Alkhedaide et al., Afr J Tradit Complement Altern Med., (2017) 14 (5): 96-103
https://doi.org/10.21010/ajtcam.v14i5.13

PROTECTIVE EFFECT OF ONION EXTRACT AGAINST EXPERIMENTAL IMMUNE-SUPPRESSION IN WISTAR RATS: BIOLOGICAL AND MOLECULAR STUDY

Adel Alkhedaide[1]; Mohamed Mohamed Soliman [1,2*], Tamer Ahmed Ismail [2,3]

[1]Department of Medical Laboratories, Faculty of Applied Medical Sciences, Taif 21944, Saudi Arabia.
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Benha 13736, Egypt.
[3]Department of Physiology,Faculty of Veterinary Medicine, Zagazig University, Zagazig 4655, Egypt.

Corresponding Author E-mail: mohamed.soliman@fvtm.bu.edu.eg

Article History
Received: 12, March. 2017
Revised Received: 07, May. 2017
Accepted: 10, May. 2017
Published Online: 01, Oct. 2017

Abstract

Background: The wrong use of drugs results in disturbances in the immunity that affect human health. These drugs have side effects that may lead to death because of lake of immunity. Human beings need to use natural products to strength the immune system and avoid such side effects. Of these products is the onion that used to strength the immune system. This study was conducted to study the protective effect of onion extract on immune-suppressed rats and its impact on the expression level of cytokines, acute phase proteins and antioxidants compared to both control and immune suppressed groups.
Materials and Methods: Forty rats were divided into four groups (10 per group) control group (CNT), immune-suppressed group (DEXA) injected with dexamethasone at a dose of 5 mg per kg intraperitoneally (IP) twice daily for 3 days, onion extract administered group (OE) given orally at a dose of 500 mg per kg for 4 weeks. Group 4 (y), was given onion extract for a week then immune-suppressed with DEXA for 3 days then continued with OE for 3 weeks. Serum and RNA were extracted for examining the biochemical and genetic changes.
Results: Injection of dexamethasone decreased number of leukocytes with increase in the number of neutrophils and the decrease of all other types of white blood cells. Moreover, a decrease in antioxidant levels such as catalase, super oxide dismutase (SOD), and reduced glutathione (GSH) with an increase in the level malondialdehyde (MDA). In parallel, a decrease in serum levels of cytokines, such as TNF and IL-6, together with immunoglobulins (IgG and IgM), were reported in DEXA injected rats that were ameliorated by prior administration of OE. Gene expression analysis revealed that dexamethasone suppressed gene expression of antioxidants together with IL-1 and 8 while increased IL-10 mRNA expression. All of these changes have been normalized to the normal level by OE administration to DEXA injected rats.
Conclusion: The present findings clearly emphasize the medical importance of onions as immune-stimulants at genetic and cellular levels, and that they are good for human health.

Key Words: Onion extract; Dexamethasone; Immune suppression; Protective effect; Gene expression.

List of abbreviations: CAT: catalase; CNT: control; DEXA: dexamethasone; GAPDH: glyceraldhyde-3-phospate dehydrogenase; GCs: glucocorticoids; GPx: glutathione peroxidase; GSH: reduced glutathione; GST: glutathione-S-transferase; HPA: hypothalamic-pituitary-adrenal, Ig: immunoglobulin; IL-: interleukin; IP: intraperitoneally; MDA: malondialdehyde; OE: onion extract; RNA: ribonucleic acid; ROS: reactive oxygen species; SEM: standard error of means; SOD: superoxide dismutase; TBE: tris-borate EDTA; TNF-α: tumor necrosis factor alpha; WBC: white blood cell; α1-AGP: alpha-1 acid glycoprotein; α2-MG; alpha-2 macroglobulin.

Introduction

The immune system of humans protect the body against several disease-promoting factors and malignant cells. It regulates the body through helper and suppressor cells and soluble products. Recently it has been shown the contents of spices and nutrients have the ability to affect immune system (Kandil et al, 1987). The relationship between the immune system and nutrients found in spices has been reviewed comprehensively. Spices enhance/stimulate immune functions (Kandil et al, 1987). Disorders of the immune system can induce inflammatory diseases, autoimmune

diseases, immunodeficiency and cancer (Coussens and Werb, 2002; O'Byrne and Dalgleish, 2001). In humans, immunodeficiency can be induced by the use of immunosuppressive medication (O'Byrne and Dalgleish, 2001).

Immunosuppressive drugs can modulate the immune system (Patil et al, 2010; 2012) and affect variable gene transcription events (Perreti and D'Acquisto, 2009; De Bosscher et al, 2008). Glucocorticoids (GCs) are the most commonly used drugs used for treatment of chronic inflammatory diseases. They mediate their actions by binding to intracellular receptors, causing alteration in protein-protein interactions and consequently regulation of gene expressions (Van der Laan and Meijer, 2008). Dexamethasone (DEXA) is one of the frequent GCs used as experimental immuno- suppressive drug. The therapeutic dose of dexamethasone is 1–2 mg/kg (Harcourt-Brown, 2002), but under experimental conditions doses up to 10 mg/kg were able to induce immune suppression (Kesavan et al, 2005).

