World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

DETECTION OF HEAT SHOCK PROTEIN 70 IN GASTROINTESTINAL ORGANS IN EXPERIMENTAL FLUOROSIS BY ELISA

Shashi A.* and Sukhmanjeet Kaur

Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147002, India.

***Corresponding Author: Dr. Shashi Aggarwal**
Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147002, India.

Article Received on 21/06/2024 **Article Revised on 11/07/2024** **Article Accepted on 31/07/2024**

ABSTRACT

Background: The essential role that inducible heat shock protein 70 plays in sustaining gastrointestinal deficits may be explained by its multiple functions as an immunomodulatory and cytoprotective protein. The present study used ELISA to detect heat shock protein 70 in the gastrointestinal organs in fluorosis and therapeutic impact of *Boerhaavia diffusa* L. **Materials and Methods:** Thirty six rats were divided randomly into six groups with six rats in each group. Group-1 (C1) received deionized water while Group-2 was administered with 600 mg NaF/kg b.w./day for 40 days via oral gavage. Group- 3 (C2) and Group- 4 (C4) were administered 250 mg and 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days respectively. While Group-5 and Group-6 received 600 mg of sodium fluoride for 40 days were post-treated with 250 and 500 mg/kg b.w./day of *Boerhaavia diffusa* L. for 20 days. The rats were sacrificed and samples from stomach, small intestine and large intestine were taken out. HSP 70 protein expression was then analyzed using ELISA. **Results:** The results revealed that in stomach, small intestine and large intestine the rats exposed to 600 mg/kg b.w./day of NaF had higher mean levels of HSP 70 as compared to control group. After post-treatment with *Boerhaavia diffusa* L. for 20 days, the tissues of fluoridated rats had comparatively lower mean levels of HSP 70. **Conclusion:** These findings demonstrated a significant relationship between the level of fluoride in gastrointestinal tissue and HSP 70 in rats. Furthermore, this research provides evidence supporting the reduction of fluoride toxicity and promotes improved well-being for animals.

KEYWORDS: *Boerhaavia diffusa* L., ELISA, Gastrointestinal organs, Heat shock protein 70, Sodium fluoride, Wistar albino rats.

INTRODUCTION

Endemic fluorosis is a public health problem in India due to high fluoride concentration in the ground water.[1] However, long term fluoride ingestion also impairs soft tissues. Sodium fluoride is a common fluoride contaminant that has been shown to have negative effects on the gastrointestinal tract as well as other physiological systems. The gastrointestinal system is thought to be the primary route of fluoride exposure, receiving various quantities of fluoride via food and water consumption on a daily basis.

The gastrointestinal tract is the main route of exposure to fluoride.[2] Early signs of fluoride toxicity include symptoms such as vomiting, abdominal pain, nausea, and diarrhea.[3,5]

One of the main causes of oxidative stress is heat stress. Heat shock promotes an increase in oxidative stress. Heat shock proteins are a class of proteins that evolve in response to a variety of physical, chemical, or biological stresses, especially exposure to the heat.[6]

HSP 70 plays a crucial role in managing various stresses such as increase temperature, chemotherapeutic agents etc. One of the myriad functions of the molecular chaperone HSP 70 is preserving cellular homeostasis[7] and acting as a strong buffer against cellular stress.[8] The gastrointestinal tract is particularly responsive to stressors which can cause a variety of changes including alteration of the normal, protective microbiota[9] and intestinal epithelium.[10]

The *Boerhaavia diffusa* L. a diuretic and laxative, is given for the treatment of ascites, jaundice and anasarca. Owing to its therapeutic properties, gastrointestinal disorders have been treated with it in particular. Roots and leaves of this plant have been widely used in the medicine to treat several illnesses including those

affecting the gastrointestinal tract such as dyspepsia and abdominal pain.[11]

Thus, the current investigation was carried out to evaluate the therapeutic effects of *Boerhaavia diffusa* L. on detrimental impacts of fluoride induced changes in HSP 70 in the gastrointestinal tract of rat.

