

## STUDIES ON SOME MEDICINAL PLANT EXTRACTS AS IMMUNOSTIMULANT IN RATS EXPERIMENTALLY INFECTED WITH *SALMONELLA TYPHIMURIUM*

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### Abstract

Aloe extract was used as diet supplementation in a group of rats experimentally infected with *Salmonella typhimurium*. It was found that rats that fed on diet with aloe extract for 30 days, then, experimentally infected with *Salmonella typhimurium* showed survival rate 92% in comparison with group of rats fed on ration without aloe extract and experimentally infected with *Salmonella typhimurium*. Histopathological examination revealed that treated group with rats of aloe extract showed few histopathological changes, while those non-treated group showed significant infiltration of liver with mature and premature leucocytic cells in hepatic tissues with severe necrotic changes. Moreover, the heart of such untreated group with aloe extract showed leucocytic inflammatory cells infiltration between the bundles of degenerated cardiac muscles.

### INTRODUCTION

Aloe is a herb native to the Mediterranean. The active principle of aloe was found to inhibit the growth of several species of pathogenic bacteria, and also, has a beneficial effect on the immune system (Harborne *et al.*, 1999).

*Salmonella typhimurium* is one of the most important bacteria causing disease condition and high mortalities, in which the immune status deficiency plays an important role in the incidence and progress of the bacteria (Harkness and Wagner, 1995 and Rofaiil, 2001). Furthermore, the possibility of preventing the disease using some dietary supplements and/or herbal medicine has attracted considerable attention (Hassan *et al.*, 2008).

Many drugs and chemicals can enhance various aspects of the immune response, immunopotentiality may occur by increasing the rate at which the immune response develops, (Rofaiil *et al.*, 2007). Interest is arising in the possible use of plant extracts as a source of naturally occurring antibiotic like substance (Aisha, 1987 and Rofaiil and Daoud, 2005).

Little effort was done on investigation of the role played by aloe extract to enhance its the therapeutic effect for controlling some infections (Laurner, 1981).

So, the aim of the present study was to throw light on the possible role of aloe extract as a protecting agent in rats experimentally infected with *Salmonella typhimurium*.

## MATERIALS AND METHODS

### Aloe extract

It was obtained as powder from Faculty of Pharmacy, mixed as 2.5% with basal ration of experimental rats.

### Ration

A balanced ration consumed by the experimental rats was examined bacteriologically and proved to be free from *Salmonella*.

### Experimental rats

One hundred and fifty healthy rats were kept in separated cages under strict hygienic conditions during the experiment.

### *Salmonella typhimurium* strain

Local isolate of *Salmonella typhimurium* used in the present study was identified through Gram staining, colonial morphology, biochemical and serological reactions as described by Forbes *et al.* (1998).

### Experimental design

Experimental rats with age of 30 days, and weight of 100 g were divided into 3 groups, (50 rats each) as follows:

**Group 1 :** Rats were fed on ration supplemented with aloe extract for a period of 30 days.

**Groups 2 and 3:** Rats were fed on ration without aloe extract supplementation for a period of 30 days.

At the end of the 30 days both groups 1 and 2 were experimentally injected peritoneally with 0.5 ml of twenty-four hours broth culture of *Salmonella typhimurium* containing  $1.5 \times 10^8$  CFU/ml. Such rats were observed daily after experimental infection, and mortalities were recorded. Smears from internal organs of freshly dead rat were subjected to bacteriological examination for reisolation of the organism. Reisolation of the organism was done as described by Forbes *et al.* (1998). The rats of group 3 were left as negative control.

For histopathological examination, tissue samples from the liver and heart of dead rats post-experimental infection were collected and fixed in 10% neutral formalin, washed by tap water, dehydrated in graded ethanol, embedded in paraffin, sectioned at 4-5 microns and stained by hematoxylin and eosin following the standard technique of Bancroft *et al.* (1994).

## RESULTS AND DISCUSSION

This work has been designed to investigate the protective effect of aloe extract supplementation on the ration of rats against artificial infection with *Salmonella typhimurium*.

Aloe is a herb that was reported to have an immunostimulant effect. The extract of such plant contained several ingredients with potential value. It is composed of glucosides as aloin, sabarin and paraolin, organic acids as simanec. Also, it contains Amadin and anthradion (Mueller *et al.*, 1999).

