IMMUNOSTIMULATING EFFECTS OF **PELARGONIUM REINFORME/SIDOIDES** EXTRACT (**KALOBIN**®) ON MICE INFECTED WITH **PROHEMISTOMUM VIVAX**

**ABSTRACT:**
The immunostimulating effect of *Pelargonium reinforme/sidoides* extract (Kalobin®) against *Prohemistomum vivax* infection in mice was investigated. Five groups of mice were constructed. The first group was not treated nor infected and considered as control, the second group; control infected, was not treated but orally infected with 150 P. vivax metacercariae. The third, fourth and fifth groups received 100 µg/dl, 200µg/dl and 400 µg/dl of *Pelargonium reinforme/sidoides* extract in drinking water, respectively. The extract was given to the experimental mice two weeks before and two weeks after oral infection with 150 P. vivax metacercariae. The results revealed that the worm burden in non-treated infected group was significantly higher than that in treated groups. Meanwhile, the group received 200 µg/dl was the least in respect to worm burden. Total serum protein, serum albumin and albumin/globulin (A/G) ratio were significantly decreased in non-treated infected group compared with control group. The anti-parasitic immunoglobulin level indicated that *Pelargonium reinforme/sidoides* administration induced significant IgG and IgA immunoglobulaenemia compared with infected non-treated group.

**Key words:**
Immune - stimulant, *Pelargonium reinforme / sidoides*, *Prohemistomum vivax*, mice, IgG and IgA.

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**INTRODUCTION**

*Prohemistomum vivax* (Sonsio, 1892) (Trematoda: Cyathocotylidae) is an intestinal parasite of piscivorous (El-Naffar and Khalifa, 1975; Fahmy *et al*., 1976; Shalaby, 1988, 1992; Khalil *et al*., 1998). The animals as well as human acquire the infection upon consumption of raw and/or undercooked fresh water fish flesh, harbouring the infective metacercariae (Nasr, 1941; Khalil *et al*., 1998). Strong pathological alterations as well as perturbation of cytokines expression were recorded during *P. vivax* infection in mice (Helal *et al*., 1998; Mahfouz *et al*., 2005), which may lead to disturbances in the physiological milieu of the host. In addition, the parasite is able to induce recognizable immune responses in the hosts (Amer 1992 & 2005).

Chemotherapy is an effective tool for the control of parasitic infections. However, development of drug resistance in the parasite population(s) (Molyneux, 2000), as well as toxicity for the host remains the most serious problems. Therefore, there is increasing interest in drugs of natural origin, especially those having immunotropic activity. They may be effective directly as antimicrobial agents and/or complementary to the chemotherapy, increasing cellular and humeral immunity of the organism. In the theme, mistletoe extract enhanced the cellular and humeral responses in mice (Stein *et al*., 2002). Datta *et al*. (1999) reported that protein CI-1; isolated from the leaves of *Cajanus indicus* enhanced the humoral and delayed type hypersensitivity responses in mice. In addition, Alchinal (a complex preparation consisting of three substances *Echinacea purpurea* extract, *Allium sativum* extract and coca) proved to be effective against *Trichinella spiralis* infection in mice (Bany *et al*., 2003).

*Pelargonium reinforme/sidoides* is a medicinal plant known to generations of Khoi/San descendants and Xhos (native tribes of South Africa) traditional healers for its health giving properties in curing stomach ailments, dysentery, blood in stool and the like. This species of *Pelargonium* is indigenous to the Eastern Cape of South Africa and grows wild; send out long bulbous roots deep into the ground. The medicinally active ingredients are found in the bitter testing root of the plant. *Pelargonium sidoides* preparation (EPs 7630) was superior in efficacy compared with placebo in the treatment of adults with acute bronchitis and treatment of acute non-group A beta hemolytic streptococcus (non-GABHS) tonsillopharyngitis in children (Bereznoy *et al*., 2003; Matthys *et al*., 2003; Chuchalin *et al*., 2005). Kalobin® is a natural extract of *Pelargonium reinforme/sidoides* roots (Marcyrl Pharmaceutical Industries El Obour City, Egypt). It is prescribed as an effective medication in respiratory infections as well as immunopotentiatior (Chuchalin *et al*., 2005). The bioactive ingredient in *P. sidoides* are the tri- and tetra-oxygenated cumarine, gallic acid and gallic acid methyl ester (polyphenols), various flavenoids, as well as significant level of calcium and silica. Gallic acid and its methyl esters are present in large amount and identified as the prominent immunomodulatory principle for this herbal medicine (kayser and Kolodzeiji, 1997). The Polyphenols, cumarin like substances, were reported to be effective in case of infections of sinus, throat and respiratory tract as well as tonsillopharyngitis. In addition, gallic acid, as an anti-Leshemian drug, affects through the activation of macrophage functions for production of TNF alpha and iON.
MATERIALS AND METHODS