Preparation of plant extract

The leaf extract of *Boerhaavia diffusa* L. was prepared by the method of Narendhirakannan *et al*.[12]

MATERIALS AND METHODS

Experimental design

Wistar albino rats weighing 150-200 g were housed in polypropylene cages with stainless steel grill tops, fed with standard commercial rat pellet diet (Hindustan Lever Limited, Mumbai, India) and water was given *ad libitum*.

After acclimatization for one week, the animals were divided into six groups having six rats in each. Group 1 served as control (C1) receiving deionized water. Group II was orally administered with 600 mg/kg b.w./day of sodium fluoride for 40 days. Group III (C3) and Group IV were administered with 250 and 500 mg/ kg b.w./day of *Boerhaavia diffusa* L. for 20 days respectively. Group V and Group VI were firstly exposed to 600 mg of NaF and then post-treated with 250 and 500 mg of leaf extract for 20 days.

Quantitative detection of HSP 70 by ELISA

The estimation of heat shock protein 70 in the gastrointestinal tissue of control, fluoride and leaf extract of *Boerhaavia diffusa* L. treated rats was done by using ELISA kit (Elabscience).

Statistical analysis

Results were expressed as Mean ± SD. All analysis was performed using SPSS 20.0 statistical software (IBM). The parameters were analyzed by one-way analysis of variance (ANOVA) followed by Post hoc Bonferroni multiple comparison test. The results were considered significant at P<0.05. The relationship between level of gastrointestinal tissue fluoride and HSP 70 was determined by Pearson's bivariate correlation and simple linear regression.

RESULTS

Stomach

The mean level of heat shock protein 70 in the gastric tissue of fluoridated rats was significantly (F= 130.421, P< 0.0001) elevated by +82.421% as compared to control. The level of HSP 70 decreased significantly (P< 0.0001) by -23.217% and -49.265% in fluorotic rats treated with 250 mg/kg b.w./day and 500 mg/kg b.w./day leaf extract of *Boerhaavia diffusa* L. respectively (Fig. 1).

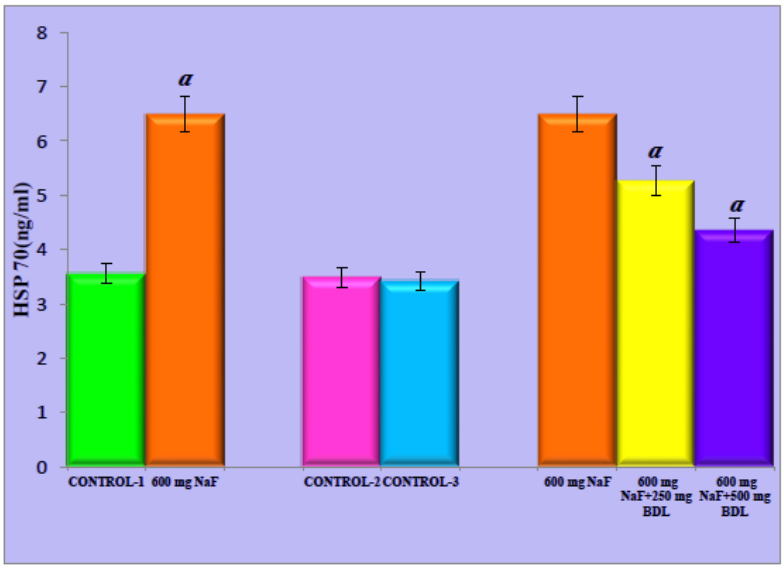

Fig. 1: Mean level of HSP 70 (ng/ml) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.0001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

Post-hoc Bonferroni multiple comparison test after ANOVA indicated a significant (P< 0.0001) increase in the level of HSP 70 in stomach tissue between and within groups (95%CI= -3.423 to -2.447) treated with fluoride for 40 days and decline in post-treated groups (95%CI= -3.500 to -0.432).

