

## Comparative studies between Gum Arabic recognized as a natural prebiotic and *Bifidobacterium* as probiotic as potential cure for experimental bacterial infection in mice

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**Abstract:** Gum Arabic as natural prebiotic is food ingredients that stimulate the growth of useful bacteria which lives in the large intestine of the human being or animal and beneficial to the digestive system, body's immunity, disposal of poisons, fats and excreta. Probiotics are beneficial bacteria that can be found in various foods, or in the form of dietary supplements. Common strains of probiotics are *Lactobacillus* and *Bifidobacterium* families of bacteria. So present study aimed to investigate the effect of Gum arabic as natural prebiotics in comparison with *Bifidobacterium animalis* sub sp. *lactis* (Bb12) as probiotic and combination of prebiotic and probiotic when administrated as food additive when challenged the experimental mice with *salmonella typhimurium*. The results indicated that there were symbiosis between prebiotic and probiotic, gum Arabic is good for health as natural prebiotic especially in the large dose, *Bifidobacterium lactis* Bb12 also good for health and immune status while the combination between gum Arabic as prebiotic and *Bifidobacterium* as probiotic (5 g GA+ Bb12) was better whether the combined application of PRO and PRE (synbiotics) has synergistic and additive significant effects, they give good results than each one alone and improved the health conditions, physiological and immune response; cellular and humeral for challenged mice with *salmonella typhimurium*.

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**Key words:** Gum Arabic; *Bifidobacterium*; phagocytic activity; lysozyme; AST; ALT; Uric acid; Creatinine.

### Introduction:

Gum arabic, known as acacia gum, *chaar gund*, *char goond*, or *meska*, is a natural gum made of hardened sap taken from two species of the acacia tree; *Senegalia senegal* and *Vachellia seyal*. The gum is harvested commercially from wild trees throughout the Sahel from Senegal to Somalia, although it has been historically cultivated in Arabia and West Asia. It is a complex mixture of glycoproteins and polysaccharides. It was historically the source of the sugars arabinose and ribose, both of which were first discovered and isolated from it (Renard *et al.*, 2006; Calame, *et al.*, 2008).

Gum Arabic as Prebiotic is food ingredients that stimulate the growth of useful bacteria which lives in the large intestine of the human being or animal since birth and beneficial to the digestive system, body's immunity, disposal of poisons, fats and excreta (Da Silva *et al.*, 2013). Besides it negates the effects of harmful bacteria thus protecting from the diseases, cancer, diabetes and obesity. It is pointed out that the gum-Arabic, extracted from Acacia Senegal trees which is more common in Sudan than anywhere in the world, is one of the natural sources of the prebiotics similar to breast milk and the processed inulin (Gerstenzang *et al.*, 2007; Calame, *et al.*, 2008). Although very few studies have tested the health effects of acacia fiber, there's some evidence that it may offer certain benefits Chloé and Fabien (2014).

The intestinal microbiota is essential for triggering local and systemic responses favorable to the host health. The intestinal microbiota is composed of about 100 trillion bacteria and encompasses more than 1,000 species (Kassinen *et al.*, 2007; Fletcher *et al.*, 2009; Garrett *et al.*, 2010; Shinohara *et al.*, 2010; Kamada *et al.*, 2013). It plays an important role in protection against pathogenic microorganisms, development and homeostasis of immune cells, digestion of polysaccharides that is indigestible by human enzymes and fat metabolism, among other functions (Holmes *et al.*, 2011; Da Silva *et al.*, 2013).

Present study aimed to investigate the effect of Gum arabic as natural prebiotics in comparison with *Bifidobacterium animalis* subsp. *lactis* (Bb12) as probiotic and combination of prebiotic and probiotic when administrated as food additive when challenged the experimental mice with *salmonella typhimurium*.

### Material and methods:

#### Experimental animals and design:

A total number of 100 white albino 6-weeks old male mice, they were prepared for the experiment and acclimated for 1 week. They were divided into five groups each 20. First group was subjected Gum arabic in a concentration 5mg /200 ml, 2<sup>nd</sup> group was subjected Gum arabic in a concentration 15 g/200 ml, 3<sup>rd</sup> group administrated to *Bifidobacterium animalis* subsp. *lactis* (Bb12) ( $1 \times 10^5$  colony forming unit (cfu)/g diet, 4<sup>th</sup> group administrated a combination

between *Bifidobacterium animalis* subsp. *lactis* (Bb12)  $1 \times 10^5$  cfu /g diet and Arabic gum in a concentration 5gm/, while 5<sup>th</sup> group was used as a control group subjected skimmed milk only without Gum Arabic. All experimental groups administrated skimmed milk with Gum arabic, (Bb12) and combination (Gum arabic 5 gm/200 ml) two times daily for 14 successive days before the challenge.

