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PROTECTIVE ROLE OF LACTOBACILLUS ACIDOPHILUS IN HYPOTHALAMUS- PITUITARY-ADRENAL (HPA) AXIS PERFORMANCE IN HEAT STRESSED RATS

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ABSTRACT

The present study was undertaken to study the protective role of Lactobacillus acidophilus against deleterious changes of Hypothalamus pituitary adrenal (HPA) axis in heat stress, adult female (n=24) Albino Wister rats were divided into four equal groups; 1st control (C) group maintained at (22-25˚C), 2nd (HS) group exposed to high ambient temperature (35 - 40˚C) for 6 hours daily, 3rd (HS-LBA). group administered L acidophilus (5 × 108 CFU) and exposed to heat stress, 4th (LBA) group administered L. acidophilus (5 × 108 CFU). Results revealed that heat stress disorders of, hyperthermia, anxiety and decreased relative growth rate RGR were ameliorated by administration of Lactobacillus acidophilus. Further-more, the typical biomarkers for hypothalamus–pituitary–adrenal axis hyper sensitivity and sympathetic system activity (cortisole, adrenocorticotropic, adrenalin, & noradrenalin) were elevated in HS group, and return to semi normal level in HS-LBA and LBA groups. Analysis of brain tissue and duodenum sections showed marked inflammatory and irregularity of histopathological changes. These changes disappeared in groups with L. acidophilus. In conclusion, the present study demonstrated for the first time that L. acidophilus improves deleterious deviation in HPA axis hypersensitivity and sympathetic system activity caused by heat stress. These changes confirmed the success of heat stress resistance through a positive role of L. acidophilus in gut-brain-axis. This study demonstrated for the first-time efficacy of L. acidophilus in reducing heat stress effects mediated by gut-brain axis.

Key words: Lactobacillus, Anxiety acidophilus, HPA axis, Heat stress

INTRODUCTION

Gut microbiota can affect brain activity through neural circuits involved the enteric nervous system, and the sensory arm of the autonomic nervous system in addition to the neuroendocrine secretion, forming a network termed as the microbiota-gut-brain axis (Cryan and Dinan, 2015; Mayer et al., 2014; Sang et al., 2009) and vasa versaas gut-brain axis (Isolurai et al., 2017). Lactobacillus acidophilus, can enhance the gut mucosa against harmful agents and strengthen mucosal barrier in addition to avoiding gastro-intestinal tract disease such as ulcerative colitis, irritable bowel disease and infectious disease, and they may be of nutrient values (Floch, et al., 2011; Alqayim and Bkhit 2016; Sherman, et al., 2009), encouraging using them as alternative treatments than antibiotics. Under stressful conditions such as high ambient temperature, the hypo-thalamus–pituitary adrenal axis seeks to regulate the body homeostasis by over stimulation, increased basal HPA axis activity markers measured as blood corticosterone or ACTH levels (Neufeld et al., 2011, Gareau, et al., 2011, Clarke, et al., 2013, Beleen et al., 2012).This triggering of HPA in stress will activate the autonomic nervous system (sympathetic) and cortisol secretion resulting in intestinal higher permeability and loosing of coherence of mucosal cells in addition to increasing production of proinflammatory cytokines. Consequently, causing intestinal inflammation and prognoses gastrointestinal disorders (Brzozo-wski, et. al., 2016; Grenham et al., 201; Cryan and Dinan, 2012) and higher production of HSPs is (Mari-Edith and Jean-Paul, 2016). Tolerance ability of Lactobacillus strains to face stress have received attention, in vitro study that L. kefiranofaciens can defend cells against heat stress by increased synthesis of stress proteins (Ming-Ju et al., 2017). Lacto-

bacilli bacteria revealed a capability to produce many stress proteins make them good choice to be relied on against heat stress effects in gastrointestinal tract (Grosu-Tudor et al., 2016). Currently in this area of research the use of materials maintaining the barrier of the intestinal mucosa became one of the strategies for avoiding heat stress effects. Lactobacillus acidophilus is the most nominated because of its positive qualities in this side. Accordingly, the present study is designed to investigate the protective effects of Lactobacillus acidophilus on the hyper sensitivity of the hypo-thalamus –pituitary-adrenal (HPA) axis in normal and heat stressed conditions and shed light on its bidirectional role in the brain –gut axis and vise versa.

