have also demonstrated the protective effect of *O. sanctum* leaf extract on paracetamol induced hepatic damage in rats as evidenced by significant reduction in the elevated serum enzyme levels at the dose of 200 mg/kg body wt.\(^\text{31}\). Extracts of *B. diffusa* leaves has also been evaluated in a previous study for its antioxidant and hepatoprotective properties in the acetaminophen-induced liver damage. Pretreatment with aqueous and ethanolic extracts decreased the activities of alkaline phosphatase, lactate dehydrogenase, SGOT, SGPT and the level of bilirubin in the serum that were elevated by acetaminophen\(^\text{32,33}\).

The results of this study provide an insight into the alternative herbal remedies which could be used for effective treatment of the visceral leishmaniasis without any harmful effects. Thus, this combination can emerge as a prospective antileishmanial therapy for its nontoxic, and additionally, its ability to induce protective immunity.

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References


