International Journal of Pharmacology

ISSN 1811-7775
Antitumoral Effect of *L. inermis* in Mice with EAC

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**Abstract:** In recent years, prophylactic usage of natural products and tendency to resort to alternative medicine has increased rapidly. Henna (*Lawsonia sp.*) has been used not only cosmetically but also medicinally in Turkish population. Among the studies of henna’s antifungal, anti-microbial, tuberculous and antitumoral effects come on the science. In this study, we planned to research the effect of *Lawsonia inermis* that is an oxidant agent against development of cancer, by constituting peritonitis carcinomatous with Ehrlich ascites. The animals were divided to three groups and *Lawsonia inermis* extract and tap water were given to mice for 5 days. After 5 days, all of animals were decapitated by cervical dislocation and their liver tissues were sampled to measure reduced glutathione (GSH) level. Mean Survival Time (MST) and Average Survival Time (AST) were calculated; peritoneal liquid pH was measured; Ehrlich Ascites Carcinoma (EAC) cells were counted with hemocytometer. At the result, the longest life period was detected on the group which was given 10 mg/kg/day *Lawsonia inermis*. In group 2 and 3 which were given *Lawsonia inermis* following to forming Ehrlich ascites carcinoma, total number of cancer cell decreased. The scaled pH levels belonging to group 2 and 3 changed into alkaline compared to that of group 1 (pH = 6.2). Glutathione levels of liver tissue were determined to decrease in group 2 and 3 in comparison with group 1. In conclusion, *Lawsonia inermis* may lead cells to apoptosis related to deficiency in detoxification of intracellular radicals.

**Key words:** *Lawsonia inermis* L., ehrlich ascites tumor, antitumoral, extract

**INTRODUCTION**

Henna (*Lawsonia sp.*) has been used not only cosmetic but also medicinal in Turkish population, becomes re-popular again among public. Henna which extract of *Lawsonia sp.* used as hair dye and nail dye in many cultures as decorative dye at centuries. Its medical properties of henna are present except the chemical use. Henna has antifungal, anti-microbial, tuberculous and antitumoral effects were reported on the science (Curreli et al., 2001; Dasgupta et al., 2003; Habbal et al., 2005; Kok et al., 2005; Malekzadeh, 1968; Sharma, 1990; Singh and Pandley, 1989).

Furthermore, at the reports presented as case reports, it said that usage of *L. inermis* in children has serious oxidant effects. The GPDH deficient was determined at biochemical evaluation in these children (Devecioglu, 2001; Raupp et al., 2001; Soker, 2000; Zinkham and Osiki, 1996).

In another study, topical application of henna reduced severity of Hand-Foot Syndrome caused by a therapeutic agent as capetitabine used for cancer treatment and so no necessary to use lower dose of therapeutic agent or to cut down. This effect of henna reported that it have anti-inflammatory, anti-pyretic and analgesic activities (Yucel and Gorullu, 2007).

During the transformation from healthy cells to cancer cells excessive DNA synthesis occurs. For this situation, ribonucleotide reductase enzyme and as correlated with this enzyme are necessary. Thus, we planned that binding H’ ion to injured cells develop to cancer cells in which excessive DNA synthesis by benefiting from oxidant effect of *L. inermis* and accelerating apoptosis by exposed excessive oxidant stress to these cells. For that purpose, we planned to research about the effect of *L. inermis* that is an oxidant agent against development of cancer, by constituting peritonitis carcinomatosis with Ehrlich ascites cells.

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MATERIALS AND METHODS

This project was conducted in Medical Research Laboratory, University of Cukurova during April 2007-August 2008.

Animals: One hundred forty Swiss albino mice (10 weeks, 25-30 g) were from Medical Research Laboratory, University of Cukurova.

The formation of EAC: Stock Mouse with EAC was obtained from Experimental Animals Laboratory, Cerrahpasa Medical Faculty, Istanbul University. Peritonitis carcinomatosis formed by injecting i.p. 0.2 mL (1×10⁶ cells) ascites fluid doing paracentesis from peritonitis of stock mouse (Jagethia and Rao, 2006). Cells in the injected ascites liquid stained with Trypan blue and counted with hemocytometer.