Data recorded in Table 1 showed that protective effect of aloe extract supplementation in ration of rats experimentally infected with *Salmonella typhimurium*, the survival percentage among such treated group with aloe extract was recorded 92%, with a mean death time of 7 days, and a mean lesion score (+) (slight congestion). Meanwhile, in non-treated group, the survival percentage was 20% with a mean death time of 3 days and a mean lesion score (+++) (severe congestion and petechial hemorrhage). It could be stated that the action of aloe extract in reducing the mortality rate of rats infected with *Salmonella typhimurium* is due to its properties as anti-microbial agent (Laurner, 1981) and immunostimulant effect (Mueller *et al.*, 1999).

From the obtained results, the anti-microbial response produced by aloe extract is similar to that reported by Benirsckle *et al.* (1978) and James *et al.* (2002) who explained that the improvement of the immune status of rats is due to the enhancement of the natural killer cells to restore their activity.

From the pathological point of view, the group of rats fed on ration supplemented with aloe extract can resist the intraperitoneal inoculation with *Salmonella typhimurium*. They appeared nearly normal macroscopically and microscopically, the internal organs, specially, liver and heart showed few pathological changes (Fig. 1 and 2). On the other hand, the rats fed on normal ration showed friable liver with focal necrosis and congestion. The liver showed mature and premature leucocytic cells infiltration in hepatic tissues and necrotic changes as shown in Figs. 3 and 4, while, the heart showed leucocytic inflammatory cell infiltration in-between the vascular and degenerated myocardial bundles.

The histopathological changes were nearly similar to those obtained by Maronpot *et al.* (1999) and Percy and Barthold (2001) who studied the pathogenesis of *Salmonella typhimurium* in rats after injection of Bacilli and invasion to general circulation producing bacteriemia and invasion to liver and heart leading to previous pathological changes.

The control negative group did not show any pathological changes in liver or heart. So, it could be concluded that, aloe extract had significant anti-microbial activity against *Salmonella typhimurium*, thus, it could be used as ration supplementation to control microbial diseases.

Table 1. Effect of aloe extract supplementation to ration of rats experimentally infected with *Salmonella typhimurium*

Groups of rats	No. of dead rats/Total no. of rats	Mean death time (days)	Mortality rate	No. of survived rats/Total No. of rats	Survival %	Lesion score in heart and liver
(1)	4/50	7	8 %	46/50	92 %	+ (Slight congestion)
(2)	40/50	3	80 %	10/50	20 %	+++ (Severe congestion and petechial hemorrhage)
(3)	0/50	-	0 %	50/50	100 %	-

Group (1): Fed on ration supplemented with aloe extract at rate of concentration of 2.5% and infected with *Salmonella typhimurium*

Group (2): Fed on ration without aloe extract and infected with *Salmonella typhimurium*.

Group (3): Fed on ration without aloe extract and not-infected with *Salmonella typhimurium*.