MATERIALS AND METHODS

The experiment conducted at the department of physiology and pharmacology in college of veterinary Medicine, University of Baghdad from 15th of September.

Experimental animals: Female Albino Wister rats (n=24) were divided into four equal groups; the 1st control (C) group maintained at control temperature(22-25˚C), 2nd group (HS) was expo-sed to high ambient temperature(35 - 40˚C) for 6 hours daily in a temperature controlled cabinet; 3rd (HS-LBA) group administered lactobacillus acidophilus (5 × 108 CFU) and exposed to heat stress, 4th (LBA) group orally administered L. acidophilus (5 × 108 CFU) and kept in normal condition, the administration was daily by oral gavages. Heat stress was induced by placing animals in high ambient temperature 35-40˚C for 6 hours daily in a cabin equipped with thermometer and heat source according to (Satter, 2015). Blood samples were collected via retro orbital sinus from each anesthetized animal at the end of the experiment.

Body temperature, RGR and anxiety measurements: Body temperature estimated by intra rectal thermometer, RGR was calculated according to the following equation (Castell and Tiews, 1980).

$$RGR\% = \frac{(final\ body\ weight - initial\ body\ weight)}{initial\ body\ weight} \times 100 \quad (1)$$

Anxiety was determined by open field test recording grooming, rearing, latency time and ambulation frequency in three minutes.

Markers for HPA hypersensitivity: Plasma glucose measured by commercial kit provided by company of Wiesbaden Germany. Plasma cortisol and ACTH was determined using commercial kits provided by Siemens according to Immulite 2000 system.

HPLC measurement of Plasma adrenalin and noradrenalin: Aluminum oxide was used to extract adrenaline and noradrenalin from plasma samples. Separation and identification of adrenaline and noradrenalin with internal standard by HPLC was performed according to (Arun and Adesh, 2009). The separation was obtained by HPLC column connected to a pump that provide a flow rate of 0.8 ml/min and a detector used was electrochemical of a UV - Vis at 285nm. The column ODS is C18 (150×4.6mm Id) 5µm particle size, column temperature = 20-26˚C and injected volume 20µl using the Ammonium acetate 90% as mobile phase, with pH ≈ 3.2. The calculation of adrenalin concentration according to the linear equation obtained from standard curve.

Histopathlogical examination: Immediately after sacrificing animals' brain and duodenum (1cm) was removed gently and placed in phosphate formalin solution 10%. Paraffin preserved samples were sectioned by a 5 µm in thickness and stained Hematoxylin and eosin (H&E).

RESULTS

Heat stressed rats were hyperthermia and sho-wed an anxiety behavior (table -1). The behavior disorders of open field test were modulated to normal in rats administered L. acidophilus and exposed to heat stress, furthermore body temperature was declined to level of control. The significant decrease in relative growth ratio of HS rats denoted in the present study was increased significantly ($P<0.05$) in HSLBA and LBA groups when compare with control group.

Table -1: Role of L. acidophilus (5 × 108 CFU) in body temperature (Cº), and anxiety (number of grooming /3minute), rearing (number of rearing/3 minute), latency (second), ambulation frequency (number of ambulation/3minute) against heat stress for 40 days.