Onion is a member of the genus *Allium*, belonging to the family Alliaceae (Kesavan et al, 2005). Onion contains about 25 active compounds with similar therapeutic properties (Augusti, 1996). Several scientific studies confirmed that presence of onion in the diet can stimulates the immune system, reduces symptoms of diabetes mellitus and inhibits platelet aggregation (Augusti, 1996). Moreover, it prevents inflammatory processes and reduces risk of cancer of stomach and brain, reduces levels of cholesterol, triglycerides, reduces symptoms of osteoporosis, inhibits the proliferation breast cells and cancer cells of ovary and colon (Chisty et al, 1996; Sanderson et al, 1999). Therefore, the current study is conducted to check the protective effect of water extract of onion on different immune responses and on the expression of some genes related to immune response after immunosuppression of rats using DEXA.

Materials and Methods

Animals and design

All procedures of this study were approved by the Ethical Committee Office of the dean of scientific affairs of Taif University, Saudi Arabia (Project number 4737-437-1) for using animals and experimental procedures. Forty rats of Sprague Dawley rats with 8 weeks age, weighing (180–200 g) were used. Animals handled daily for adaptation and kept under eye observation for one week before the start of the experiment. The animals were kept at dark light cycle (12-h light /12-h) with free access to food and water. Animals were allocated into 4 groups, control group (CNT) without any treatment; DEXA; OE and OE + DEXA group. Groups received free access to food and water for consecutive 4 weeks. Dexamethasone was injected in a dose 5 mg per kg IP twice daily for 3 days, onion extract (OE) was administered orally at a dose of 500 mg per kg for 4 weeks. Group 4 (DEXA+OE), was given onion extract for a week then immune-suppressed with DEXA twice a day for 3 days then continued with OE for 3 weeks. Dose of DEXA was used based on study of Grace et al. (2013) while the dose of OE was stated based on previous study of Mirabeau and Samson (2012).

After 4 weeks, all rats were decapitated after inhalation of diethyl ether. Tissues and blood and were collected from decapitated rats. Blood was centrifuged for serum extraction (10 min at 4000 xg). For mRNA expression, liver samples were kept in Qiazol reagent for ribonucleic acid (RNA) extraction at -80 ˚C in deep freezer and gene expression. Serum was stored at -20 °C till biochemical assays.

Serum chemistry, Cytokines and immunoglobulins assays

Catalase, reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) activities were recorded using commercial spectrophotometric analysis kits (Bio-Diagnostic Company, Giza, Egypt) based on manufacture instruction manual as described previously (Alkhedaide et al., 2016). All procedures were carried out based on instruction manual protocols. For IL-6 measurments, we bought the kit for Rat IL-6 ELISA Kit (Interleukin-6) (ab100772) from Abcam, USA. For TNF-alpha, Rat TNF alpha ELISA Kit (ab46070). IgG and IgM were measured on serum samples using a radial immunodiffusion assay bought from Clini Lab, Al-Manial, Cairo, Egypt based on our previous study (*Soliman* et al, 2015).

Gene expression measurements

From liver tissue samples the total RNA was extracted as previously discussed (Soliman et al., 2015). The integrity of RNA was confirmed after electrophoresis in 1.5% denaturated agarose gel after staining with ethidium bromide. About 3 µg of total RNA and 0.5 ng oligo dT primer (Qiagen Valencia, CA, USA) were used for reverse transcription. Specific primers as shown in Table 1, were designed using Oligo-4 computer program, ordered and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea). All procedures for PCR analysis were explained in details in another study (Soliman et al, 2015). PCR was conducted in Bio-Rad T100^{TM} Thermal Cycler with the cycle sequence at 94°C for 5 minutes one cycle, followed by 29 cycles (Table 1). Each cycle consisted of denaturation at 94 °C for 1 min, annealing at the specific temperature (as seen in table 1) and extension at 72 °C for 1 min with an additional final extension at 72 °C for 7 min. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as a reference. Products of PCR were electrophoresed and visualized after running in 1.5% agarose (Bio Basic, Markham, ON, Canada) gel after staining with ethidium bromide in TBE buffer (Tris-Borate-EDTA) under UV light. Products of PCR were photographed by In Genius 3.0 gel documentation system (Syngene, Frederick, MD, USA). The band intensities were quantified using Image J software version 1.47 (http://imagej.en.softonic.com/).

Statistical analysis

Data are expressed as means ± standard errors of means (SEM) for different 10 rats per group. ANOVA and Fischer's post hoc test were used for this statistical analysis, with $p<0.05$ being considered as statistically significant.