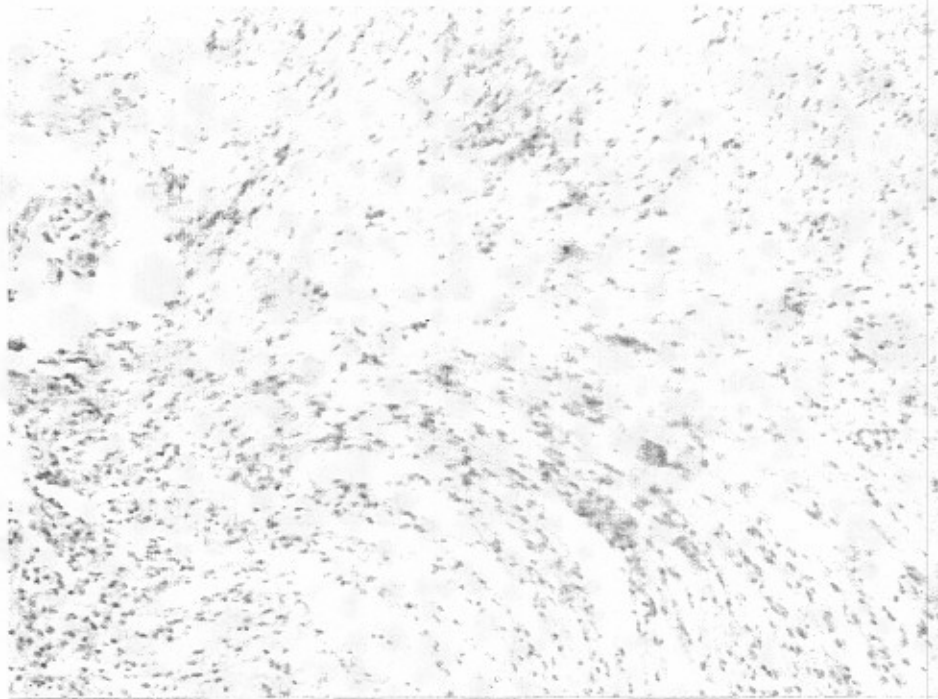


Fig. 1. Heart of rat that had aloe extract in ration and inoculated (I/P) by *Salmonella typhimurium* showing few necrotic changes (H & E, x40)

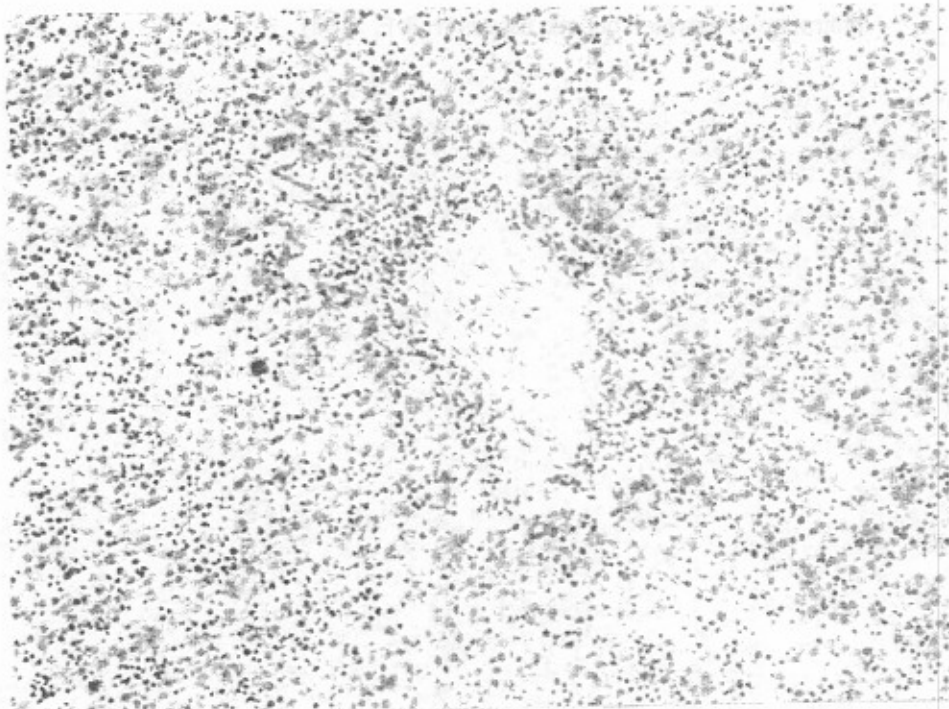


Fig. 2. Liver of rat that had aloe extract in ration and inoculated (I/P) by *Salmonella typhimurium* showing few cells infiltration and less necrotic changes (H & E, x40)