Animal Groups	Body Temp.(˚C)	RGR (%)	Anxiety			
			Grooming	Rearing	Latency p.	Ambulation
C	36.4 ±0.060C	34.1 ±4.73A	0.16±0.166B	6.3±0.802C	0.92±0.047B	13.3±0.843A
HS	38.31±0.231A	5.46 ±0.92B	3.6±1.229A	13.5±0.957A	1.7±0.147A	14.8±1.166A
HSLB	37.06±0.340B	39.36 ±3.94A	1.5±0.670B	12.33±0.666A	1.0±0.0B	15.0±0.683A
LBA	36.4±0.125B	44.23 ±7.18A	0.0±0.0B	10.83±0.703B	1.13±0.164B	14.0±0.930A
LSD	0.649	14.03	2.080	2.332	0.333	2.722

C-control group, HS- heat stressed,HSLbA-heat stress & L.acidophilus, LBA- Lactobacillus acidopilus. Capital letters denote significant differences between groups n-6, means± SE.

Biomarkers of hypothalamus pituitary adrenal axis hypersensitivity: Heat stress biomarkers of the HPA axis, the ACTH and cortisol revealed a significant increase in HS group reached statistical (P<0.05) significance (3.55±0.491, 196.5±57.590) respectively, and non-significant increase in HS-LBA (table-2).

Table-2: Role of Lactobacillus acidophilus (5 × 108 CFU) on serum cortisol (µg/dl) and ACTH (pg/dl) after 40 days.

Animal Groups	C	HS	HS-LBA	LBA	LSD
Cortisol	0.65±0.13C	3.55±0.49A	1.63±0.32B	0.81±0.17B	0.925
ACTH	31.73±1.80B	196.5±57.59A	74.64±14.00B	38.15±15.02B	90.228

C-control group, HS- heat stressed, HSL-heat stress & L.acidophilus,LBA- Lactobacillus acidopilus. Capital letters denote significant differences between groups, n-6, means± SE.

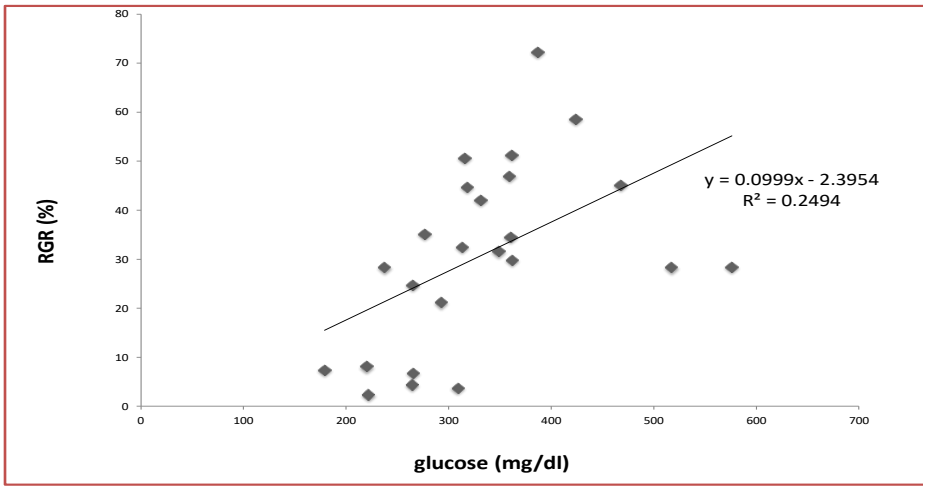

Figure -1 : The positive relationship between glucose and RGR in animal groups

Plasma adrenalin and noradrenalin: Catecholamine showed a significant increase (p 0.05) in HS group (1.042±0.218, 0.860 ± 0.334) when compared with control. The administration of L. acidophilus resulted in significant reduction in adrenaline and noradrenalin of stressed animals to semi normal levels (table-3). Results illustrated in Figure-2 revealed that all the biomarkers of heat stressed rats which related to HPA axis and

sympathetic system were obviously elevated to levels which is significantly differs than control at the same time the efficacy of L. acidophilus in modulating these biomarkers increase to levels semi normal, but the clear effects was on catechol-amine.

Table -3: Role of Lactobacillus acidophilus on adrenaline (mg/L) and noradrenalin (mg/L) after 40 days of heat stress.