Table 1: PCR conditions and primer sequence for examined genes.
Abbreviations for genes are GPx: glutathione peroxidase; GST: glutathione-S-transferase; IL-1β; interleukin-1 beta; IL-8 and 10: interleukin 8 and 10; α2-MG: alpha-2 macroglobulin; α1-AGP: alpha1- acid glycoprotein; GAPDH; glyceraldhyde-3-phosphate dehydrogenase.

Gene	Product size (bp)	Annealing (ºC)	Direction	Sequence (5'-3')
GPx	406	57	Sense	AAGGTGCTGCTCATTGAGAATG
			Antisense	CGTCTGGACCTACCAGGAACTT
GST	575	55	Sense	GCTGGAGTGGAGTTTGAAGAA
			Antisense	GTCCTGACCACGTCAACATAG
IL-1β	497	61	Sense	ATGGCAACCGTACCTGAACCCA
			Antisense	GCTCGAAAATGTCCCAGGAA
IL-10	320	57	Sense	GGAGTGAAGACCAAAGG
			Antisense	TCTCCCAGGGAATTCAAATG
IL-8	308	56	Sense	CTCCAGCCACACTCCAACAGA
			Antisense	CACCCTAACACAAAACAGAT
α2-MG	325	56	Sense	GCTCCTGTCTGTTTCCTTAGTT
			Antisense	ATTGGCCTTTCGTGGTTTAG
α1-AGP	230	55	Sense	GCTTTCCTCCTGACAACGCTG
			Antisense	GGCTTTTTGTTGTTTGCTTCTATTTC
GAPDH	309	52	Sense	AGATCCACAACGGATACATT
			Antisense	TCCCTCAAGATTGTCAGCAA

Results

Protective effect of onion extract on changes in white blood cell counts in immune-suppressed Wistar rats

As seen in table 2, dexamethasone injection decreased total white blood cell counts and OE significantly increased it. When OE administered prior to dexamethasone, it prevented this alteration. Of note dexamethasone induced neutrophilia and lymphopenia. OE administration normalized their secretion pattern (neutrophilia and lymphopenia). N/L ratio was increased in DEXA injected rats and normalized in OE+ DEXA administered group.

Table 2: Protective effects of onion extract on dexamethasone induced changes in white cell counts in immune suppressed rats.

	WBCes (10^3 x mm^3)	Neutrophil (10^3 x mm^3)	Lymphocytes (10^3 x mm^3)	Monocytes ($10^3/mm^3$)	Esinophiles ($10^3/mm^3$)	N/L ratio
Control	9.2 ± 0.5	2.55 ± 0.04	5.7 ± 0.3	0.27 ± 0.02	0.48 ± 0.03	0.45 ± 0.03
DEXA	6.3 ± 0.7*	3.80 ± 0.3*	1.6 ±0.3*	0.16 ± 0.01*	0.23 ± 0.04*	2.68 ± 0.33*
OE	13.4 ± 0.7	4.23 ± 0.18*	7.4±1.3*	0.37 ± 0.02	0.83 ± 0.29*	0.60 ± 0.09
OE + DEXA	8.9 ± 0.5#	2.70 ± 0.23#	3.7± 0.2#	0.41 ± 0.02#	0.84 ± 0.38#	0.72 ± 0.05#

Values are means ± SEM for different 10 rats per each treatment. Values are statistically significant at *$p<0.05$ Vs. control and #$p<0.05$ Vs. dexamethasone injected rats.

Protective effects of onion extract on dexamethasone induced changes in serum antioxidant levels in immune suppressed rats

DEXA injection increased serum MDA levels significantly, while it was decreased in both OE and OE+ DEXA injected groups. On parallel, DEXA injection decreased GSH, catalase and SOD activities. OE alone increased these antioxidant levels. When OE administered prior to DEXA injection it protected DEXA altered parameters as seen in table.3.

Table 3: Protective effects of onion extract on dexamethasone induced changes in serum antioxidant levels in immune suppressed rats.

	MDA (nmol/ml)	GSH (mg/dL)	SOD (U/ml)	Catalase (U/ml)
Control	16.8 ± 1.6	6.7 ± 0.3	213.1 ± 42	116.7 ± 18.6
DEXA	31.4 ± 2.9*	4.5 ± 0.1*	116.6 ± 19	56 ± 11.4
OE	18.2 ± 0.9	7.6 ± 0.4	293.6 ± 39	179 ± 18.3*
OE + DEXA	18.32 ± 1.9#	6.2 ± 0.2#	194 ± 9.6#	97 ± 6.3#

Values are means ± standard error (SEM) for 10 different rats per each experiment. Values are significant at *p<0.05 Vs. control and #p<0.05 Vs. dexamethasone injected rats.