Preparing of L. inermis solution: 0.5% solution of L. inermis get by 0.5 g L. inermis powder dissolved in 100 mL distilled water, boiled and filtered (Ozaslan et al., 2007).

Researching of antitumoral effect of L. inermis solution

• First step: EAC was developed in Swiss Albino mice, divided 6 groups (n = 10), 5, 10, 20, 50 and 200 mg/kg/day L. inermis were given to the experimental groups and tap water was given to the control group per orally (p.o.) at equal two doses in a period of 12 h for 5 days.

• Second step: The animals divided to three groups and L. inermis extract and tap water given to mice for 5 days.

Negative control group (group 1) (n = 20); not given EAC intra peritoneal (i.p.), given only tap water p.o.

Positive control group (group 2) (n = 30); given EAC i.p. and given tap water p.o.

Group 3 (n = 30); given EAC i.p. and given 10 mg/kg/day L. inermis extract p.o.

After 5 days, all of animals decapitated by cervical dislocation and their liver tissues sampled.

Survival analysis: Mean Survival Time (MST) and Average Survival Time (AST) calculated according to these formulas:

\[
MST = \frac{\text{The first death day} + \text{The last death day}}{2}
\]

\[
AST = \frac{\text{The total death days}}{\text{Total mice number}}
\]

The percentage of Increasing Mean Standard Life (IMSL) and The Percentage of Increasing Average Standard Life (IASL) calculated with these formulas (Jagethia and Baliga, 2003; Jagethia et al., 2005; Jagethia and Rao, 2006).

\[
\text{IMSL} (%) = \frac{\text{Experimental group MST} - \text{Control group MST}}{\text{Control group MST}} \times 100
\]

\[
\text{IASL} (%) = \frac{\text{Experimental group AST} - \text{Control group AST}}{\text{Control group AST}} \times 100
\]

pH in ascites liquid assays: 4cc SF i.p. inoculated to mice 2 h after giving L. inermis at the 5th day of the inoculation of EAC. Peritoneal liquid samples taken after massaging stomach during 2 min. Peritoneal liquid pH measured with pH meter without loss of time (InoLab pH meter, Germany).

Number of cancer cells in ascites liquid: The cells of peritoneal EAC liquid that collected 2 h after giving L. inermis at the 5th day of the inoculation of EAC stained with Trypan blue and counted with hemocytometer.

The preparation of liver tissue samples: After cervical decapitating the mice whose abdomen dissected by median incision, approximately 200 mg liver tissue was sampled and washed with SF. Without loss of time, tissues were taken in tubes with 8 mL 0.02 M EDTA and stored at -20°C.

The measurement of GSH: Five milliliter homogenate was prepared from liver tissue taken out tubes with EDTA, was mixed with 4 mL distilled water and 1 mL 50% trichloroacetic acid (TCA). Then it was centrifuged 3000 rpm 15 min. Two milliliter liquid of supernatant was mixed with 1 mL 0.4 M Tris buffer (pH 8.9) and 0.1 Ellman reagent [5,5’-dithiobis-(2-nitrobenzoic acid)] (DTNB) (Sigma). The absorbance at 412 nm in spectrophotometer was recorded. The result values signified as mol GSH/g tissue (Sedlak and Lindsay, 1968).

Statistical analysis: The results calculated with SPSS 12.0 package program. Student t-test used for normal dispersion data and Mann-Whitney U test for abnormal dispersion data. The limit of statistical significance was set at p<0.05.

RESULTS

In this study, to determine antitumoral effect of L. inermis different dosages life span of mice, amount of cancer cells in ascites liquid, pH of ascites liquid and GSH level in liver tissue were performed.
First step results: The longest life period was detected on group which given 10 mg/kg/day L. inermis. We found these results MST = 13.5, AST = 13.1, IMSL = 12.5% and IASL = 12.5% for this group (Table 1).

Second step results: Number of cancer cells counted in ascites liquid doing paracentesis from groups (1, 2 and 3) summarized in Fig. 1. The formation of ascites liquid in group 1 (negative control group) which not formed EAC was not encountered. Although, in group 2 (positive control group) which not given L. inermis following to formed EAC total cancer cell number was approximately 14.6±2.05, in group 3 was approximately 12.3±2.02. The difference between total number of cancer cells in group 2 and 3 was significant statistically (p<0.001).