#### Challenge experiment:

Each mouse in all groups were orally challenged with 0.2 ml of the prepared *Salmonella typhimurium* suspension ( $1.5 \times 10^8$  CFU/ml) (Silva *et al.* 2004). The feces of the mice in each group were individually collected after the administration of the last dose of skimmed milk with Gum arabic, (Bb12) and combination (Arabic gum 5 gm/200 ml) on the 2<sup>nd</sup>, 5<sup>th</sup> and 10<sup>th</sup> day post infection for detection of *Salmonella typhimurium*.

#### Strains:

*Salmonella typhimurium* ATCC 14028 kindly obtained from King Abdul Aziz Medical City (National Guard Hospital) in Jeddah, Saudi Arabia.

#### Arabic gum as prebiotic:

Arabic gum obtained from Khartoum, Sudan naturally grinded then mixed with milk powder (SM) for feeding mice along the experimental period. Gum arabic was prepared by dissolving the powdered form of Arabic gum in cold water for complete dissolving, making solutions in tow concentrations 5 gm, 15 gm in 200 ml water.

#### In vitro antagonistic activity of the prebiotic:

By a sterile swab, *Salmonella typhimurium* with a concentration of  $1.5 \times 10^8$  CFU was spread over a nutrient agar plate and inoculated at 37°C for 24 h. about 10 $\mu$ l of each concentration of Gum arabic solution was inoculated in wells in the nutrient agar and incubated at 37°C for 24 hours. The inhibition zones was measured in mm. and recorded (Jakee *et al.*, 2010).

#### Preparation of prebiotic milk:

Prebiotic milk was prepared from reconstituted skimmed milk SM (Silva *et al.*, 1999). 2 concentrations of Gum arabic were prepared and mixed with skimmed milk except of control. Skimmed milk prepared alone without Gum arabic.

#### *Salmonella typhimurium* fecal colony count:

Five grams of Pooled fresh feces were collected from each group separately; feces were diluted in sterile buffered saline (pH 7.2) viable *S. typhimurium* were determined (Jakee *et al.*, 2010).

#### Phagocytic assay:

Macrophage monolayers were obtained and prepared it were harvested from the peritoneal cavities of mice and were resuspended in Hanks' balanced salt solution (HBSS) (Sigma Chemical Co., St. Louis, MO). The number of viable cells was determined by trypan blue dye exclusion and the coverslips were

washed with HBSS. *Candida albican* particles were added to the monolayers in a 5:1 (particle: macrophage) ratio and the coverslips incubated at 37°C in humidified atmosphere. After 30 min, the coverslips were washed with HBSS, fixed in methanol, and stained with Giemsa. After drying, the coverslips were mounted on glass slides and examined microscopically. The percentage of cells with ingested particles was multiplied by the average number of particles per macrophage to calculate phagocytic index. At least 100 macrophages were counted per cover slip (Belline, *et al.*, 2004).

#### Lysozyme assay:

The lysozyme concentration in the serum of mice was determined according to Schultz (1987) on the 2<sup>nd</sup>, 5<sup>th</sup> and 10<sup>th</sup> days after oral challenge with *S. typhimurium*.

#### Determination of (AST), (ALT), Uric acid and Creatinine:

At the end of the experimental period, blood samples were collected from tail vein in sterile test tubes centrifuged for serum separation at 1500 rpm for 15 min. to estimate AST, ALT according to methods described by Kaplan and Pesce (1996) uric acid and creatinine according to Tietz *et al.* (1995).

#### Statistical analysis

Data were represented as mean  $\pm$  standard error (S.E). The data obtained in this study were analyzed statistically using a *t*-Student test. The differences were considered significant at \*  $p < 0.05$  and high significant \*\*  $p < 0.01$  as compared to the control group. The data were analyzed according to Sendecor (1964).

#### Results:

#### Antagonistic activity of Gum Arabic and Bb12 against *Salmonella typhimurium* in vitro:

Present study revealed that the antagonistic activity of Gum Arabic (prebiotic) and Bb12 (probiotic) against *Salmonella typhimurium* in vitro was recorded as the 1<sup>st</sup> group (5gGA) recorded 0.31mm inhibition zone while 2<sup>nd</sup> group recorded 0.42 mm and 3<sup>rd</sup> group recorded 9.65 mm and 4<sup>th</sup> group recorded 9.77 mm in contrast the control group revealed 0 mm inhibition zone table 1.