Protective effects of onion extract on dexamethasone induced changes in serum IL-6, TNF- alpha, IgG and IgM levels in immune suppressed rats

DEXA injection decreased both IL-6 and TNF-alpha levels as seen in table 4, while OE alone increased them confirming its immunomodulatory effect. When OE administered prior to DEXA, it inhibited this decrease and maintained normal levels of these cytokines in a way to protect rats from immunosuppressive effect of dexamethasone. Coinciding with cytokines findings, DEXA decreased IgG and IgM levels and OE counteracted this decrease when administered prior to DEXA and continued for 3 weeks (table 4).

Table 4: Protective effects of onion extract on dexamethasone induced changes in serum IL-6, TNF- alpha, IgG and IgM levels in immune suppressed rats.

	IL-6 (pg/ml)	TNF-α (pg/ml)	IgG (mg/dL)	IgM (mg/dL)
Control	116.8 ± 8.7	69.6 ± 4.2	801.3 ± 16.8	33.3 ± 18.6
DEXA	83.4 ± 3.9*	46 ± 12.4*	575.6 ± 49*	25.9 ± 2.2*
OE	125.8 ± 3.5	108.6 ± 4.8	875.6 ± 49	30.2 ± 2.2
OE + DEXA	101.2 ± 5.6#	84.2 ± 2.9#	779 ± 22.5#	31.7 ± 2.1#

Values are means ± standard error (SEM) for 10 different rats per each experiment. Values are significant at *p<0.05 Vs. control and #p<0.05 Vs. dexamethasone injected rats.

Protective effects of onion extract on dexamethasone induced changes on glutathione-S-transferase (GST) and peroxidase (GPx) expression in immune suppressed rats

As seen in figure1, DEXA down regulated mRNA expression of GST and GPx significantly compared to control rats. OE alone upregulated GST and GPx expression. Prior administration of OE protected rats from immune suppressive effect of dexamethasone.

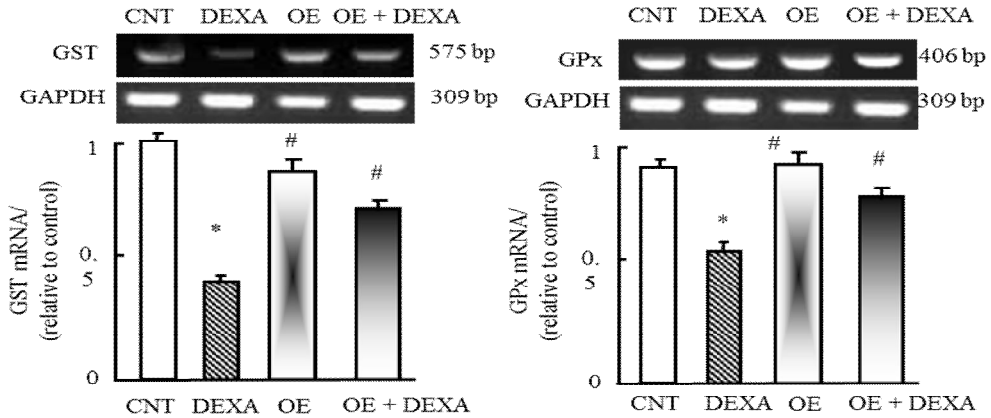

Figure 1: Protective effect of OE on changes in antioxidants expression induced by dexamethasone. RNA was extracted from liver tissues and the expressions of GST and GPx were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 10 rats. $^{*}P < 0.05$ Vs control group; $^{\#}P < 0.05$ Vs DEXA group. Upper panels are mRNA expression of examined genes. Lower columns are densitometric analysis of gene expression.

Protective effects of onion extract on dexamethasone induced changes on cytokines expression in immune suppressed rats

Next, we examined the protective effect of OE on variations on cytokines expression associated with DEXA injection. As seen in figure 2, DEXA induced down expression of IL-1β (Figure 2A) and upregulation in IL-8 and IL-10 (Figure 2 B and C). OE alone upregulated IL-1β and down regulated IL-8 and not alters IL-10 mRNA expression level. Prior administration of OE to DEXA administered rats ameliorated these changes induced by dexamethasone.

