

ORIGINAL ARTICLES

Antitoxin effect of *Streptococcus thermophilus* and *Lactobacillus delbrekii ss. bulgaricus* against benzo[a]pyrene in rats.

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ABSTRACT

Benzo[a]pyrene is a probable human carcinogen, commonly used as an environment indicator for polycyclic aromatic hydrocarbons (PAHs). Benzo[a]pyrene have various toxicological effects, widespread availability in the environment and great potential for human exposure, therefore this study investigated the effect of lactic acid bacteria strains against toxicity induced by benzo[a]pyrene in rats. Six animals groups were used in this study, the first group received basal diet (negative control), the second group received basal diet contaminated with 50ppm benzo[a]pyrene (positive control), the third group fed on basal diet supplemented with strain 1 of lactic acid bacteria (*Streptococcus thermophilus*), the fourth group fed on basal diet supplemented with strain 2 of lactic acid bacteria (*Lactobacillus delbrekii ss. bulgaricus*). The other two groups received basal diet contaminated with 50 ppm benzo[a]pyrene and supplemented with strain1 and strain 2 for 6 weeks. The results showed that positive control gave a significant increased in liver functions alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), kidney functions (creatinine and urea);significantly decreased glutathione content (GSH), glutathione peroxidase (GPx), total protein (TP), albumin and blood hemoglobin. These results indicate on the toxicity of benzo[a]pyrene. On the other hand lactic acid strain 1 of lactic acid bacteria (*Streptococcus thermophilus*) and strain 2 of lactic acid bacteria (*Lactobacillus delbrekii ss. bulgaricus*) supplemented to contaminated diet benzo[a]pyrene significantly improved liver and kidney functions and increased the depletion in GSH, GPx,TP, albumin and blood hemoglobin. It was noticed that the group fed on strain2 *Lactobacillus delbrekii ss. Bulgaricus* showed better results than strain 1 *Streptococcus thermophilus*. The results also revealed that the group received basal diet supplemented with strain1 and strain 2 showed significant beneficial health effects. It could be concluded that the tested strains have a protective action against benzo[a]pyrene toxicity as well as their beneficial health effects and may thereby enable the development of nutritional strategies of protection against toxins and occurrence of cancer.

Key words: PAHs, Benzo[a]pyrene, lactic acid bacteria,rats

Introduction

Benzo[a]pyrene (BaP) is a well known member of the polycyclic aromatic hydrocarbons (PAHs) family, which includes more than 100 different compounds formed during the incomplete combustion of fuels and other organic materials such as motor oils, gasoline, tobacco, cooking, oils, butter, margarine, charbroiled meat and other food (HSDB, 2004). PAHs are widely distributed in the environment and thus contaminated food. It has been established that there are two major sources of PAHs formation in foods, the first source is mainly due to the methods of food preparation, such as char-broiling, grilling, roasting, frying or baking (Yabikn *et al.*, 1993). The other major source of contamination of foodstuffs is by deposition of airborne or in area with high traffic (Kazerouni *et al.*, 2001; RAS, 2004). Because of the geochemical process and atmospheric deposition of air pollution particulate on the crops, it would be possible to generate these naturally occurring PAHs in food (Kazerouni *et al.*, 2001). It has been reported that PAHs are present in cereals, grains, flour, bread, vegetables, fruits, fish, meat, processed or pickled foods and contaminated cow's milk or human breast milk (FSA, 2002; Falco, 2003).

Benzo[a]pyrene is classified as an animal carcinogen and a probable human carcinogen (NIOSH, 2002). The non-carcinogenic toxicity of BaP has been studied in animals by a number of investigators; the major effects attributable to BaP appeared to occur in the liver, the hematopoietic immunosuppression, reproductive systems and kidney (Silkworth *et al.*, 1995; De Jong *et al.*, 1999; Knuckles *et al.*, 2001). Severity of the effects

depends on dose, administration of dose (route and vehicle of administration) and animal species, age and genotype. (ATSDR, 1995; NIOSH, 2002).