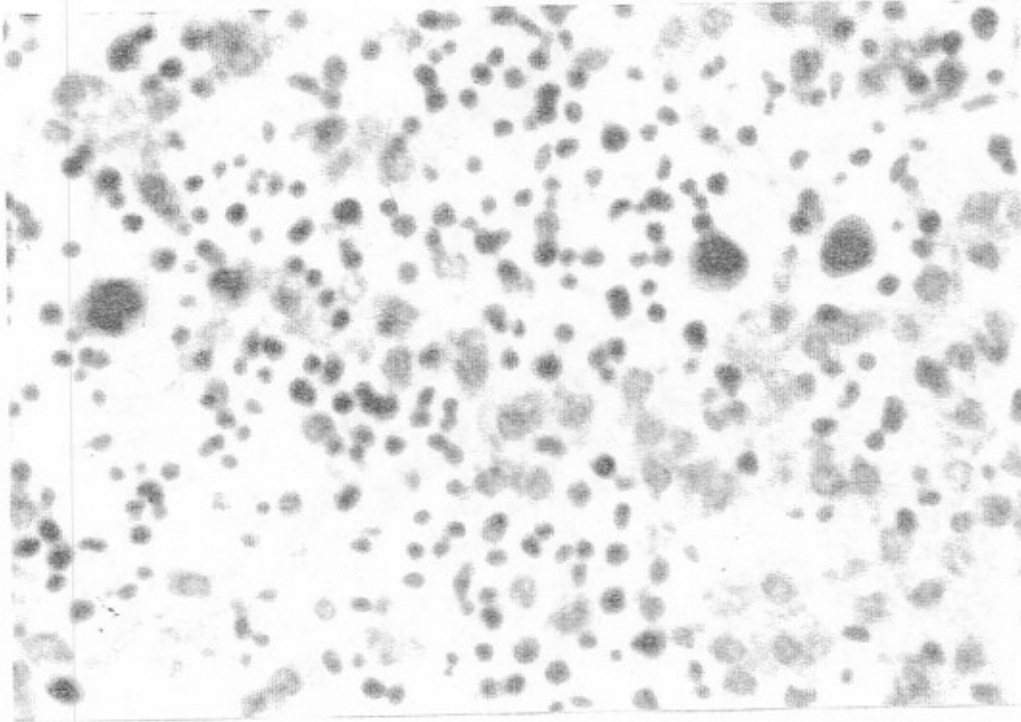


Fig. 3. Liver of rat that had not aloe extract in ration and inoculated (I/P) by *Salmonella typhimurium* showing mature and premature leucocytic cells infiltration in-between hepatic tissues (H & E, x160)

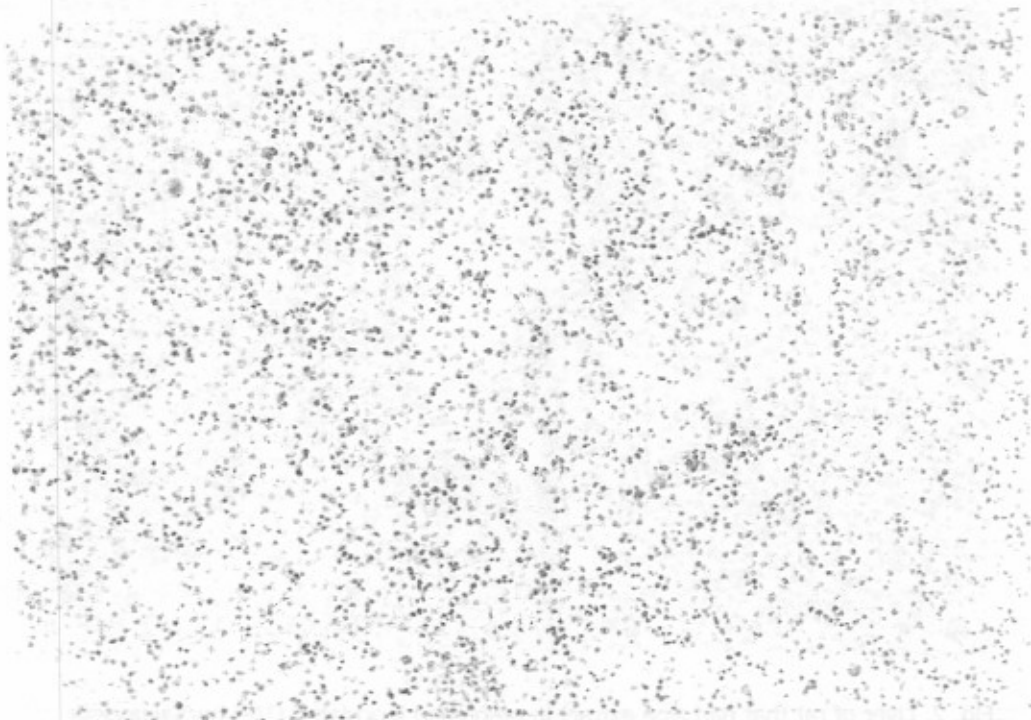


Fig. 4. Liver of rat that had not aloe extract in ration and inoculated (I/P) by *Salmonella typhimurium* showing hepatocellular necrosis and the mononuclear cells infiltration (H & E, x40)