Small intestine

The mean level of HSP 70 in the small intestine of fluorotic rats rats was significantly (F= 124.376, P< 0.0001) elevated by +37.647% as compared to control. The level of HSP 70 in the small intestine showed a significant decrease (P< 0.0001) by -22.429% and -

33.221% in fluorotic rats post-treated with leaf extract of *Boerhaavia diffusa* L. (Fig. 2).

Post-hoc Bonferroni multiple comparison test after ANOVA indicated a significant (P< 0.0001) increase in the level of HSP 70 in small intestine between and within groups (95%CI= -3.452 to -2.467) treated with 600 mg NaF/kg b.w. for 40 days and revealed significant decline in post-treated groups (95%CI= -3.508 to -0.223).

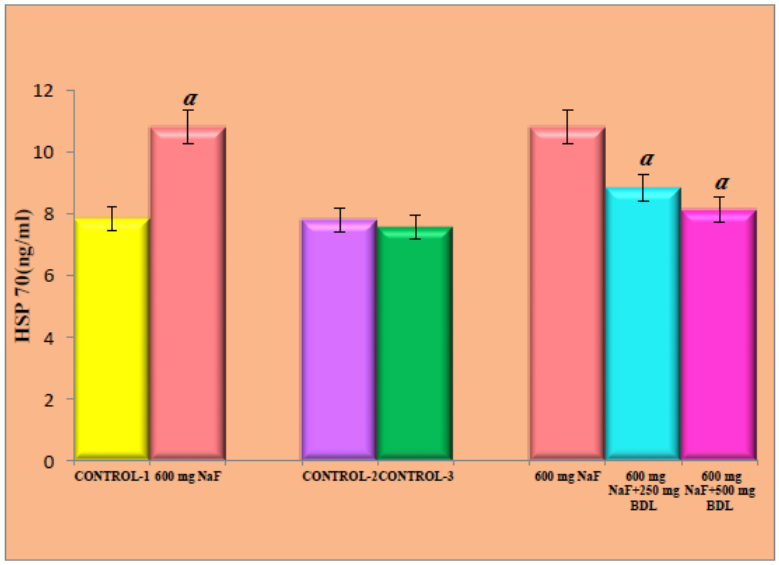

Fig. 2: Mean level of HSP 70 (ng/ml) in small intestine of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.0001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

Large intestine

The mean level of heat shock protein 70 in the large intestine of fluoridated rats showed significant (F= 21.138, P< 0.0001) increase by +41.780% as compared to control. The level of HSP 70 in large intestine displayed a significant decline (P< 0.0001) with mitigation of 250 mg/ kg b.w./day and 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. by -30.716% and -25.311% (Fig. 3) in fluorotic rats.

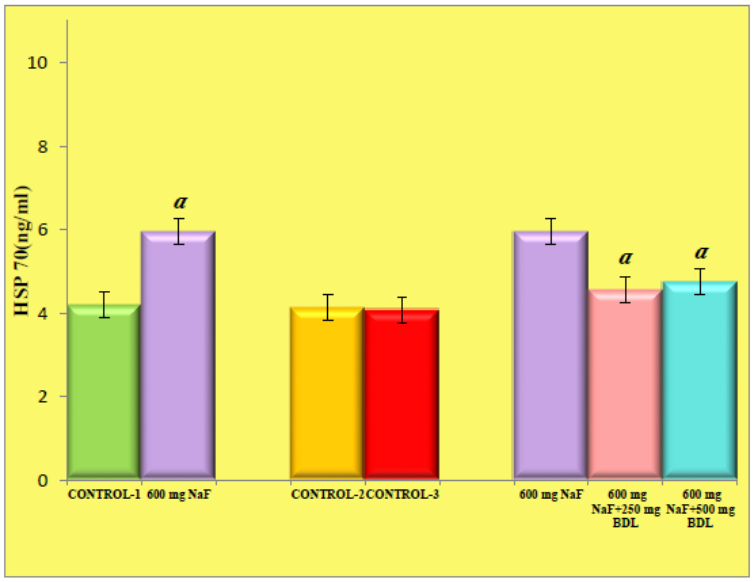

Fig. 3: Mean level of HSP 70 (ng/ml) in large intestine of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.0001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups.One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

