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The effect of hydro alcoholic extract of garden sage on liver enzymes in mice under heat stress

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ABSTRACT

Using pharmaceutical plants in reducing stress can play key role in preventing damages of heat stress. The goal of this study was investigating the anti-stress effects of garden sage (Salvia officinalis)in mice. Fifty mature mice were studied in five groups including control, 0, 100, 200, and 400 mg/kg weight of body of hydro alcoholic extract in drink water. Mice were under heat stress (36±0.5° C) for four hours a day. At end of period, blood samples were prepared and liver enzymes AST, ALT, and ALP were studied. According to results, ALP was decreased in zero groupswhereas100 mg/kg compensated the reduction (p< 0.05). AST amount was not different in treatment groups. The amount of ALT was decreased in zero group but 200 and 400 mg/kg doses compensated the reduction (p< 0.05). On the whole, hydro alcoholic extract of garden sage could reduce negative effects of heat stress on liver enzymes does dependently.

Keywords: heat stress, garden sage, AST, ALT, ALP

INTRODUCTION

Heat stress is one of ranchers especially in tropical regions. High evaporation from the surface of the body is the main mechanism of defense against rising temperatures. Whenthe temperaturesuddenly or more than tolerance level of animalrises, homeostasis of body will be lost,appetiteand reproduction will be decreased and even death may be occurred [1].

Increase in temperature and humidity in summer can cause physiological and behavior changes in the animal. There aremanymanagement techniques to deal withheat stress including proper nutritionandinclusion ofvitamins andantioxidants andmineralsin the diet [2].

Using pharmaceutical plants in reducing stress can play key role in preventing damages of heat stress. These plants have been used from ancient times and the benefits of them are obvious.

Garden sage (*Salvia officinalis*) from labiatcaefamily is a pharmaceutical plant with anti-stress effects. Leaves of this plant are tonic and energy provider due to having essence and tannin. Furthermore, it is diuretic, anticonvulsant, antipyretic, facilitatesdigestion, and lowers bloodsugar. Anti-oxidant properties of garden sage are ascribed to phenolic compounds like touon, sineol, and camphor[3]. The extract of this plant is used in external use to heal and disinfect wounds. The highest essence is obtained in flowering time and the most important components of essence are: Alpha terpineol11.13%, linalin acetate 23.08%, and linalool 30%. Also, beautifulleaves andflowersof this plantand high smell atflowering stage,and considering phenolic compoundswiththe ability toactas anelectrondonorand the ability tochelatemetalionsproduced,these compounds can be absorbed or inhibit free radicals andantioxidant activity ofsage is possibly due to itsphenolic compounds [4].The mostwell-knowncomponentsof this plantareT-cadinolandCaryophylleneoxide[5].

Heat stress increases free radicals and these radicals cause various diseases. Free radicals are atoms with one electron in outer ring,high oxidation power and abilityfor damage to proteins, DNA and vital components of cell including lipids and enzymes. Reactive oxygen species are from free radicals which areproduced in unbalanced intracellular redox reactions. Accumulation of these matters is an important reason of appearance of many diseases [6]. Live organisms have complicated anti-oxidant system to defend against active species of free radicals and reducing their destructive effects. Anti-oxidant system acts as enzymatic and non-enzymatic antioxidant defense system. Anti-oxidant compounds which are extant in foods are vitamins, poly phenols and carotenes[7].

According to our searches, there is no scientific study about the effects of this plant on liver enzymes under heat stress. Therefore, this research was carried out to investigate the effects of garden sage's extract on prevention of changes in liver enzymes under heat stress.

MATERIALS AND METHODS

Fifty female mice by mean weight of 30 to 35 g were prepared and kept for 15 day to adapt to environment.

Flowering branches of garden sage were dried and grinded. Hydro alcoholic extract was prepared to use in 100, 200 and 400 mg/kg of body weight doses.

Mice were divided in five groups (with 10 members in each group) including: control group to determine the base levels of liver enzymes, zero group which experienced the heat stress without any extract, and three treatment groups which received 100, 200 and 400 mg/kg extract in drink water.

Heat stress was enforced using electrical heaters and temperature was adjusted about 36.5±0.5$^{\circ C}$. Control group was under 22-24 °Ctemperature. After 30 days, blood samples were taken in heparin tubes and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) enzymes of plasma were measured in laboratory.

- **Statistical analysis**

Obtained data were analyzed using SPSS program and LSD test at 5% probability level.

RESULTS

ALP amount was different significantly ($p<0.05$) in control and zero groups. Using 400 mg/kg of extract decreased ALP ($p<0.05$) but 100 mg/kg compensated this reduction (Figure 1).

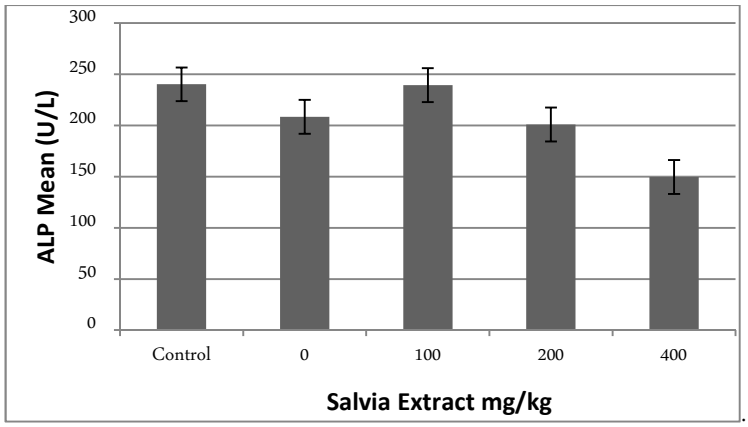

Fig 1: Mean comparison of ALP in all groups

AST amount was not different in various treatment groups at 5% probability level (Figure 2).