Fig. 5. Heart of rat that had not aloe extract in ration and inoculated (I/P) by *Salmonella typhimurium* showing leucocytic cells infiltration in-between the degenerated myocardial bundles (H & E, x40)

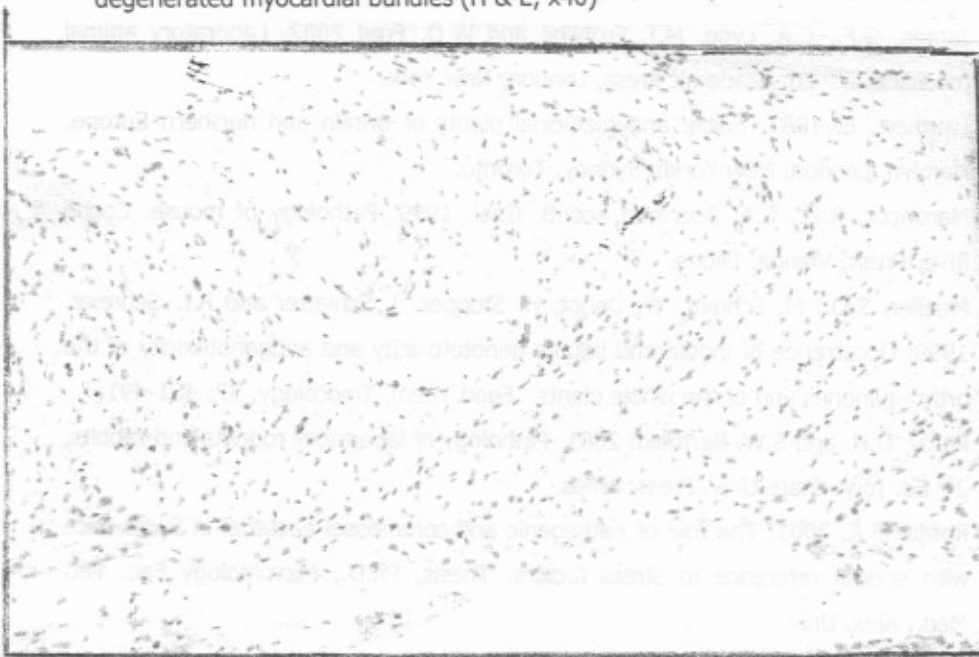


Fig. 6. Heart of rat that had not aloe extract in ration and inoculated (I/P) by *Salmonella typhimurium* showing hyperemic myocardial blood vessels (H & E, x40)

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دراسات على بعض مستخلصات النباتات الطبية كمنشط مناعى فى الجرذان المعدية  
تجريبياً بالسالمونيللا تيفيموريوم

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أجريت هذه الدراسة لمعرفة التأثير الوقائى لمستخلص نبات الصبار على الجرذان المعدية تجريبياً بالسالمونيللا تيفيموريوم وأثبتت النتائج أن هناك تأثيراً مناعياً واضحاً فى الجرذان التى تم عداها بالسالمونيللا تيفيموريوم سبق معالجتها بخلاصة الصبار فى العليقة حيث كانت نسبة الجرذان التى لم تنفق ولم تتأثر بالعدوى ٩٢% بالمقارنة بالمجموعة الغير معالجة التى كانت فيها نسبة النفوق ٨٠%، علاوة على خلو الجرذان المعالجة من الميكروب بالفحص البكتريولوجى. كما لوحظ عدم وجود أية تغيرات هستوباثولوجية فى المجموعة المعالجة بخلاصة الصبار بينما المجموعة الغير معالجة بتلك الخلاصة ثبت وجود تغيرات واضحة فى الكبد وهى عبارة عن تكسر فى الخلايا وارتشاح متجمع من خلايا الدم البيضاء فى النسيج الكبدى . وفى القلب ظهرت تغيرات عبارة عن ارتشاح متجمع من خلايا الدم البيضاء بين الأوعية المتخللة لعضلة القلب مع احتقان بالأوعية الدموية.