Lactic acid bacteria are a group of bacteria characterized by their ability to synthesize lactic acid, bacteriocins, etc. and are widely used in food manufacturing for their beneficial technological properties. They comprised of about 20 genera; *Lactobacillus* is the largest of these genera comprising around 80 recognized species (Axelson, 2004). Numerous investigations identified that lactic acid bacteria have beneficial health effects in humans (Saxelin *et al.*, 2005). It has been also reported that lactic acid bacteria removed cyanotoxins (Meriluoto *et al.*, 2005; Nybom *et al.*, 2007), heavy metals (Halttunen, 2007, Salim *et al.*, 2011a), cyanotoxins (Meriluoto *et al.*, 2005; Nybom *et al.*, 2007) and mycotoxins (Haskard *et al.*, 2001; Turbic *et al.*, 2002; Salim *et al.*, 2011b) from aqueous solution in vitro. However very few investigation were performed in vivo. Report on microbial degradation of PAHs appear increasing numbers, but such investigation tend to be focused on soil or aquatic microorganisms (Luning and Pritchard., 2002; Story *et al.*, 2004), while the activity of microorganisms associated with food fermentation has less investigated (Abou-Arab *et al.*, 2010). In addition there was a paucity of information about the effect of microorganisms on toxicity of PAHs in rats, therefore the present study was concluded to investigate the effect of some strains of lactic acid bacteria against toxicity induced by benzo[a]pyrene.

Materials and Methods

Chemicals:

All chemicals used in this study were obtained from sigma Company (St. Louis, USA). Commercial kits were purchased from BioMerieux Company (L'Etoile, France) and from Eagle Diagnostics (Dollas, TX, USA).

Media:

MRS broth and MRS agar were obtained from Oxoid Ltd. Eade Road, Basingstoke, U.K.

Organisms:

The two strains of lactic acid bacteria strain1 (*Streptococcus thermophilus* CH-1) and strain2 (*Lactobacillus delbrekii* ss *bulgaricus* CH-2) used in this study were obtained from the agent of Chr. Hansens Laboratory Denmark

Animals:

Forty two albino male rats with an average weight 130 ± 10 g were obtained from the Animal House Colony, Giza, Egypt. Rats were housed on a 12h light-12h dark schedule, and fed with water ad-libitum for 6 weeks. All rats were adapted for three days on the control diet before the beginning of the experiment.

Activation of tested bacterial strains:

Strain1 (*Streptococcus thermophilus* CH-1) and strain2 (*Lactobacillus delbrekii* ss *bulgaricus* CH-2) were activated according to DeMan *et al.*, (1960)

Preparation of bacterial strains:

Lactic acid bacteria strain 1 and strain 2 were prepared at National Research Center, Egypt in vitro as the following: 5.0 ml of the activated tested bacteria were added to 500 ml of MRS broth and anaerobically incubated at the optimum temperature (37°C) for 24 hr. Then the activated tested bacteria were centrifuged at 3000 r.p.m at 4°C for 20 min. to harvest the cells. Dehydrated cells were obtained by addition 50 g of defatted soy proteins to cells in big Petri dishes and the cells were incubated under vacuum incubator at 40°C overnight until it seemed like as thin slice or skins. The viability of the cells was tested on MRS agar plates then the strains were chopped and made as a powder containing 10^9 of bacteria/g.

Experimental design:

The basal diet was prepared according to the description of the National Research Council, (1978). The rats were randomly divided into six groups of 7 animals each and treated 6 weeks as follows: the first group received basal diet (negative control), the second group received basal diet contaminated with 50ppm Benzo[a]pyrene

(positive control), the third group fed on basal diet supplemented with strain 1 of lactic acid bacteria (*Streptococcus thermophilus*), the fourth group fed on basal diet supplemented with strain 2 of lactic acid bacteria (*Lactobacillus delbrekii ss. bulgaricus*). The other two groups received basal diet contaminated with 50 ppm benzo[a]pyrene and supplemented with strain1 and strain 2.

Biochemical analyses:

At the end of the experiment rats were fasted overnight and anesthetized with diethyl ether. Blood samples were collected in clean dry centrifuge tubes from hepatic portal vein. All blood samples were centrifuged for 15 min at 3000 rpm to separate the serum. The clear serum was kept frozen at -20C till analysis. The toxicity of benzo(a)pyrene and the protective effect of lactic acid bacteria strain 1 and 2 against benzo(a)pyrene toxicity were evaluated by assayed the following parameters: liver functions included serum transaminases, alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities according to the method of Reitman and Frankel (1957), alkaline phosphatase (ALP) by the method of Roy (1970). Enzymatic determination of urea was performed according to the method of Fawcett and Scott (1960) and creatinine was analysed by kinetic method kits described by Bartels *et al.*, (1972) tested as kidney functions. Colorimetric determination of albumin using bromocresol green at PH 4.2 according to the method of Doumas *et al.*, (1971) and total protein (TP) was estimated according to the method of Gornall *et al.*, (1949). Glutathione (GSH) activity and glutathione peroxidase (GPx) were measured by the method of Paglia and Valentine (1967). Blood hemoglobin was determined according to Jacobs *et al.*, (2001).