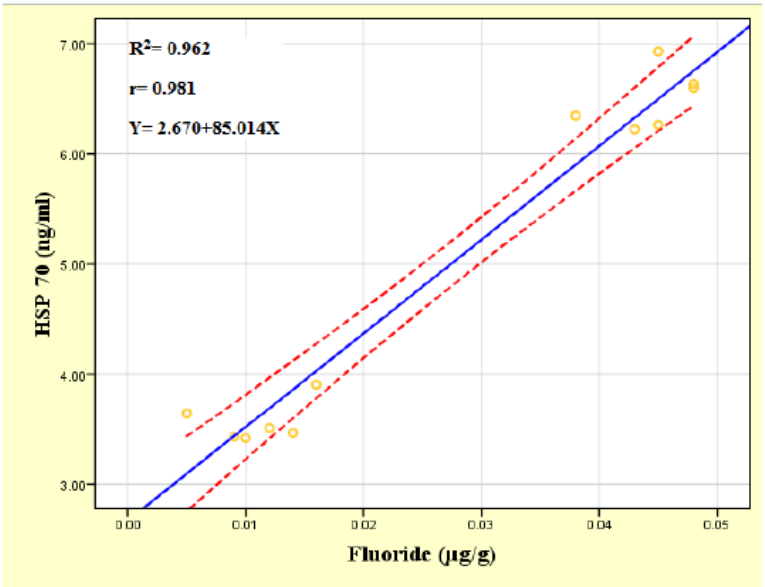

Fig. 4: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of stomach fluoride (µg/g) and HSP 70 (ng/ml) in test rats after 40 days of fluoride treatment.

Post-hoc Bonferroni multiple comparison test after ANOVA exhibited significant ($p<0.0001$) increase in the level of HSP 70 in large intestine between and within groups (95%CI= -2.442 to -1.058) treated with fluoride for 40 days and exhibited a significant fall in post-treated groups (95%CI= -2.503 to -0.887).

Correlation analysis

Pearson's bivariate correlation and simple linear regression analysis demonstrated significant ($P< 0.0001$) positive relationship between levels of fluoride and HSP 70 in stomach (R^2= 0.962, Pearson r = 0.981; Y= 2.670+85.014X; Fig. 4), small intestine (R^2= 0.952, Pearson r = 0.976; Y= 6.618+91.000X; Fig. 5) and large intestine (R^2= 0.962, Pearson r = 0.981; Y= 3.620+48.604X; Fig. 6) of rats after 40 days of fluoride exposure.

