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The Effects of Co-administration of Immobilization Stress and *Aloe vera* on Serum Carcinoembryonic Antigen in Rats

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Background: Immobilization has been used extensively and accepted widely for studying stress-induced alterations. To determine the protective effects of *Aloe vera* leaf extract, we evaluated the effects of co-administration of immobilization stress and *Aloe vera* leaf extract on serum level of carcinoembryonic antigen (CEA) tumor marker in male rats.

Materials and Methods: In this experimental study, 45 male Wistar rats were randomly divided into 9 groups of 5 rats in each including: 1) control, 2) normal saline receiving, 3) *Aloe vera* extract receiving, 4) acutely immobilized, 5) chronically immobilized, 6) acutely immobilized+*Aloe vera* extract, 7) chronically immobilized+*Aloe vera* extract, 8) acutely immobilized+normal saline and 9) chronically immobilized+normal saline. The animals were exposed to chronic or acute immobilization stress for 2 h/day or 8 h/day for a period of 3 weeks or one week, respectively. *Aloe vera* extract (300 mg/kg/day) was fed by gavage feeding orally. Blood samples were collected and following serum collection, CEA level was determined by radioimmunoassay method. Data were compared statistically between groups using ANOVA.

Results: Serum CEA level was significantly increased in acutely (0.640±0.025 ng/mL) or chronically immobilized (0.647±0.023 ng/mL) rats compared with control animals group (0.564±0.014 ng/mL) ($p<0.001$). However, there was no significant difference between serum CEA levels of acutely or chronically immobilized *Aloe vera* extract received animals compared with control rats (0.622±0.027 ng/m and 0.616±0.044 ng/mL, respectively).

Conclusion: Our findings indicate that immobilization stress enhances serum CEA level, however, intake of *Aloe vera* extract can withstand against.

*Keywords:*Carcinoembryonic antigen; Immobilization; *Aloe vera*; Rat

1. Introduction

Tumor markers are substances found in the blood, urine, stool or tissues of cancer patients. They are produced by tumor cells or in some cases by the body in response to certain benign conditions [1]. CEA (carcinoembryonic antigen), a surface glycoprotein which was first identified in 1965 is a well-studied onco-fetal antigen [2]. It has been shown that rat CEA has the tissue distribution and physicochemical properties similar to human CEA [3]. Enhanced serum CEA levels occur in a large number of epithelial malignancies, including colorectal cancer [4], gastrointestinal malignancies [5], pancreatic and lung adenocarcinoma [6].

Elevated CEA levels can also occur in patients with non-cancerous conditions, including inflammatory bowel disease, pancreatitis and liver disease [7, 8], ageing [9] and alcohol drinking [10]. Many different types of stress trigger changes in the immune system, according to which, there is some link between stress and developing certain kinds of cancer as well as increased serum level of tumor markers. Immobilization is one of the most common performed stresses on animals [11]. Among the various stress models, acute or chronic immobilization has been used extensively and accepted widely for studying the association between stress and pathophysiological alterations [12]. Immune system suppression in laboratory animals in response to stresses [13], including immobilization stress, may prove this association. Repeated immobilization stress causes structural remodeling in areas of the brain responsible for emotional memories and regulation of the stress response [14]. However, there are studies showing that acute stress enhances immune function but chronic stress suppresses the immune system [15].

Considering botanical agents as medical remedies [16, 17], there are several plant species reported to have potential to cure cancers [18] including the oldest and most popular medicinal plant, *Aloe vera* [19]. Scientists have discovered over 150 nutritional ingredients in *Aloe vera*. Ten main areas of chemical constituents of *Aloe vera* include: amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharide, polysaccharides, salicylic acid, saponins and sterols [20]. Studies suggest that *Aloe vera* extract may play an immunomodulatory role against cancer or pathological conditions [21].

Although serum tumor markers assay, in particular CEA, has an important role in screening a healthy population

or a high risk one for the presence of cancer [9], there are few studies examining the relationship between environmental factors and elevated serum CEA levels. There are also few studies examining the antitumorigenic potential of *Aloe vera*. The reports, however, have established pathophysiological and tumorigenic effects of immobilization stress on animals [22]. In this respect, the present study was exerted to determine the effects of immobilization stress on serum CEA level and possible protective effects of *Aloe vera* leaf extract against pathophysiological effects of immobilization stress on serum CEA level.

2. Materials and Methods

In this experimental study, 45 adult male Wistar rats weighting 190±10 g were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran). The temperature was at 20-25°C and animals kept under a schedule of 12 h light: 12 h darkness (light on at: 8:00 am) with free access to water and standard laboratory chow. Care was taken to examine the animals for general pathological symptoms. Food was withheld for 12-14 h before operation or death. In all experiments, attention was paid to the regulation of local authorities for handling laboratory animals and the Ethical Guidelines for investigation of immobilization stress in rats [23]. This work was conducted in laboratory complex of IAU-SR (Tehran, Iran).

In this study, 45 male Wistar rats were randomly divided into 9 groups of 5 rats in each including: 1) control, 2) normal saline receiving, 3) *Aloe vera* extract receiving, 4) acutely immobilized, 5) chronically immobilized, 6) acutely immobilized+*Aloe vera* extract receiving, 7) chronically immobilized+*Aloe vera* extract receiving rats, 8) acutely immobilized+normal saline receiving and 9) chronically immobilized+normal saline receiving rats. Animals in the control group received no treatment of any kind. In all *Aloe vera* extract received groups, *Aloe vera* leaf extract administered orally at a dose of 300 mg/kg.