Statistical analysis:

Data were subjected to analysis of variance (ANOVA) and computing using the SAS General Linear Model producer (SAS, 1990). Differences with $P \leq 0.05$

Results and Discussion

The results in Fig (1) showed the effect of different treatments on rats' serum liver function enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Benzo[a]pyrene treatment caused significant increased in enzyme activities ($P \leq 0.05$) compared with rats fed on basal diet (control). However, lactic acid strain1 *Streptococcus thermophilus* and strain 2 *Lactobacillus delbrekii ss. Bulgaricus* showed significant improved in liver functions ($P \leq 0.05$). The affected liver functions by benzo[a]pyrene is similar to those reported by Knuckles *et al.*, (2001) who found that benzo[a]pyrene significantly increased activities of ALT, AST and ALP in rats.

The results in Fig (2) illustrated significant alleviation ($P \leq 0.05$) in kidney functions, creatinine and urea in rats treated by lead comparing to the control. Both tested lactic acid bacteria strains supplemented to benzo [a]pyrene treated group showed a significant improvement in both parameters. Cosan *et al.*, (2006) indicated that liver and kidney functions impaired with benzo(a)pyrene in rats.

Serum albumin and total protein (TP) were significantly decreased ($P \leq 0.05$) in rats treated with benzo[a]pyrene (Fig 3). The decreased in both parameters was significantly improved by tested lactic acid strains. The smoke from the mesquite coils (generate benzo[a]pyrene) produced significant increased in the levels of total proteins and albumin in rats exposed to smoke for 14 days (Abubakur and Hassan, 2007).

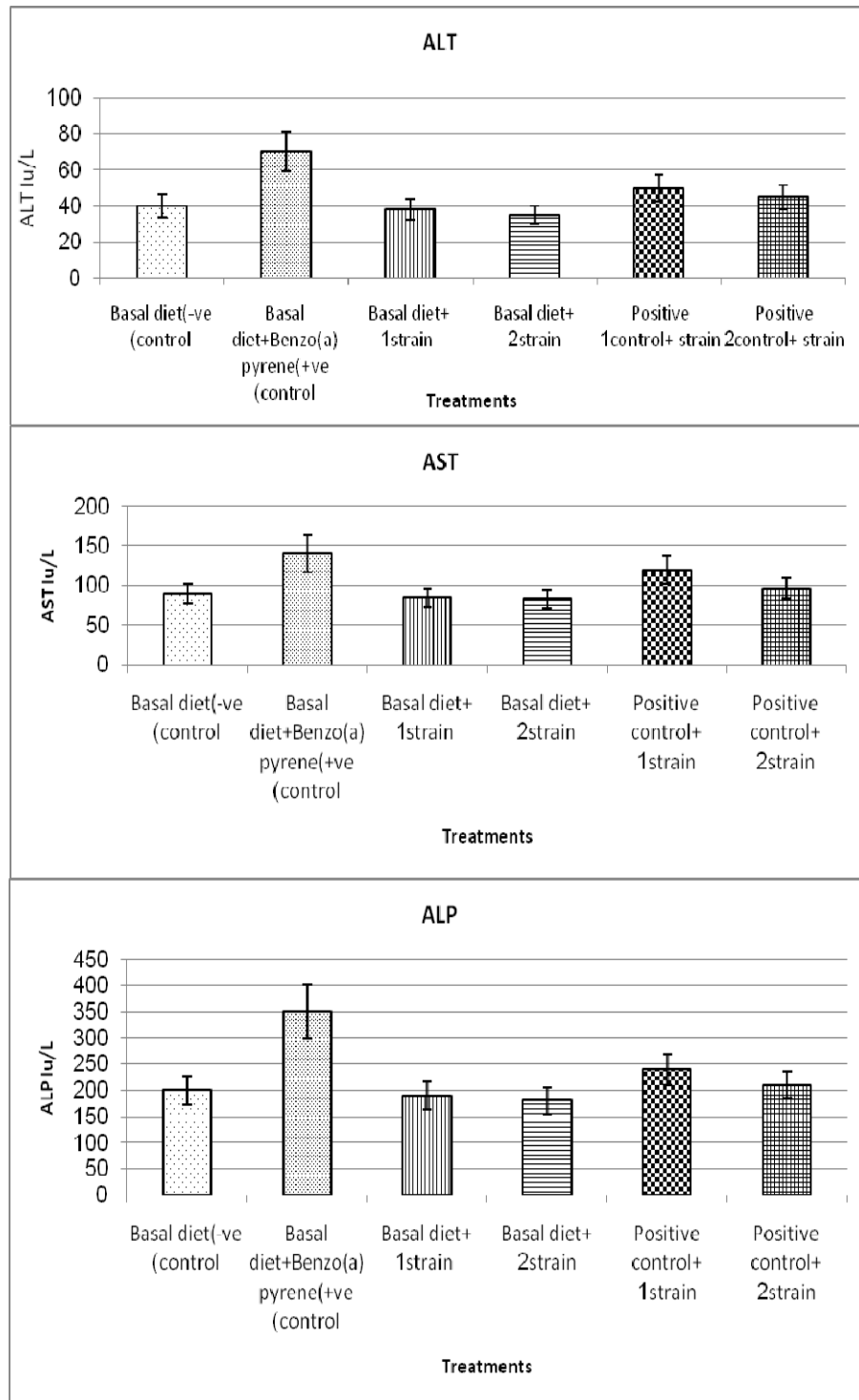
The results in Fig (4) illustrated that benzo[a]pyrene treatment depleted glutathione content (GSH), this depletion was significantly improved by strain1 *Streptococcus thermophilus* and strain 2 *Lactobacillus delbrekii ss. Bulgaricus* treatments. Glutathione plays important roles in antioxidant defense, nutrient metabolism and regulation of cellular events. Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many disease including , kwashiorkor, seizure, alzheimers' disease, liver disease, HIV, AIDS, cancer, heart attack and diabetes (Wu *et al.*, 2004).