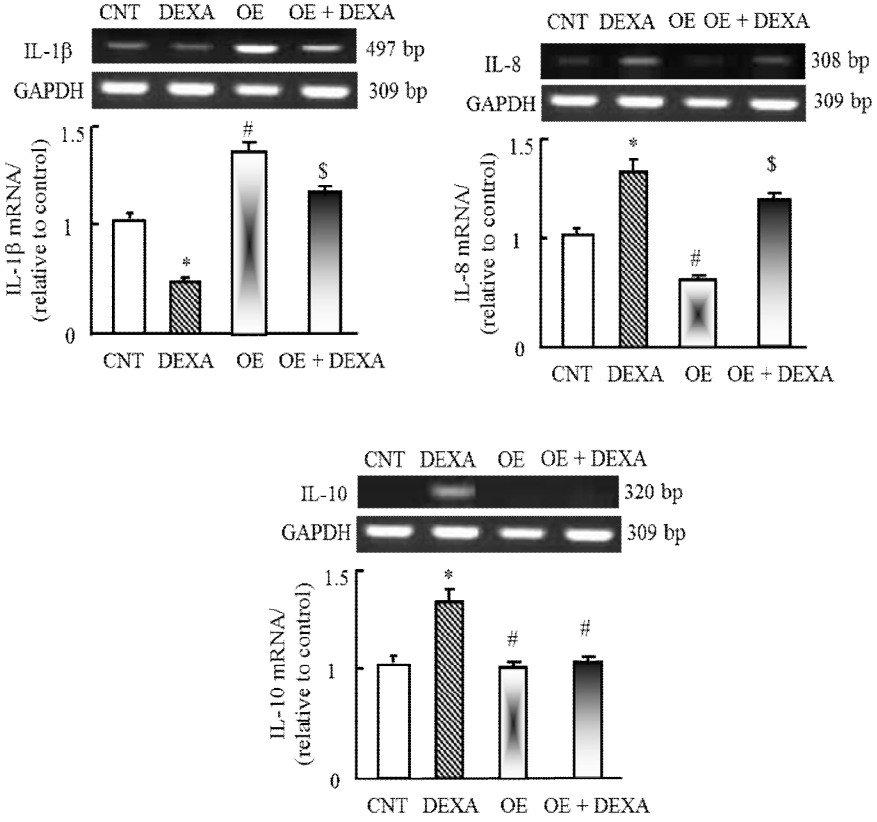

Figure 2: Protective effect of OE on changes in cytokines expression induced by dexamethasone. RNA was extracted from liver tissues and the expressions of IL-1β, Il-8 and IL-10 were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 10 rats. $^{*}P < 0.05$ Vs control group; $^{\#}P < 0.05$ Vs DEXA group and $^{\$}P < 0.05$ Vs OE group. Upper panels are mRNA expression of examined genes. Lower columns are densitometric analysis of gene expression.

Protective effects of onion extract on dexamethasone induced changes on acute phase proteins expression in immune suppressed rats

Finally, we checked the protective effect of OE on alpha-1-acid glycoprotein (α1-AGP) and alpha-2 macroglobulin (α2-MG) expression associated with DEXA injection. DEXA down-regulated mRNA expression of AGP in parallel with OE. When OE administered prior to DEXA, it is partially counteracted the most suppressive effect of DEXA (figure 3). Regarding α2-MG expression, DEXA upregulated it while OE decreased this increase in mRNA expression (Figure 3).

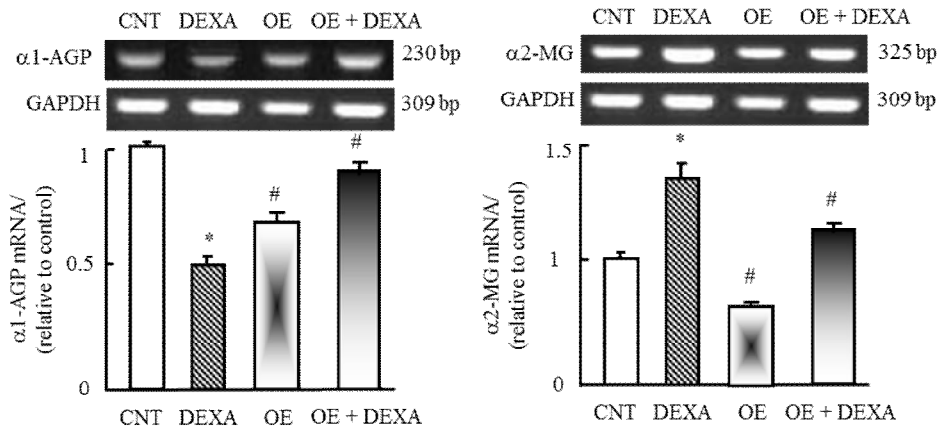

Figure 3: Protective effect of OE on changes in acute phase proteins expression induced by dexamethasone. RNA was extracted from liver tissues and the expressions of α1-AGP, and α2-MG were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 10 rats. $^{*}P < 0.05$ Vs control group; $^{\#}P < 0.05$ Vs DEXA group. Upper panels are mRNA expression of examined genes. Lower columns are densitometric analysis of gene expression.

Discussion

The results of this study demonstrated that onion extract ameliorated the immunosuppressive effects of dexamethasone on white blood cell counts, antioxidants and cytokines expression. As known, the immune system is a highly developed system that helps the body to resist the invaders and diseases. It is basically consisted of lymphoid tissue, bone marrow, lymph nodes, thymus, tonsils, spleen and gastrointestinal tract that play vital roles in human immunity (Anafi et al, 2014). Stress causes immunosuppression, affects the immune functions through activation of the hypothalamic-pituitary-adrenal axis (Schwab et al, 2005). Immunosuppression can occur through the direct effects of viral replication on lymphocyte functions although other blood parameters such as neutrophils and white blood cell (WBC) count may also be involved (Anafi et al, 2014).