Experimental animals:

Eight weeks old male BALB/C mice, weighting about 21.0 g at the start of the experiment, were obtained from the animal house of Theodore Bilharz Research Institute, Giza, Egypt. Age and weight matched mice were allocated into five experimental groups, 5 mice each. The first group was not treated nor infected and served as control group. The second group, control infected, was not treated but orally infected with *P. vivax* metacercariae. Groups from third to fifth were infected with *P. vivax* provided with 100, 200, and 400 µg/dl of *Peloragion* extract; respectively, in drinking water two weeks prior to infection and along the course of the experiment. Animals were housed at room temperature (25 + 3° C) in plastic cages with wire tops. Sawdust bedding was changed daily after day during the course of experiment. Powdered casein-based diet, (Table 1) to meet National Research Council requirements for laboratory mouse, was formulated after Shi et al. (1998). Food and water were available ad Libitum.

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>Minerals</th>
<th>Vitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (30%)</td>
<td>Calcium Carbonate 0.28</td>
<td>Vitamin A palmitate (250,000U/g) 160.0</td>
</tr>
<tr>
<td>Fat (10%)</td>
<td>Phosphorus 0.63</td>
<td>Ferric Citrate 6.06</td>
</tr>
<tr>
<td>Carbohydrate (60%)</td>
<td>Sodium Chloride 74.0</td>
<td>Vitamin B-12 100.0</td>
</tr>
<tr>
<td>Water (10%)</td>
<td>Potassium Citrate 28.0</td>
<td>Ferrous Sulfate 16.06</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Calcium pantothenate 160.0</td>
<td>Pyridxin hydrochloride 70.0</td>
</tr>
<tr>
<td>Water (5%)</td>
<td>Sucrose 200.0</td>
<td>Niacin 6.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Corn starch</td>
<td>Menadione 0.5</td>
</tr>
<tr>
<td>Water (10%)</td>
<td>Cellulose 30.0</td>
<td>α-Tochopherol (250 U/g) 2000.0</td>
</tr>
<tr>
<td>Vitamins (1%)</td>
<td>Choline chloride 2.0</td>
<td>ZnSO 4.7H2O</td>
</tr>
<tr>
<td></td>
<td>Chromium Potassium Pulphate 0.032</td>
<td>Vitamin K 0.132</td>
</tr>
</tbody>
</table>

| Table (1): Composition of experimental diet, minerals and vitamins mixture. |

Drug administration:

*Kalobin* (water extract of *Peloragion reniforme/sidoides*) was used as immunostimulant, for 2 weeks prior to infection and along the course of experiment. The drug was provided in distilled water for the animals, *ad libitum*, at three doses, 100 µg/dl, 200 µg/dl and 400 µg/dl of *Peloragion* extract, respectively. The glass bottles used for providing water or drug were washed daily and fresh mixture was provided on daily basis. Control mice were provided with distilled water.

Parasite and infection:

*P. vivax* metacercariae were obtained from the skeletal muscles of fresh water cat-fish *Claris lazera*, collected from the local markets at Kafr El-Sheikh city, Egypt. The metacercariae were counted using stereomicroscope. Mice were deprived of food for 12 hours before the oral infection with 150 metacercariae/mouse. Food was provided 4 hours after infection. At necropsy, some flukes were collected, processed for light microscopy using aceto-alum carmine stain (Drury et al., 1976), and identified as *P. vivax* (Yamaguti, 1971)

Collection of samples:

Two weeks after infection (WAI), all mice were sacrificed by cervical dislocation. Individual blood samples were collected in Eppendorf tubes, after coagulation and centrifugation; sera were collected and stored at –20°C until use.

Determination of worm burden:

At necropsy, the whole intestine of each mouse was removed, opened at the mesentery border and immersed in cold phosphate buffered saline (PBS) for 60 min. Liberated worms were counted by the aid of stereomicroscope. Percentage of worms recovered was calculated according to the following equation:

Worm recovery rate = \( \frac{\text{No of worms recovered}}{\text{Initial dose of infection}} \) %

RESULTS

Fig. (1) indicates that the treated mice harbored significantly (*P< 0.05*) fewer worms than those in non-treated ones. While the worm burden markedly decreased as the dose of the drug increased up to 200 µg/dl, further drug dosage increase had no further effect.

