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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, tuberculostatic 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 cells. 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 group1 (pH = 6.2). Glutathione levels of liver tissue were determined to decrease in group 2 and 3 in comparison with group1. 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, tuberculostatic 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 G6PD deficient was determined at biochemical evaluation in these children (Devecioglu, 2001; Raupp *et al*., 2001; Soker, 2000; Zinkham and Oski, 1996).

In another study, topical application of henna reduced severity of Hand-Foot Syndrome caused by a therapeutic agent as capecitabine 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 Gonullu, 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 (1×10^6 cells) ascites fluid doing paracentesis from peritonitis of stock mouse (Jagethia and Rao, 2006). Cells in the injected ascites liquid stained with Tryphan blue and counted with hematocytometer.

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:

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

$$\text{AST} = \frac{\text{The total death days}}{\text{Total mice number 5}}$$

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 Tryphan blue and counted with hemocytometer.

The preparation of liver tissue samples: After cervical decapitating the mice whose abdomen dissectioned 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% trichloroaceticacid (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.

Table 1: Life span period related with *L. Inermis* different dosages

Parameters	Eac+ tap water (Group-1)	EAC+ 5 mg kg^{-1} *L.inermis* (Group-2)	EAC+ 10 mg kg^{-1} *L.inermis* (Group-3)	EAC+ 20 mg kg^{-1} *L.inermis* (Group-4)	EAC+ 50 mg kg^{-1} *L.inermis* (Group-5)	EAC+ 200 mg kg^{-1} *L.inermis* (Group-6)
MST	12	12.5	13.5	12.5	11.0	8.5
IMSL (%)	-	4.2	12.5	4.2	-8.3	-29.7
AAST	11.6	11.6	13.1	12.5	9.8	6.4
IASL	-	10.3	12.9	7.6	-15.5	-44.8

EAC: Erlich ascites carcinoma

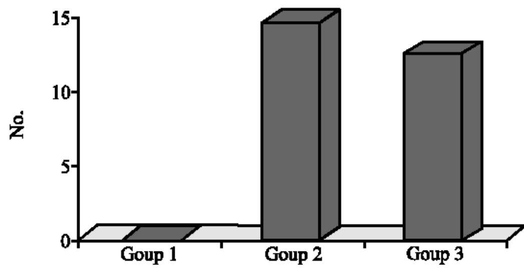

Fig. 1: Cancer cell number in ascites liquid

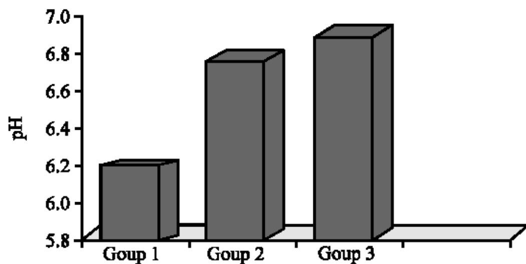

Fig. 2: pH levels in ascites liquid

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 = 12.9% 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 group1 (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 14.61±2.05, in group 3 was approximately 12.34±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 group1 (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 group2 was 6.81±0.09, pH level of group 3 was 6,90±0,08'dir (Fig. 2). The difference between pH levels of these two groups was significant statistically ($p<0.05$).

Table 2: The effect of l.inermis 10 mg/kg/day dose application on life span

Parameters	Tap water (Negative control group) (group-1)	EAC+tap water (Pozitive control group) (group-2)	EAC+*L.inermis* (group-3)
MST	-	10.0	11.0
IMSL (%)	-	-	10.0
AAST	-	10.1	11.5
IASL (%)	-	-	13.9

EAC:Erlich ascites carcinoma

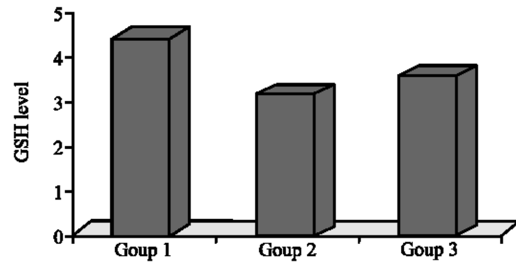

Fig. 3: GSH levels of liver tissue (μmol g^{-1})

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 group2 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 arised 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 giveng *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 0.73 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 H_2O_2 and being stress on detoxification of H_2O_2. 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.

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