Animals groups	C	HS	HSL	LBA	LSD
Adrenaline	0.01±0.000 B	1.042±0.218 A	0.06±0.014 B	0.006±0.085 B	0.228
Noradren.	0.012±0.000 B	0.860±0.334 A	0.166±0.069 B	0.004±0.000 B	0.203

C-control group, HS- heat stressed, HSL-heat stress & L. acidophilus, LBA- Lactobacillus acidopilus. Capital letters denote significant differences between groups, n-3, means± SE.

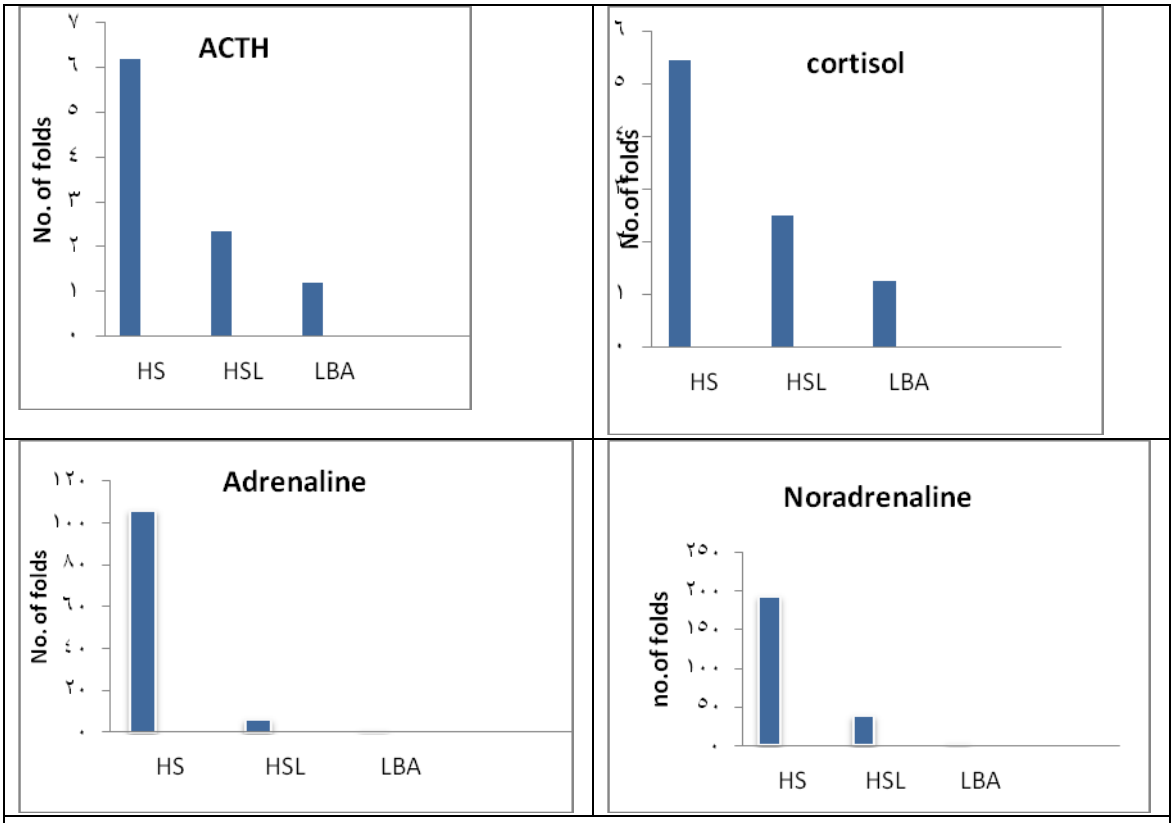

Figure 2: Role of Lactobacillus acidophilus on hypersensitivity of HPA axis and sympathetic activity biomarkers in heat stress. C. control, HS: heat stress, HSL: heat stress and L.acidophilus and LBA: L.acidophilus