![Fig. (1): Recovery rate of *P. vivax* from mice administered different doses of *Peloragion reniforme/sidoides* extract 2 weeks after infection.](http://www.egyptseb.org)

The changes in total serum protein and serum protein fractions are illustrated in Table (2). As indicated in the table, *Prohemistomum vivax* infection resulted in a pronounced hypoproteinemia in non-treated mice. Although treatment at a dose to 100 µg/dl ameliorated the recorded hypoproteinemia it still significantly lower than that in non-infected mice. Notably no significant changes were recorded in mice treated at a dose 200µg/dl and 400µg/dl compared with that in non-treated ones.

| Table (2): Means ± standard error of total protein, albumin, globulin and A/G ratio |

Measurement of serum albumin indicated that the infection induced significantly hypoalbuminemia in infected non-treated mice than that recorded in the infected and treated groups. Those groups showed more or less serum albumin level similar to that in non-infected mice. The level of serum globulin

**References**

Kaye et al., 2001; Kolodziej et al., 2003. However, little is known about its effect during helminthes infections. Therefore, the present study is devoted to elucidate the effect of treatment with *Kalobin* on the *Prohemistomum vivax* infection in mice.
as well as albumin/globulin ratio in infected mice fluctuated around the levels in non-treated ones.

Figures (2) and (3) indicate that infected treated mice mounted high anti-P. vivax IgG and IgA immunoglobulinemia than infected non-treated ones. The IgG immunoglobulinemia responses were pronounced in mice treated with 200 µg/dl than those in non-treated mice. Furthermore, those mice (treated with 200 µg/dl) showed higher IgA responses than and than that in all groups.

![Fig (2): IgG values in sera of mice administered different doses of Pelargonium reinforme/sidoides extract 2 weeks after P. vivax infection.](image2)

![Fig (3): IgA values in sera of mice administered different doses of Pelargonium reinforme/sidoides extract 2 weeks after P. vivax infection.](image3)

**DISCUSSION**

The present study provides evidence regarding the anti-parasitic effect of *Pelargonium reinforme/sidoides* extract (Kalobin<sup>®</sup>) during Prohemistomiasis in mice. Considering the worm burden as the end point, treatment of mice with *Pelargonium* extract significantly reduced the number of worms recovered at 2 WAI compared to that in non-treated infected mice. In agreement with the recorded observation, mice treated with herbal extract Alchinal (a complex preparation consisting of three substances *Echinacea purpurea* extract, *Allium sativum* extract and coca) harbored significantly fewer T. spiralis larvae (Bany et al., 2003).

Noteworthy, the results of the present study indicated that non-treated infected mice suffered from pronounced hypoproteinaemia and hypoalbuminemia compared to non-treated ones. Hypoproteinaemia as well as hypoalbuminemia may be a feature associated with gastrointestinal parasitosis (Hollmes and Maclean, 1971; Sinski, 1975; Sinski and Bezubik, 1980; Amer, 1992, and 2005, Amer et al., 2002a&amp;b; Amer and Osman 2004). *Prohemistomum vivax* infection results in significant hypoalbuminemia in rats and mice (Amer, 1992 and 2005). These findings may be attributed to hypophagia, malnutrition, and malabsorption in infected animals (Dunn and Svergien, 1998). Moreover, loosing of plasma proteins through the inflammatory areas as well as feeding of parasite on the oozing host fluids may be contributing factors (Kramar et al., 1974). Treatment of mice with *Pelargonium* extract ameliorated the level of serum protein and albumin. Although the exact mechanism of the extract to exert its action remains obscure, reduction of worm burden, improvement of intestinal milieu for digestion and absorption or improving the activity of liver for production of albumin may be possible mechanisms. It is well established that humoral immune responses play an important role in limiting parasitic infections. In agreement with this view, the results reported by Amer (2005) indicated that *Prohemistomum vivax* infection in mice resulted in IgG and IgM and IgA immunoglobulinemia. The results reported herein indicated that treatment of mice with *P. reinforme* extract augments anti-Prohemistomum vivax IgG and IgA production.

Similarly, the role of herbal extract in enhancement antibody production to sheep RBCs was documented in mice treated with *Booheavia diffusa* (Mungantiwar et al., 1999), *Andrographis paniculata* (Puri et al., 1993) and *Cajanus indicus* (Datta et al., 1999). Oral administration of aqueous extract of *Epimedi herba* in mice enhanced the total serum level of IgG, IgG1, IgG2a and IgM as well as production of cytokines (Kim et al., 2001).