Our findings reported that a decrease in total white leukocyte count with a decrease in lymphocyte and increase in neutrophils. Lymphocytes constitute about 60% of total leukocytes count in blood (Jibrin et al, 2006; Stebbing et al, 2005). The significant reduction of WBC and lymphocytes counts observed in this study following administration of dexamethasone was similarly reported by Anafi et al. (2014); Ohkarura et al. (2010) and Yasuhiko et al. (2010).

Reduction in MDA levels as oxidative biomarker by onion extracts involves a decrease in generation of reactive oxygen species (ROS) and a reduction in ROS-mediated induction of GST activity. Teyssier et al (2001) concluded that onion has the potential to protect against cancer via a modulation in GST activity. The enhancement in SOD and CAT activities probably reduce oxidative injury in liver as confirmed by our genetic and serum analysis. Early phase of free radical generation is associated with an increase in GST activity and that is coincided with increased levels of MDA (Ola-Mudathir et al, 2008). Tatfeng and Samson (2012) reported that onion extract has immuno-stimulating properties and is good marker for immune status of rats.

Most of cytokines are regulatory proteins, which in turn may cause dysregulation of innate and/or adaptive immune responses. Resident macrophages and mast cells (known as resident cells) initiate inflammation at the site of injury and cause a release of pro-inflammatory mediators such as TNF-α, IL-6 and IL-1β Coutinho and Chapman (2011). These cause at the site of injury a vasodilation and an increase in capillary permeability together with emigration of leukocytes into injured tissues and or organs. All lead felling pain, heat, redness and swelling of inflamed area and a generation of a chemotactic gradient to activate and guide recruited cells to the site of injury (Coutinho and Chapman, 2011). Therefore, during inflammation their secretion and expression is increased in a way to control inflammation. Dexamethasone is anti-inflammatory drug, as reported here, it decreased the secretion of IL-6 and TNF-α and downregulated the mRNA expression of IL-1β and this is coincided with other reports (Coutinho and Chapman, 2011). OE ameliorated this suppression and normalized altered cytokines in a way to counteract the side effects associated with DEX injection. Glucocorticoids (dexamethasone) downregulate transcription of key enzymes involved in the initiation and/or maintenance of the host inflammatory response, chemokines, pro-inflammatory cytokines and cell adhesion molecules (Barnes, 1998; Perretti and Ahluwalia, 2000; Smoak and Cidlowski, 2004). Interleukin-8 (IL-8) is a chemoattractant cytokine produced by a variety of tissue and blood cells. Unlike many other cytokines, it has a distinct target specificity for the neutrophil, with only weak effects on other blood cells. Interleukin-8 attracts and activates neutrophils in inflammatory regions (Bickel, 1993). IL-8 is an important mediator of host and inflammation (al-Dalaan et al, 1995). It possesses diverse functions as a neutrophil activator and a chemoattractant for T cells,

neutrophils, and basophils (Wang et al, 1997). IL-8 is produced by monocytes/macrophages, T cells, neutrophils, endothelial cells, fibroblasts, and keratinocytes (Ozoran et al, 1995 and Wang et al, 1997).

We reported that DEXA upregulated mRNA expression of IL-10 and onion normalized it. Clark (2007) reviewed the anti-inflammatory actions of glucocorticoids. IL-10, a potent immunomodulatory and anti-inflammatory cytokine (Couper et al, 2008) is included in this action. Kozan et al. (2016) confirmed that DEXA regulated acute lung injury through an increase in IL-10 secretion. IL-10 is included in negative regulation of corticosterone synthesis in the adrenal gland (Koldzic-Zivanovic et al, 2006), suggesting a homeostatic mechanism to end hypothalamic-pituitary-adrenal (HPA) axis activation after resolving inflammation. IL-10 is a cytokine that regulates immune functions and inflammation. It participates on downregulation in the expression of Th1 cytokines and enhancement in B cell proliferation and survival and antibody production. In current study, DEXA increased IL-8 and 10 expressions. Therefore, onion normalized IL-8 and10 expression after DEXA injection to control B cell survival and antibody secretion.

It has been reported that some medications mediate their action by α1-AGP during hepatic toxicity and inflammation in some tissues (Anderson et al 1999). Our findings confirmed that DEXA decreased α1-AGP expression and OE controlled AGP to be within normal ranges. In parallel, α2-MG is downregulated after DEXA injection and normalized by onion extract. Onion extract normalized and increased α2-MG as a counteract mechanism to contribute to the pathogenesis of several diseases (Savary et al, 2001). Current finding concluded that onion extract has immunomodulatory activity and has the potential to regulate immunosuppressive effects of dexamethasone at cellular and genetic levels.