Aqueous extract of *Aloe vera* leaves was prepared according to previous studies with slight modifications [24]. Briefly, fresh *Aloe vera* leaves weighing between 500-600 g with approximate length 60-80 cm were collected and the thick epidermis was removed. Fleshy mucilaginous pulp (parenchymatous tissues) was selectively scraped out, homogenized and centrifuged at 6000 g for 15 min to remove the fibers. The supernatant was lyophilized. A known amount of extract was suspended in sterilized distilled water used once per day.

In the present study, an immobilization system that allows rats free intake of feed and water while restraining their movement was established. Animals assigned to stress groups underwent immobilization using restraining device. Rats were immobilized but not compressed, pinched, or screaming/screeching. According to previous studies [25], immobilization stress was performed as chronic or acute. In chronically immobilized groups, animals were immobilized 2 h/day for 3 weeks. In acutely immobilized groups, animals were immobilized 8 h/day for one week.

Blood samples were collected using cardiac puncture technique after anesthetizing animals by ether. Following serum collection, CEA level was determined by radioimmunoassay method using commercially available kits (Immunotech A Beckman Coulter/ Ref.2121).All results are presented as mean±SD. Data were analyzed using SPSS-19.0 software. The significance of differences between groups was determined by analysis of variance (ANOVA). Games-Howel test was used for post-hoc comparisons. Differences were considered significant when $p<0.001$.

3. Results

Table 1 represents serum CEA levels in different groups of our study. Our findings indicated that there was no significant difference in serum CEA levels of normal saline received rats compared with control animals. Therefore, feeding method used for extract administration had no significant impact on the results of our study. Furthermore, there was no significant difference in serum CEA levels of *Aloe vera* extract received animals compared with control rats. Serum CEA level was significantly increased in acutely and chronically immobilized rats compared with control animals ($p<0.001$). However, there was no significant difference in serum CEA levels between acutely immobilized and chronically immobilized normal saline received animals compared with control rats. Also, serum CEA levels was lower in chronically and acutely immobilized *Aloe vera* extracts received rats than chronically and acutely immobilized animals, respectively ($p<0.001$).

Table 1. Serum CEA levels in control and experimental groups

Animals	CEA (ng/mL) (mean±SD)	p-Value
Control	0.564±0.014	-
Normal saline received	0.535±0.039	N.S.
***Aloe vera* extract (300 mg/kg/day)**	0.535±0.036	N.S.
Chronically immobilized	0.647±0.023	<0.001
Chronically immobilized+*Aloe vera* extract (300 mg/kg/day)	0.616±0.044	N.S
Acutely immobilized	0.640±0.025	<0.001
Acutely immobilized+ *Aloe vera* extract (300 mg/kg/day)	0.622±0.027	N.S.
Chronically immobilized+normal saline	0.641±0.018	<0.001
Acutely immobilized+normal saline	0.642±0.026	<0.001

N.S. indicates non significant difference.

4. Discussion

Our findings clearly indicated that immobilization stress results in enhanced serum CEA level whether the stress is acute or chronic. Consistent with our finding, there are reports suggesting that stress can increase tumor growth and tumor marker expression [26, 27]. It has also been shown that chronic stress increases susceptibility for developing pancreatitis in rats, which involves TNF-α (tumor necrosis factor-alpha) sensitization of pancreatic acinar cells to undergo injury [28]. However, in contrast to our finding there are studies indicating that chronic stress can strengthen the immune system [15], thereby may cause a resistance to tumorogenesis and forestall against elevating of serum tumor marker levels.

Our findings have also shown that despite significant increasing of serum CEA levels in chronically and acutely immobilized rats, there was no significance difference in serum CEA levels of chronically or acutely immobilized *Aloe vera* extract received rats compared with control animals. This finding suggests that administration of *Aloe vera* leaf extract withstands against increasing of CEA level caused by immobilization stress, indicating the protective role of *Aloe vera* leaf extract against potentially tumorigenic effects of immobilization stress. Our data highlight for the first time, to our knowledge, the protective effects of *Aloe vera* leaf extract on serum CEA level enhancement induced by immobilization stress. In line with this finding, studies have found that *Aloe vera* extract has a protective role in different organs [29].

The main mode of mechanism of action, however, in one hand, can be thought to be on the reducing effect of immobilization stress on cytotoxic T lymphocytes and natural killer cells (NK cells) [30] which makes body susceptible to numerous diseases and may result in pathological outcomes followed by increased serum level of tumor markers [31]. On the other hand, *Aloe vera* plant has more than 60 medicinal active elements [32] and anti-cancer components such as anthraquinones [33] which can suppress tumor progression [34] and forestall against increasing of tumor markers levels in blood.

In conclusion, our findings indicate that immobilization stress has tumorigenic potential to increase serum CEA level and *Aloe vera* leaf extract administration can withstand against, suggesting the protective effects of *Aloe vera* leaf extract against tumorigenic conditions. However, further clinical research is required to confirm this finding.

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Authors' Contributions

This work is a product of the intellectual environment of the whole team; and that all members have contributed in various degrees to the analytical methods used, to the research concept, and to the experiment design. All authors have contributed to, seen and approved the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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