Histopathological changes: Examination of H&E stained brain sections in figure-3, revealed nor-mal architecture with no clear lesions, and nor-mal basophilic neurons and oriented axonal fiber. Heat stressed brain rats showed intracellular odematous neurons appearance with necrotized, empty lacuna and signs of inflammation and alzahamer cells characterization. In addition, there were signs of hyperactivity, proliferated astrocytes (astrocytosis) and oligodentrocytes, also there were aggregation of microglia cells forming microglial nodules Blood vessels were congested and filled with inflammatory cells as well as peri-vascular edema. L. acidophilus treated group sho-wed normal histological appearance, further-more, there were obvious appearance of myelin sheath-oriented neurons lines and activity of microglia cells in spite of a single astrocytes. The analysis of the photographs obtained from H&E stained intestinal sections.

Figure 4 referred to sloughing vili, mild desquamation of columnar epithelial, infiltrated of lamina proporia with PMN with thick, more eosinophilic granules of mucosa, indication stimulation exertion increases in goblet cells with extending of infiltration in sub mucosa. Enlarged goblet cells with musin threads, submucosal infiltration of PMN, macrophages, congested of lamina proporia slough of brush border and mild vacuolar degeneration. These changes were dis-appeared in intestinal sections of control group dark with normal size goblet cells, mild mono-nuclear cell filtration in lamina proporia and normal brush border. In HSL group, enlarged vili, hyperplacia of epithelial mucosa, enlarged vili due to hyperplastic changes of columnar epithelia, intact brush border and normal number of cells. In LBA group normal epithelial vili, long twisted vili, moderate mononuclear cells infiltration many lymphpocytes.

DISCUSSION

In the present study the highest frequency of rearing and grooming and ambulation per duration of time at the day 40 of experiment indicated the hyperactive and aggressiveness of rats in chronic heat stress, these changes are well correlated with physiological variables and attributed to changes in brain neurotransmitters levels important for transmission of neural signals (Dey 1998; Mathew et al., 2008), and related to endocrine system disorders in exposure of rats to high ambient temperature a stressor factor (Rakesh and Amit, 2004). In normal conditions the behaviors are controlled by the activity of neuro-endocrine cells within central nervous system, these cells synthesis and secret neurotransmitter and other synaptic-related proteins, synaptophysin, modulation of these proteins expression or turnover mediate motor and behavior control (Diaz Heijtz et al., 2011). Hypothalamus release corticotropin releasing factor (CRF) which initiating cascade of biological events by activating the CRF receptors (CRFR1) either in the hypothalamus or in the other regions of the brain, activation of the CRFR1 responsible for sympathetic out flow thermoregulation, motor activity and endocrine function.

The mechanism and pathophysiology underlying the behavior disorders and anxiety caused by heat stress mediated by the CRFR1 over activation alleviate the stress-related pathologyies (Yael et al., 2016) and via down regulation and attenuation of extracellular signals pathways caused by neurotrophins (Wang and Huanlin, 2017). Heat stressed rats suffered from sharp decrease in the RGR, this could be contributed to increase in catabolic and decrees anabolic activity of tissues are the characteristic effects of heat stress, increasing the catabolism activity of the heat shock protein, which is produced at the time of heat stress denatures the other protein, which may have a body building function (Sheridan and Bickford (2011), also the appetite and food intake adversely affected by the long term stress, (Sapolsky et al., 2000). Since the current study aimed to verify the efficiency of L. acidophilus in reducing these heat stress response, results of anxiety, clinical signs, hyperthermia, animal beha-vior and RGR of rats exposed to heat stress in the present study were modulated. This may be directed in to two mechanisms. First possible mechanism based on possible role of probiotics produced by neural circuits, gut microbiota can affect brain activity through neural circuits involved the enteric nervous system, and the sensory arm of the autonomic nervous system may be a route whereby gut microbiota induce these effects in addition to the neuroendocrine secretion, forming a network termed as the microbiota-gut-brain axis (Cryan and Dinan, 2015; Mayer et al., 2014; Sang et al., 2009). Gut microbial colonization specifically Lactobacillus in the intestine process initiates signaling mechanism that affect neuronal circuits involved in motor anxiety behavior (Diaz Heijtz et al., 2011; Tanida and Nagai, 2011). Lactobacillus species feeding changed the concentration of neurotransmitters that related to excitation inhibition balance in central nervous system (Janik et al., 2016), which make it a therapeutic approach for depression treatment, and other nervous system dysfunction namely thro-ugh modulation of inflammation and the activity of the hypothalamus- pituitary adrenal axis (Kang and Yue, 2017). The second mechanism is via a gut microbiota on intestinal mucosal barrier.