Furthermore, Rivera et al. (2003) reported that Ginseng extract augmented the antibody responses of pigs to viral (porcine parvovirus vaccine) and bacterial (*Erysipelothrix rhusiopathiae*) infection antigens. Moreover, herbal extract can normalize the immune responses in stressed or immune suppressed animals (Sharma et al., 1997; Bodinet et al., 2002; Amer et al., 2004).

The precise mechanism by which *Pelargonium* extract exerts its anti-Prohemistomum effect remains unclear at present. However, it is thought that plant extracts may exert their effects through activation of antigen presenting cells as well as antibody production (Wang et al., 2001). The previous studies proved the role of gallic acid (the main constituent of *Pelargonium* extract) as anti-leishmanial effect. In the theme, macrophage activation was reported in association with treatment with *Pelargonium* extract through the induction of TNF alpha, iNO and INF gamma production (Kayser et al., 2001). In agreement, Bloksma et al. (1979) reported that the cellular response to sheep red blood cells (SRBC) was augmented after intracutaneous immunization with antigen and different doses of mistletoe extract (Iscador). The IgM plaque forming cell response was accelerated and followed by an augmentation of the IgG and IgA plaque forming cell responses in Iscador treated mice than in non-treated mice. Cytokines are important factor for enhancement of immune responses and for combating microbial infections (Barbieri, 2006). Kim et al. (2001) reported that *Epimedi herba* extract augmented IL4 and IFN gamma production in mice. In addition the aqueous ethanolic extract of herbal mixture, *Thujae summitates Baptiseae tinctoriae rodix, Echinaceae purpureae rodix and Echinaceae pallida* rodix enhanced the cytokines production in normal mice and restore the normal milieu in immunosupressed one (Bodinet et al., 2002). Moreover, ginseng is reported to stimulate the production of TNFα (Assinewe et al., 2002) and support the phagocytic activity of bovine polymorphonuclear leukocytes and human alveolar macrophages (Scagione et al., 1994; Hu et al., 1995).

In conclusion, Kalobin<sup>®</sup> proved to be effective as immunopotentiator in case of Prohemistomiasis in mice. However, more studies are prompted to elucidate the exact mode and mechanism(s) of action of the extract.

**REFERENCES**


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أظهرت النتائج أن عدد الديدان في المجموعة المصابة غير المعالجة كان أعلى من المجموعات المصابة والمعالجة. وفي نفس الوقت كانت المجموعة المصابة 200 ميكروجرام من المستخلص لكل 100 مل من الماء الأقل في عدد الديدان موجودة.

هذا وقد أظهرت تباين العصبية والثبات الفيسيولوجي في المجموعة المصابة وغير المعالجة مقارنة بالمجموعة المصابة والثبات. لعامة المحاولات الطفيلية في مصل الفئران المصابة أظهرت الدراسة أن إعطاء المستخلص كان له تأثير إيجابي في زيادة الأجسام المضادة من النوع IgA و IgG في المولدة ضد طفيل البرهوموسوم.

المحفور

التأثير المناعي المحفر لمستخلص البلاروجونيم رنينفروم/ سيدرو بسد (كالوين) على الفئران المصابة ببرهوموسوم فيفاكس

يتم في هذه الدراسة اختبار التأثير المناعي المحفر لمستخلص البلاروجونيم رنينفروم/سيدرو بس (كالوين) على الفئران المصابة ببرهوموسوم فيفاكس حيث تم تكمن خمس مجموعات من الفئران: المجموعة الأولى لم يتم إصابتها ولم يتم معالجتها واعترف كمجموعة ضابطة. المجموعة الثانية تم دفعها بعد 150 ميكروجرام للفئران الفيماكس عن طريق الفم، ولم يتم معالجتها أما المجموعة الثالثة والرابعة والخامسة فقد تم دفعها بعد 150 ميكروجرام للفئران الفيماكس وتم معالجتها بالم่าง من المستخلص البلاروجونيم رنينفروم/سيدرو بس (كالوين) بتركيز 100 ميكروجرام لكل 100 مل من الماء. 200 ميكروجرام لكل 100 مل من الماء و400 ميكروجرام لكل 100 مل من الماء على النحو التالي. هذا وفق نتائج المستخلص لمدة أسبوعين قبل وبعد العدوى.

المحكم:

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أ. د. إبراهيم بكر هلال

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