The affected GSH by benzo[a]pyrene is similar to those reported by Kumer *et al.*, (2012) who found that a single dose of Bap (125mg/kg bw orally) decrease the levels of endogenous antioxidants such as superoxidase (SOD), glutathione reductase (GR) and glutathione (GSH) significantly in mice.

The results in Fig (5) showed that benzo[a]pyrene significantly decreased in glutathione peroxidase (GPx) activity (used as marker of oxidative stress in liver) as compared to negative control. The decreased in GPx due to benzo[a]pyrene was significantly reduced when diet supplemented with strain1 and strain2. A single dose Bap (125mg/kg b.w) in normal mice significantly decreased the levels of endogenous antioxidants such as glutathione peroxidase (GPx) and glutathione (GSH) (Sehgal *et al.*, 2011).

The level of blood hemoglobin was significantly decreased ($P \leq 0.05$) in positive control as compared to negative control (Fig 6), indicating that benzo[a]pyrene causing anemia. The decreased in blood hemoglobin was significantly improved by strain 1 *Streptococcus thermophilus* and strain 2 *Lactobacillus delbrekii ss.*

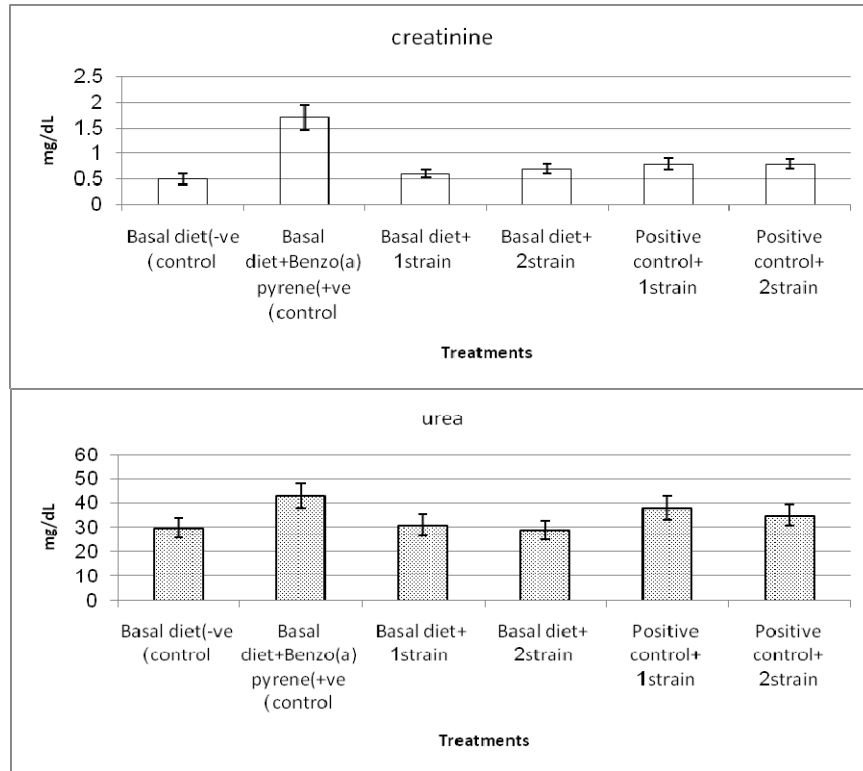
Bulgaricus supplemented to Bap treated group. The results are agree with Rowland and Gangolli, (1999) who concluded that there was some experimental evidence that administered LAB decreased the amount of carcinogens reaching the blood in rats.