The gut epithelium functions as a physical barrier that separates the septic condition in the gut space from the aseptic condition in circulating blood. However, the permeability of the gut epithelium is increased during intense exercise and heat stress exposure by weakening of the tight junctions and increasing bacterial translocation into the intestinal wall, increased microbial load in the colonic tissue, excessive cytokine release (Bartoze et al., 2016). Improve general and specific health conditions to promote gut barrier protection and appropriate immune will be the good strategies to avoid heat stroke dangerous and preventing hyperthermia (Lim and Suzuki, 2017). Our previous work denoted that administration of L. acidophilus improved as intestinal barrier enhancement and tight junction strength, stimulate secretion of mucous barrier and up regulated the Xcd2 gene for intestinal mucosa proliferation (Alqayim and Bakhit, 2016, Alqayim and Jabbar, 2015) as well as the protective proinflammatory role of yogurt containing Mix-LAB reducing cytokine-induced intestinal epithelial barrier dysfunction (Zeng et al., 2016). Other types of probiotics improved gut microbiota enhanced intestinal integrity, which may be responsible for inhibiting the invasion of pathogen bacteria (Panwang, et al., 2107)

Biomarkers of HPA and sympathetic activity: During heat stress the hypersensitivity of the HPA axis aimed to increase energy level by different mechanism, either by directly effects of glucocorticoids (cortisol) on the liver increase glyciogenolysis and the gluconeogenesis from proteins of or indirectly by stimulation of adrenalin synthesis increased gluconeogenesis from lipolysis. Continuous heat stress increase in hole body glucose utilization and fuel requirement for sustain heat stroke response in dairy cows (Quan et al., 2016), the storage form of energy, glycogen stored in liver and lipid tissues will be limited for glucose (Thomas, et al., 2017) lead to adequate glucose level in the serum, further- more the sharp decrease in RGR of heat stressed rats support this proposed explanation for decreased glucose denoted in the present experiment. On the other hand, the harmful effect of heat stress on gastrointestinal mucosa denoted by histopathological changes caused decreased absorption of glucose, the reduction of farm animals production in heat stress attributed to alteration in post absorptive carbohydrate, lipid, and protein metabolism independently of reduced feed intake through coordinated changes in fuel supply and utilization by multiple tissues (Lance and Robert., 2013). Role of gut microbiota on glucose homeostaisi is completely opposite based on type of micro biota. The modulation effects of lactobacillus on intestinal barrier integrity enhance the absorptive action of gut for glucose and affect insulin sensitivity. Secretion of glucagon-like peptide-1(GLP-1) with the pointed role in regulation of inflammatory cytokines, make lactobacillus bacteria played role in metabolic parameters of obesity (Yu-chun et al., 2016). There are growing evidenced data about the interaction of guy micro biota and the host, the modulation of brain activity in normal and stress condition by administration of lactobacillus is obvious in the present study, represented by normal levels of adrenalin and noradrenalin. Gut microbiota can communicate with host brain because they can segregate hormones and neurotransmitters from the brain, also bacteria have receptors for the host neurotransmitters (Bailey, 2014).