Acknowledgements

This study was supported by a grant in aid for the Deans of Scientific Research Affairs of Taif University, Saudi Arabia in a grant for Mohamed Mohamed Soliman (Project # 4737-437-1).

Declaration: Authors declare that this research presents no conflict of interests.

References

1. Al-Dalaan A, al-Sedairy S, al-Balaa S, al-Janadi M, Elramahi K and Bahabri S, et al: Enhanced interleukin 8 secretion in circulation of patients with Behcet's disease. J Rheumatol 22: 904-7, 1995.
2. Alkhedaide, A., Soliman, M.M., Salah-Eldin, A.E., Ismail, T.A., Alshehiri, Z.S., Attia, H.F. (2016). Chronic effects of soft drink consumption on the health state of Wistar rats: A biochemical, genetic and histopathological study. Mol Med Rep. 13(6):5109-17.
3. Anafi, S.B., Kwanashie, H.O and Anuka, J.A. (2014). Study on the effect of dexamethasone as an immunosuppressive agent on some blood parameters in wistar rats. Nig. Journ. Pharm. Sci., 13 (2): 20-27.
4. Anderson, S.P., Cattley, R.C. and Corton, J.C. (1999). Hepatic expression of acute-phase protein genes during carcinogenesis induced by peroxisome proliferators. Mol Carcinog 26:226–238.
5. Augusti, K.T. (1996). Therapeutic values of onion and garlic. Ind J Exp Biol 34: 634-640.
6. Barnes, P.J. 1998. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin. Sci. (Lond) 94, 557–572.
7. Bickel M. (1993). The role of interleukin-8 in inflammation and mechanisms of regulation. J Periodontol. 1993, 64(5 Suppl):456-60.
8. Chisty, M., Quddus, R., Islam, B. and Khan B. (1996). Effect of onion extract on immune response in rabbits. Bangladesh Med Res Council Bull. 22: 81-85.
9. Clark, A.R., 2007. Anti-inflammatory functions of glucocorticoid-induced genes. Mol. Cell. Endocrinol. 275, 79–97.
10. Couper, K.N., Blount, D.G., Riley, E.M., 2008. IL-10: the master regulator of immunity to infection. J. Immunol. 180, 5771–5777.
11. Coussens, L. M and Werb, Z. (2002). Inflammatory Cells and Cancer. J Exp Med 193(6): F23-26.
12. Coutinho. A. E and Chapman, K. E. (2011). The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol. 335(1):2-13.
13. De Bosscher, K., Van Craenenbroeck, K., Meijer, O. C and Haegeman G. (2008). Selective trans-repression versus transactivation mechanisms by glucocorticoid receptor modulators in stress and immune systems. Eur J Pharmacol 583(2-3):290 – 302.
14. Grace, U., Steve, O., Tedd, E. and Shakirat, B. (2013). Immunostimulatory and biochemical effects of ethanolic extract of *mangifera indica* stem bark on dexamethasone-induced immunosuppressed male rats. Inter J Phar Pharmaceut Sciences, 2: (3).
15. Harcourt-Brown F. (2002). Therapeutics. In: Harcourt-Brown, F. (Ed.), Textbook of Rabbit Medicine. Reed Educational and Professional Publishing Ltd., Oxford, pp. 94–120.
16. Jibrin, Y.B., Yusuph, H., Garbati, M.A., Gashau, W., Abja, UMA. (2006). Should absolute lymphocyte count be used as a surrogate marker for CD4+ count in patient with HIV/AIDS? Africa Journal of Medicine. 35, 9-13.