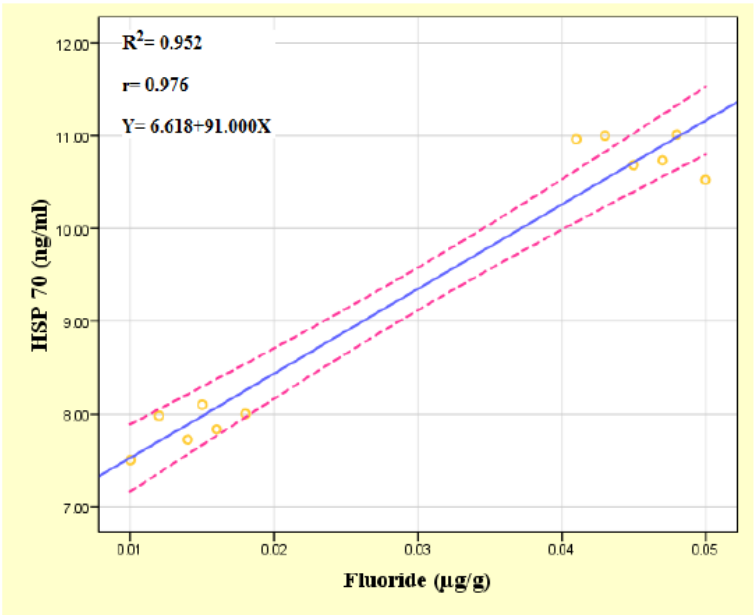

Fig. 5: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of small intestine fluoride (µg/g) and HSP 70 (ng/ml) in test rats after 40 days of fluoride treatment.

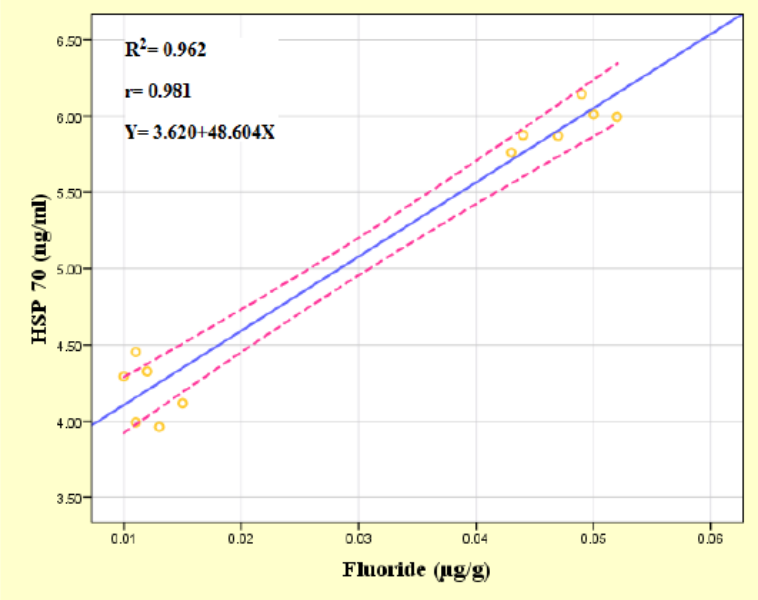

Fig. 6: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of large intestine fluoride (µg/g) and HSP 70 (ng/ml) in test rats after 40 days of fluoride treatment.

DISCUSSION

The present study demonstrated that the expression of HSP 70 in the gastrointestinal organs was upregulated in fluoridated rats as compared to control in response to stress. These results are in agreement with the study of Gu *et al*.[13]

One of the HSP family's most prevalent and well-studied proteins is HSP 70. Many endogenous physiological or environmental stresses, including heat, ethanol, caloric restriction, and bacterial infection in gastrointestinal epithelial cells, increase the expression of HSP 70.[14,16]

Heat shock protein 70 plays a crucial role in preserving cell integrity and in preventing and repairing damage caused by various stressors to cells and organs. Its expression is activated by structural damage to cell proteins, primarily due to thiol oxidation and general disturbances in the cellular redox status. HSP 70 is vital for cellular defense against different stresses, including heat shock, oxidative stress, and exposure to toxins and infections.

The heat stress greatly raised the expression of HSP 70 mRNA in the rat's digestive system. The intestine is vulnerable to environmental variables such as hypoxia, heat stress, and others that can harm the mucosa.