The scaled pH levels belong to group 2 and 3 changed into alkaline than of group 1 (pH = 6.2). It was detected that pH 7 level scaled for group 3 which given L. inermis was more alkaline than for group 2 which not given L. inermis. While pH level of group 2 was 6.8±0.09, pH level of group 3 was 6.9±0.08'dir (Fig. 2). The difference between pH levels of these two groups was significant statistically (p<0.05).

The GSH levels of liver tissue were determined to decrease in group 2 and 3 comparison with group 1. Although, GSH level of group 2 was measured as 3.04 μmol g⁻¹, GSH level of group 3 as 3.45 μmol g⁻¹ (Fig. 3). The difference between these measured GSH levels was significant statistically (p<0.05). Life span periods belong to group 1, 2 and 3 summarized in Table 2. While in group 2 MST = 10, AST = 10.1 were found, in group 3 MST = 11, AST = 11.5, IMSL = 10% and IASL = 13.9%.

**DISCUSSION**

Although, cancer takes part among top of death list in recent years, its treatment is still very complicated and has great pre-conditions. In this complicity, numerous natural agents which were used traditionally have significant role in fighting cancer such as protective, preventive and curative consolidated immune system. The substances extracted from plants and suggested as active were tested in experimental cancer model studies (Jagethia and Baliga, 2003; Jagethia et al., 2005; Jagethia and Rao, 2006).

Raupp et al. (2001) reported that topical application of L. inermis caused hemolytic effect in children with G6PD enzyme deficient (Raupp et al., 2001).
G6PD is one of the main H+ ion sources via NADPH for cell. These findings were supported that in children with G6PD enzyme deficient, a little oxidant effect aroused from L. inermis may cause significant alterations on H+ ion balance.

Our study composed of two experimental steps: in first of them, mice transplanted EAC were divided six groups (one control and five experimental) and in this step was determined as the most curative and the longest life span dose was 10 mg/kg/day than others. In second step, life span period was determined to get longer in group 3 which was transplanted EAC and then given p.o. L. inermis and to be decreased cancer cell number in ascites liquid. This difference was significant statistically (p<0.001). GSH level was determined to decreased in groups transplanted EAC than its in groups not transplanted EAC. GSH level was higher in group 3 giving L. inermis orally than its in group 2 not given L. inermis, belong to groups transplanted EAC (p<0.05). Our result was correlated with the study of Dasgupta and colleagues (Dasgupta et al., 2003). They investigated the effect of L. inermis against stomach and skin papillomatous tumor and found the result of L. inermis inhibited tumor progression and also they reported that L. inermis increased GSH and SOD levels (Dasgupta, 2003).

It was known that during the transformation from healthy cells to cancer cells excessive DNA synthesis occurs. In cell, increased DNA synthesis causes increased H+ usage. This case creates pH increasing in the cell. Austin and Wray et al. (1993) reported that 0.75 fold of each difference occurred in extracellular pH causes variation in intracellular pH that to tolerate variations in tissue pH level. This study supported our result of being increased pH levels in group 3 shows not only excessive DNA synthesis increased H+ consumption in cancer cells but also SOD activity increased by oxidant effect of L. inermis occurred H2O2 and being stress on detoxification of H2O2. Thus, L. inermis may lead cell to apoptosis related to deficient in detoxification of intracellular radicals.

Although, a study performed that L. inermis inhibited papillomatous tumor progression (Dasgupta et al., 2003), it was not encountered a report about the effect of L. inermis on EAC progression. This report is first because of researching the antitumoral effect of L. inermis on experimental tumor model EAC and the relationship between L. inermis and pH in the immune system.

The net conclusions of this study were (1) GSH level in tissue increased (2) SOD activity increased by oxidant effect of L. inermis, (3) it inhibited EAC progression. It is necessary to investigate detailed biochemical parameters by studying with bigger experimental groups to explain completely effect mechanism of L. inermis on apoptosis and intracellular free radicals together. Thus, it may be clarified clearly inhibitor effect of L. inermis against cancer metabolism.

REFERENCES