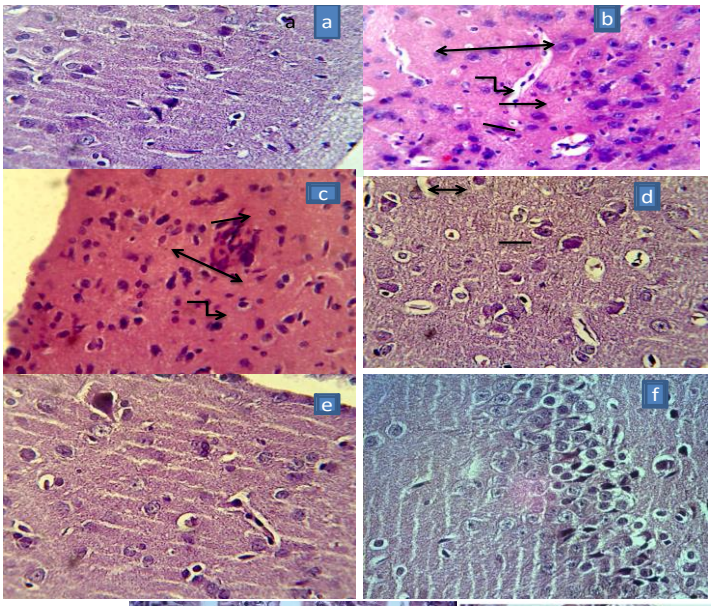

Fig-3: Light microscopic image of H&E stained brain(cerebrum) sections of a:control,b,c,d :HS, e: HSL , f:LBA groups. At 400X Oligodentrocytes= Microglia = Astrocytes = prevascular edema=

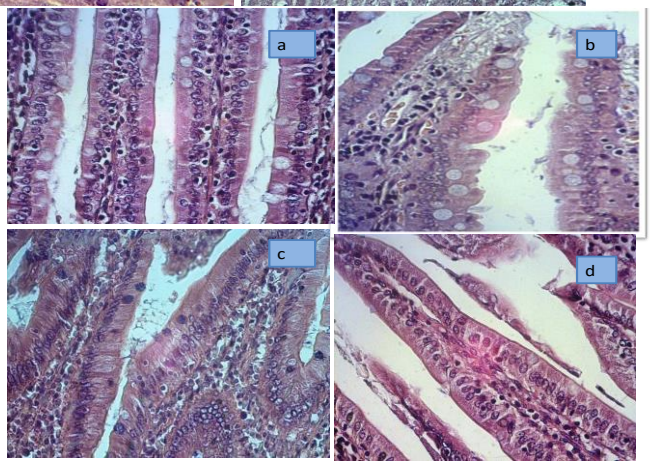

Fig-4: Light microscopic image of H&E stained duodenum sections of a:control,b :HS, c: HSL , d:LBA groups. At 400X

Another mechanism for the good modulation of the HPA axis and sympathetic system achieved by the lactobacillus in the present study could be due to immunomodulatory and anti-inflammatory action of this bacteria, research on contribution of gut microbota to physiological function and disorders of the central nervous system reviewed by Katarzyna, et al., 2016. Recently, found that gut micro-biota is necessary for main-tains central nervous system homeostasis by regulating the immune function of blood brain barrier integrity (Pauline et al., 2017) There are strong evidence about the modulator role of some Lactobacillus spp. between proinflammatory T helper and antiinflammatory T regulatory lymphocytes shifting towards T regulatory furthermore it reduced the dendritic cells proinflammatory responses (Lee et al., 2011). Previous results of our experiment state the modulation effects of L. acidophilus between the pro-inflammatory and anti-inflammatory cytokines release in healthy and colitis rats (Alqayim and Bakhit, 2016).

CONCLUSION

It is concluded that hyperthermia and anxiety caused by heat stress correlated with a deviation in the hypothalamus–pituitary adrenal axis and duodenum structure referring to a brain- gut axis involvement. Also, it is indicated for the first time a positive role for Lactobacillus acidophilus in correction of the gut deviation in combination with hormones related to the HPA axis confirmed the success of heat stress resistance through a positive role of L. acidophilus in gut-brain-axis.

Ethical approval: This research was approved by the ethical clearance committee of the physiology and pharmacology depart-

ment, college of Vet. Med., University of Baghdad.

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