Strain 1 = *Streptococcus thermophilus*

Strain 2 = *Lactobacillus delbrekii ss.bulgaricus*

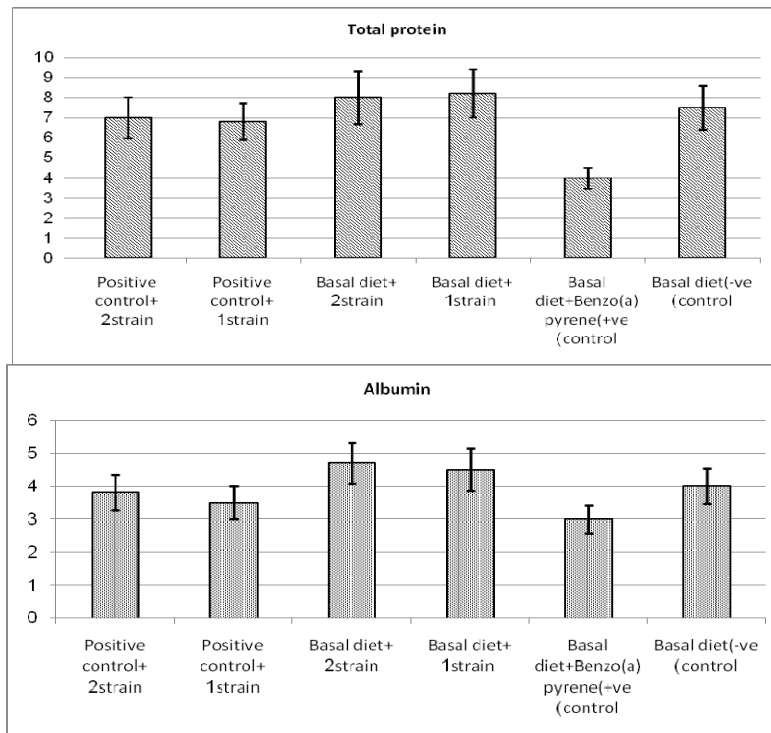
Fig. 1: Effect of lactic acid bacteria on ALT, AST and ALP in different treatment groups



Strain 1 = *Streptococcus thermophilus*

Strain 2=*Lactobacillus delbrekii ss.bulgaricus*

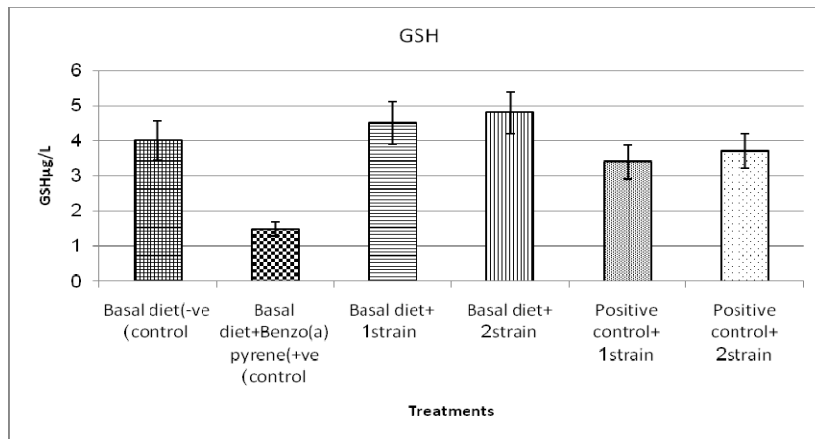
Fig. 2: Effect of lactic acid bacteria on creatinine and urea in different treatment groups