After transportation, rats exhibit increased levels of HSP 70 in their intestines.[17] This increase in HSP70 expression is a protective response to the stress caused by transport. The production of heat shock proteins can be triggered by various chemical and physical stressors.[18] Weaning has been demonstrated to result in abberrent spatial and temporal patterns of HSP 70 expression in the gastrointestinal tract of pigs.[19]

HSP 70 reduces the structural and oxidative damage of the intestinal mucosa brought on by heat stress, which raises the intake of HSP 70 while lowering the protein content. As the effects of heat stress intensifies, high levels of HSP 70 expression are produced, which boosts the activity of digestive enzymes while reducing lipid peroxidation in the intestinal mucosa.[20]

The distribution of HSP 70 may be correlated with the molecular chaperones protective role.[21,22] The level of ATP in cells considerably affects the expression of HSP 70. The deleterious effects of ATP depletion on cell homeostasis are well-known. Protein aggregation, cytoskeleton collapse, and loss of ionic equilibrium are some of these impacts. The result of the stress reaction and subsequent HSP 70 buildup is an ATP-sparing effect.[23]

Hence, HSP 70 is essential for maintaining mitochondrial function, integrity, and capacity for ATP synthesis, all of which are crucial for determining whether or not a cell will survive when exposed to damaging stress.

CONCLUSION

In conclusion, the level of heat shock protein 70 was increased in stomach, small intestine and large intestine of rats and the study implies that the administration of leaf extract of *Boerhaavia diffusa* L. plays an important role in inhibiting fluoride induced gastrointestinal damage.

ETHICAL ASPECTS

The experimental protocols were performed under the approval of Institutional Animal Ethical Committee of Punjabi University, Patiala (Animal maintenance and Registration No. 107/GO/ReBi/S/99/CPCSEA/2017-41).

ACKNOWLEDGEMENT
This work was supported by a grant from National Fellowship for Scheduled Caste Students (NFSC) program, University Grants Commission, Govt. of India.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