17. Kandil, O.M., Abdellah, T. H and Elkadi, A. (1987). Garlic and the immune system in humans: its effects on natural killer cells. Federal Procedures. 46:441.
18. Kesavan, A.K., Mendez, S.E., Hatem, C.L., Lopez-Molina, J., Aird, K., Pitt, ML., Dannenberg, Jr, AM., and Manabe, Y.C. (2005). Effects of dexamethasone and transient malnutrition on rabbits infected with aerosolized Mycobacterium tuberculosis CDC1551. Infect Immun 73, 7056–7060.
19. Koldzic-Zivanovic, N., Tu, H., Juelich, T.L., Rady, P.L., Tyring, S.K., Hudnall, S.D., Smith, E.M., Hughes, T.K. (2006). Regulation of adrenal glucocorticoid synthesis by interleukin-10: a preponderance of IL-10 receptor in the adrenal zona fasciculata. Brain Behav. Immun. 20, 460–468.
20. Kozan, A., Kilic, N., Alacam, H., Guzel, A., Guvenc T and Acikgoz M. (2016). The Effects of Dexamethasone and L-NAME on Acute Lung Injury in Rats with Lung Contusion. Inflammation. 39 (5):1747-56.
21. Mirabeau, T.Y, and Samson, E.S. (2012). Effect of Allium cepa and Allium sativum on some immunological cells in rats. Afr J Tradit Complement Altern Med. , 9(3):374-379, 2012.
22. O'Byrne, K.J and Dalgleish A. G. (2001). Chronic Immune Activation and Inflammation as the Cause of Malignancy. Br J Cancer, 85 (4): 473–483.
23. Ohkarura, Y., Arairo, N., Ohno, H., Sato, S., Sakakibara, Y., Suzuki, H., Aritoshi, S., Akimoto, S., Ban, T., lhnihata, J., Thchiyashiki, K. and Imaizumi, K. (2010) Acute and Subacute Effects of Dexamethasone on the Number of White Blood Cells in Rats. J. health Science. 56 (2) 215-220.
24. Ola-Mudathir KF, Suru SM, Fafunso MA, Obioha UE, Faremi TY. (2008). Protective roles of onion and garlic extracts on cadmium induced changes in sperm characteristics and testicular oxidative damage in rats. Food Chem Toxicol. 46(12):3604-11.
25. Ozoran K, Aydintug O, Tokgoz G, Duzgun N, Tutkak H and Gurler A: Serum levels of interleukin-8 in patients with Behcet's disease. Ann Rheum Dis; 54: 610, 1995.
26. Patil, U.S., Jaydeokar, A.V. and Bandawane, DD. (2012). Immunomodulators: A Pharmacological Review Int J Pharm Pharm Sci, 4 (Suppl 1), 30-36.
27. Patil, V.V., Bhangale, S.C and Patil, Vijay R. (2010). Studies on Immunomodulatory activity of Ficuscarica. Int J Pharm Pharm Sci, 2(4):97-99,
28. Perreti, M. and D'Acquisto, F. (2009). Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. Nat Rev Immunol 9 (1): 62-70.
29. Perretti, M. and Ahluwalia, A. (2000). The microcirculation and inflammation: site of action for glucocorticoids. Microcirculation 7, 147–161
30. Sanderson, J., Mclauchlin, W. and Williamson, G. (1999). Quercetin inhibits hydrogen peroxide-induced oxidization of the rat lens. Free Rad Biol Med 26(6): 639-645.
31. Savary, I., Debras, E., Dardevet, D., Rambourdin, F., Vasson, M.P, Obled, C. and Grizard J. (2001). Evidence for an alteration of plasma and liver proteins response to dexamethasone in aging rats. Mech Ageing Dev. 122(1):105-20.
32. Schwab, C.L., Fan, R., Zheng, Q., Myers, L.P., Herbert, P., Pruett, S.B. (2005). Modeling and Predicting Stress-induced Immunosuppression in Mice using Blood Parameters. Toxicology Sciences. 83(1), 101-113.
33. Smoak, K.A. and Cidlowski, J.A. (2004). Mechanisms of glucocorticoid receptor signaling during inflammation. Mech. Ageing Dev. 125, 697–706.
34. Soliman MM, Baiomy AA and Yassin MH: Molecular and histopathological Study on the Ameliorative Effects of curcumin against lead acetate induced hepatotoxicity and nephrototoxicity in Wistar Rats. Biol Trace Elem Res 167(1):91-102, 2015.
35. Stebbing, J., Sawleshwarker, S., Michailidis, C., Jones, R., Bower, M., Mandalia, S., Nelson, M., Gazzard, B. (2005). Assessment of the efficacy of total lymphocyte counts as predictors of AIDS defining infections in HIV-1 infected people. Post Graduate Medical Journal. 81(959), 586-8.
36. Tatfeng Y. M and Samson S. E. (2012). Effect of allium cepa and allium sativum on some immunological cells in rats. Afr J Tradit Complement Altern Med. 9(3):374-379.
37. Teyssier, C., Amiot, M.J., Mondy, N., Auger, J., Kahane, R. and Siess, M.H. (2001). Effect of onion consumption by rats on hepatic drug metabolizing enzymes. Food Chem. Toxicol. 39, 981-987.
38. Van der Laan, S., and Meijer, O.C. (2008). Pharmacology of glucocorticoids: beyond receptors. Eur J Pharmacol 585 (2-3): 483- 491.
39. Wang, L.M., Kitteringham, N., Mineshita, S., Wang, J.Z., Nomura, Y. and Koike Y et al (1997). The demonstration of serum interleukin-8 and superoxide dismutase in Adamantiades-Behcet's disease. Arch Dermatol Res 289: 444-7, 1997.
40. Yasuhiko, O., Natsuko, A., Hitoshi, O., Shogo, S., Yuko, S., Hiroko, S., Shoko, A., Shunta, A., Takamasa, B., Jun, T., Kaoru, T., Kazuhiko, I. (2010). Acute and Subacute Effects of Dexamethasone on the Number of White Blood Cells in Rats. J Health Sci. 56(2), 215-22.