Strain 1 = *Streptococcus thermophilus*

Strain 2=*Lactobacillus delbrekii ss.bulgaricus*

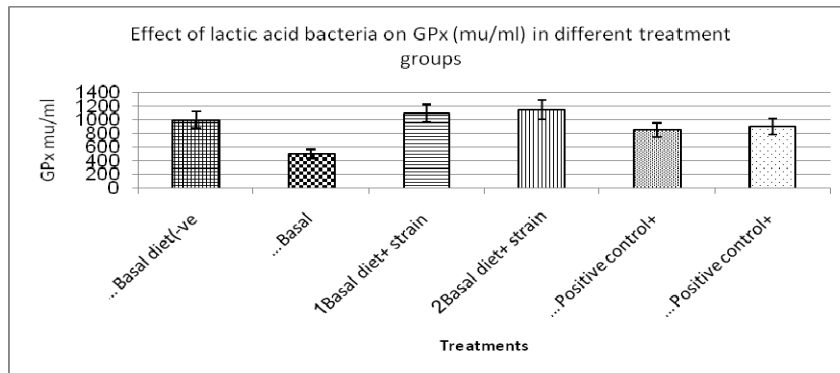
Fig. 3: Effect of lactic acid bacteria on Total protein and Albumin in different treatment groups.



Strain 1 = *Streptococcus thermophilus*

Strain 2=*Lactobacillus delbrekii ss.bulgaricus*

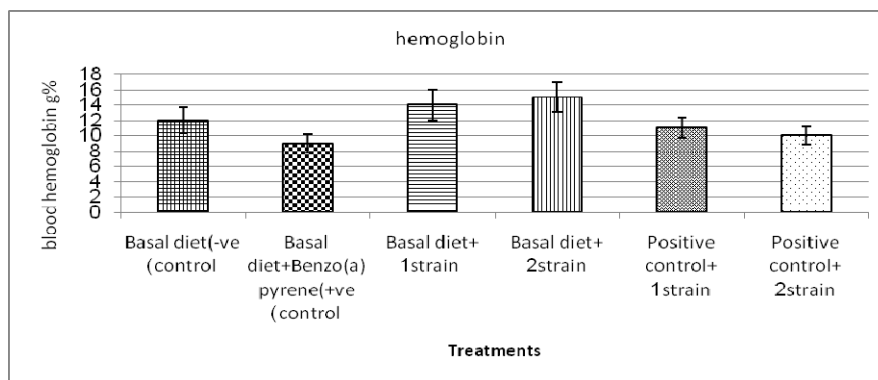
Fig. 4: Effect of lactic acid bacteria on glutathione (GSH) in different treatment groups



Strain 1 = *Streptococcus thermophilus*

Strain 2=*Lactobacillus delbrekii ss.bulgaricus*

Fig. 5: Effect of lactic acid bacteria on glutathione peroxidase (GPx) in different treatment groups



Strain 1 = *Streptococcus thermophilus*

Strain 2=*Lactobacillus delbrekii ss.bulgaricus*

Fig. 6: Effect of lactic acid bacteria on blood hemoglobin in different treatment groups

It was noticed from the results that strain 2 *Lactobacillus delbrekii ss. Bulgaricus* showed better results than strain 1 *Streptococcus thermophilus*. Lactic acid bacteria have been observed to be strain dependent and have reported a wide range of genus, species and strain specific binding toxins capacity (Halttunen, 2007; Nybom *et al.*, 2007).

It could be concluded from the results that dietary supplementation of lactic acid bacteria strain 1 *Streptococcus thermophilus* and strain 2 *Lactobacillus delbrekii ss. Bulgaricus* improved liver and kidney functions and alleviated GSH, GPx, blood hemoglobin, albumin and total protein depletion induced by

benzo[a]pyrene. One possible mechanism for this effect involves a physical binding of the mutagenic compounds to LAB bacteria (Orrhage *et al.*, 1994) and the capacity to adhere or bind different targets and inhibiting their absorption (Nybom *et al.*, 2007; Dalie, 2010).

In addition detoxification activity of LAB bacteria are believed to be attributed to improve the immunity by activation antioxidant enzymes such as glutathione (GSH) and glutathione peroxidase (GPx) and preventing oxidative damage. LAB could also treat some diseases by activation antioxidant enzymes (Jain *et al.*, 2009; Korche and Dilimi, 2010). Some LAB strains may enhance the SOD, GSH and GPx activities and prevent oxidative damage (Tsai *et al.*, 2009). The tripeptide glutathione (GSH) is a major antioxidants and detoxification of PAH metabolisms (Nakamura *et al.*, 2012).

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