REFRENCES

1. Shashi, A., Kumar, M. and Bhardwaj, M. 2008. Incidence of skeletal deformities in endemic fluorosis. Trop. Doc., 38(4): 231-233. https://doi.org/10.1258/td.2008.070379
2. Zheng, Y., Wu, J., Ng, J. C., Wang, G. and Lian, W. The absorption and excretion of fluoride and arsenic in humans. Toxicol. Lett., 2002; 133(1): 77-82. https://doi.org/10.1016/s0378-4274(02)00082-6
3. Susheela, A. K., Kumar, A., Bhatnagar, M. and Bahudur, R. Prevalence of endemic fluorosis with gastrointestinal manifestations in people living in some North-Indian villages. Fluoride, 1993; 26: 97–104. https://doi.org/10.13141/RG.2.2.26997.17122
4. Das, T.K., Susheela, A.K., Gupta, I.P., Dasarathy, S. and Tandon, R.K. Toxic effects of chronic fluoride ingestion of the upper gastrointestinal tract. J. Clin. Gastroenterol., 1994; 18(3): 194-199. https://doi.org/10.1097/00004836-199404000-00004
5. Sharma, J.D., Jain, P. and Sohu, D. Gastric discomforts from fluoride in drinking water in Sanganer Tehsil, Rajasthan, India. Fluoride, 2009; 42(4): 286-291.
6. Ganter, M. T., L. B. Ware, M. Howard, J. Roux, B. Gartland, M.A. Matthay, M. Fleshner, and J. Pittet. Extracellular heat shock protein 72 is a marker of the stress protein response in acute lung injury. Am. J. Physiol. Lung Cell. Mol. Physiol., 2006; 291: 354–361. https://doi.org/10.1152/ajplung.00405.2005
7. Mallouk Y, Taussat M, Bonventre J. and Polla B. Heat shock protein 70 and ATP as partners in cell homeostasis. Int. J. Mol. Med., 1999; 4(5): 463-474. https://doi.org/10.3892/ijmm.4.5.463
8. Murphy, M. E. The HSP 70 family and Cancer. Carcinog., 2013; 34(6): 1181-1188. https://doi.org/10.1093/carcin/bgt111
9. Burkholder, K.M., Thompson, K.L., Einstein, M.E., Applegate, T.J. and Patterson, J.A. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella enteritidis* colonization in broilers. Poult. Sci., 2008; 87: 1734-1741. https://doi.org/10.3382/ps.2008-00107
10. Quinteiro-Filho, W.M., Ribeiro, A., Ferraz-de-Paula, Pinheiro, M.L., Sakai, M., Sa, L.R.M., Ferreira, A.J.P. and Palermo-Neto, J. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poult. Sci., 2010; 89: 1905-1915. https://doi.org/10.3382/ps.2010-00812
11. Akhter, F., Hashim, A., Khan, M.S., Ahmad, S., Iqbal, D., Srivastava, A.K. and Siddiqui, M.H. Antioxidant, α-amylase inhibitory and oxidative DNA damage protective property of *Boerhaavia diffusa* (Linn.) root. South Asian Journal of Botany, 2013; 88: 265-272. https://doi.org/10.1016/j.sajb.2013.06.024
12. Narendhirakannan, R.T., Subramanian, S. and Kandaswamy, M. Biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin induced diabetes in experimental rats. Clin. Exp. Pharmacol., 2006; 33: 1150-1157. https://doi.org/10.1111/j.1440-1681.2006.04507
13. Gu, X. H., Hao, Y. and Wang, X. L. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. Poult. Sci., 2012; 91: 790-799. https://doi.org/10.3382/ps.2011-01628
14. Musch, MW., Sugi, K., Straus, D. and Chang, E.B. Heat-shock protein 72 protects against oxidant-induced injury of barrier function of human colonic epithelial Caco2/bbe cells. Gastroenterol., 1999; 117: 115–122. https://doi.org/10.1016/s0016-5085(99)70557-3
15. Ren, H., Musch., M.W., Kojima, K., Boone, D., Ma, A. and Chang, E.B. Short-chain fatty acids induce intestinal epithelial heat shock protein 25 expression in rats and IEC 18 cells. Gastroenterol., 2001; 121: 631–639. https://doi.org/10.1053/gast.2001.27028
16. Wada, I., Otaka, M. and Jin, M. Expression of HSP 72 in the gastric mucosa is regulated by gastric acid in rats—correlation of HSP 72 expression with mucosal protection. Biochem. Bioph. Res. Co., 2006; 349: 611–618. https://doi.org/10.1016/j.bbrc.2006.08.088
17. Wan, C., Yin, P., Xu, X., Liu, M., He, S., Song, S., Liu, F. and Xu, J. Effect of simulated transport stress on the rat small intestine: a morphological and gene expression study. Res Vet. Sci., 2014; 96: 355–64. https://doi.org/10.1016/j.rvsc.2014.01.008
18. Morimoto, R.I. Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. Genes Dev., 2008; 22: 427–1438. https://doi.org/10.1101/gad.1657108
19. David, J.C., Grognet, J.F. and Lalles, J.P. Weaning affects the expression of heat shock proteins in different regions of the gastrointestinal tract of piglets. J Nutr., 2002; 132: 2551–2561. https://doi.org/10.1093/jn/132.9.2551
20. Hao, Y., Gu, X. H. and Wang, X. L. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 1. Intestinal structure and digestive function. Poult. Sci., 2012; 91(4): 781-789. https://doi.org/10.3382/ps.2011-01627
21. Arvans, D.L., Vavricka, S.R. and Ren, H.Y. Luminal bacterial flora determines physiological expression of intestinal epithelial cytoprotective heat shock proteins 25 and 72. Am. J. Physiol.

Gastrointest. Liver Physiol., 2005; 288: 696–704. https://doi.org/10.1152/ajpgi.00206.2004
22. Yu, J.M., Bao, E.D., Yan, J.Y. and Lei, L. Expression and localization of Hsps in the heart and blood vessel of heat-stressed broilers. Cell Stress Chaperones, 2008; 13: 327–335. https://doi.org/10.1007/s12192-008-0031-7
23. Kabakov, A.E. and Gabai, V.L. Heat shock proteins and cytoprotection: ATP-deprived mammalian cells. Austin: RG Landes, 1997.