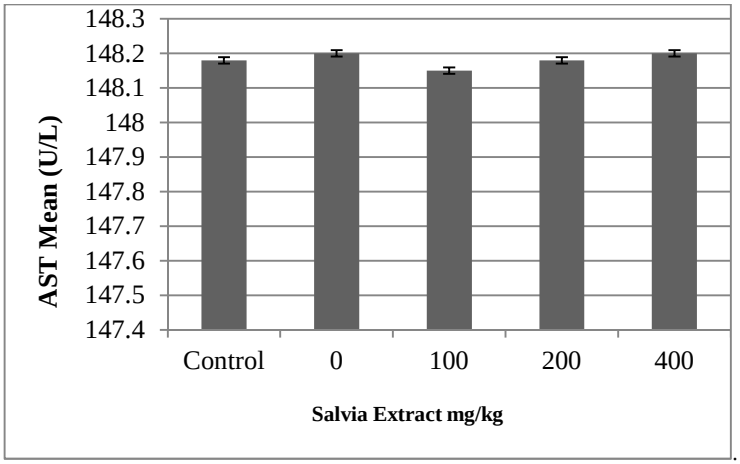

Fig 2: Mean comparison of AST in all groups

Difference of ALT levels in control and zero groups was significant ($p<0.05$). The amounts of this enzyme in 200 and 400 mg doses were significantly different from zero doses and were close to control group (Figure 3).

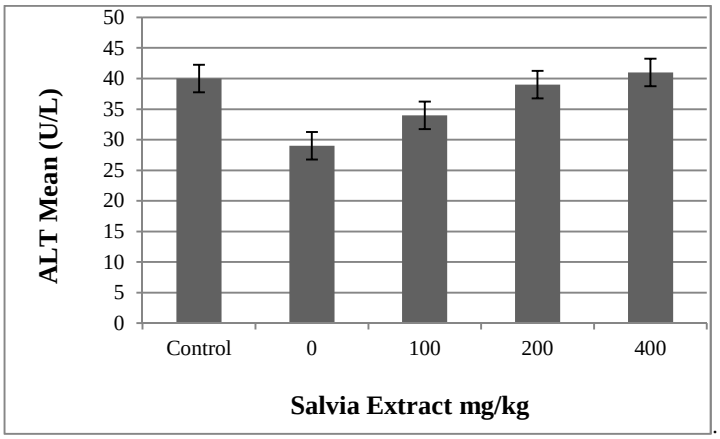

Fig 3: Mean comparison of ALT in all groups

DISCUSSION

The difference between control and zero groups for ALP enzyme indicates that heat stress reduced this enzyme. Also, 200 and 400 mg doses were significantly different from control group ($p<0.05$) and between these twogroups,the doseof400reduce theALP more.The difference between 100 and200mg/kgdoses withzerodose groupswas not significant, but the difference was significantbetween thedoses of 400mg/kg andzero ($P<0.05$)and reducedALP. Thedifferencesareshownin Figure 1.

AST amount was not different in various experimental groups thatmean heat stress didn't have a direct effect on this enzyme changes.

Increase in liver enzymes has been introduced as reason of damages to liver cells. Increase is AST is a sensitivespecific indicator of liver cells injuries [8]. ALT enzyme is more specific indicator of liver injuries[9], and high levels of ALP, and AST enzymes and bilirubin are symptoms of liver injuries [10].

ALT is a gluconeogenicenzyme. Along diabetes, which insulin signal is reduced this enzyme will be increased. This increase can even not be related to liver injuries [8]. Law etal. researches showed that flavonoids reduce plasma sugar.

Phenolic compounds control the activity of HMG-COA reductasewhich leads to reduction of liver cholestrole and prohibits fattyliver;therefore reduce liver enzymes levels in plasma [11].

Live organisms have wide complicated anti-oxidant system to defend against active species of free radicals and reducing their destructive effects. Enzymatic defense system like superoxide dismutase, glutathione peroxidase, catalase and non-enzymatic system such as food anti-oxidants (vitamins, poly phenols and carotenes) are from the most important defense systems of body to adjust the amount of reactive oxygen species under stress [7]including hydrogen peroxide, oxygen active forms (ROS), superoxide radicals, hydroxyl radicals. These matters which are derived fromthe metabolism ofoxygenin theintracellularredoximbalance, can damage large molecules of cells like proteins, fats and DNA severely [6].

Oxidative stresses in biosystems were proposed in 1995 by Cayseet al. which announced oxidative stress is caused by an imbalance in the pro oxidant/ antioxidant system [7]. Anti-oxidant properties of garden sage are ascribed to phenolic compounds like touon, sineol, and camphor [1].

ALT is a specific enzyme which is increased only in liver diseases and AST is increased in Liver parenchymal injuries as well as damage to the heart muscle. When the permeability of liver cells membranesis increased due to injuries, these enzymes will be releasedin bloodmoreand more [8].

CONCLUSION

Whenever heat stress cause lower movement and metabolism, protein catabolism and gluconeogenesis will be decreased and the amount of these enzymes will be reduced. Results showed that garden sage's extract compensated this deficit.

Considering that AST amount was not changed significantly in this study it can be said that liver tissue was not damaged by heat stress.

Significant ALT reduction showed reduction in liver metabolism which this difference was increased in 200 and 400 doses of garden sage and this dose of extract caused more metabolisms and shows compensation the reduction.
The difference between ALP amount of zero group and control group was significant which shows again lower general metabolism of liver.On the whole, garden sage extract in 400 mg/kg dose had the best effect on reducing negative effects of heat stress on liver enzymes and could return these enzymes to normal metabolismof body.

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