Table 1: Results of Antagonistic activity of Gum Arabic (prebiotic) and Bb12 (probiotic) against *Salmonella typhimurium* in vitro

group	Inhibition zone
gp1 (5gGA)	0.31 mm
gp2 (10gGA)	0.42 mm
gp3 (Bb12)	9.65 mm
gp4 (5 g GA+ Bb12)	9.77
gp5 (Saline control)	0 mm

**Salmonella typhimurium colony count from fecal samples of mice:**

From the obtained results it was noticed that in 2<sup>nd</sup> day after challenge the lowest bacterial colony count was recorded in the 4<sup>th</sup> group (SM + 5 g GA+ Bb12)  $9 \times 10^4$  followed by 3<sup>rd</sup> group (Bb12)  $12 \times 10^4$  followed by 2<sup>nd</sup> group (SM+10g GA)  $17 \times 10^4$  while the

highest bacterial colony count was recorded in the 1<sup>st</sup> group (SM+5g GA)  $23 \times 10^4$  in comparison with the control group. With the time after 5 days and 10 days, bacterial colony count decreased and *Salmonella typhimurium* bacteria completely diminished after 10 days in 4<sup>th</sup> group (SM +5 g GA+ Bb12) and 2<sup>nd</sup> group (SM+10 gGA) table 2.

Table 2: Results of *Salmonella typhimurium* colony count from fecal samples of challenged mice in the experimental groups

group	before challenge	2 days	5 days	10 days
gp1 (SM+5gGA)	0	$23 \times 10^4$	$7 \times 10^{4***}$	$4 \times 10^{4**}$
gp2 (SM+10gGA)	0	$17 \times 10^{4*}$	$4 \times 10^{4**}$	0**
gp3 (SM+Bb12)	0	$12 \times 10^{4*}$	$5 \times 10^{4**}$	$1 \times 10^{4**}$
gp4 (SM+5 g GA+ Bb12)	0	$9 \times 10^{4**}$	$2 \times 10^{4**}$	0**
gp5 (SM control)	0	$33 \times 10^4$	$29 \times 10^4$	$24 \times 10^4$

\*significance; \*\*high significance

**Daily weight gain of the examined mice:**

In all examined groups there is no significant increase of daily weight gain 2,5 day post

administration while there is significant increase after 10 and 20 day particularly in 4<sup>th</sup> group, 3<sup>rd</sup> group and 2<sup>nd</sup> group table 3.

Table 3: The daily weight gain of the examined mice groups in the experimental groups

group	2day	5 day	10day	20 day
gp1 (SM+5gGA)	19.3±4.33	22.3±5.34	23.8±3.14	24.7±4.89
gp2 (SM+10gGA)	19.8±3.12	25.5±3.38	28.4±6.36*	29.1±5.45*
gp3 (SM+Bb12)	19.0±5.34	23.4±4.89	27.2±3.23	29.0±5.35*
gp4 (SM+5 g GA+ Bb12)	19.9±2.23	24.4±5.27	28.5±4.57*	31.6±4.23**
gp5 (SM control)	20.3±4.56	22.1±4.78	25.7±5.68	24.3±4.15

mean ± S.E; \*significance; \*\*high significance

**Results of estimation of phagocytic activity:**

The results displayed that phagocytic activity day 2 after oral challenge was significant recorded for group 3 and 4 only in comparison with the control group, while at day 10 after challenge was significant only for 2 groups 2<sup>nd</sup> ( $77.34 \pm 6.13$ ) and 3<sup>rd</sup>

( $78.67 \pm 3.24$ ) groups while 4<sup>th</sup> group was high significance ( $85.67 \pm 6.78$ ) in comparison with the control group furthermore, Day 15 after oral challenge significance was for 3<sup>rd</sup> ( $76.56 \pm 5.23$ ) group and high significance for 4<sup>th</sup> ( $79.25 \pm 6.21$ ) group table 3.

Table 3: Results of detection of phagocytic activity of Gum Arabic in mice.

groups	Day 2 after oral challenge	Day 10 after oral challenge	Day 15 after oral challenge
gp1 (SM+5gGA)	$75.44 \pm 6.56$	$73.23 \pm 3.23$	$74.78 \pm 7.23$
gp2 (SM+10gGA)	$75.12 \pm 5.21$	$77.34 \pm 6.13^*$	$73.35 \pm 2.34$
gp3 (SM+Bb12)	$75.56 \pm 4.53^*$	$78.67 \pm 3.24^*$	$76.56 \pm 5.23^*$
gp4 (SM+5 g GA+ Bb12)	$78.94 \pm 3.87^*$	$85.67 \pm 6.78^{**}$	$79.25 \pm 6.21^{**}$
gp5 (SM control)	$74.65 \pm 4.57$	$75.14 \pm 1.90$	$74.55 \pm 4.44$

mean ± S.E; \*significance; \*\*high significance

**Lysozyme concentrations:**

The results displayed that lysozymal concentrations in the serum, day 2 after oral challenge was significant recorded for group 3 and 4 only in comparison with the control group, while at day 10 after challenge was significant only for 2 groups 2<sup>nd</sup>

( $77.34 \pm 6.13$ ) and 3<sup>rd</sup> ( $78.67 \pm 3.24$ ) groups while 4<sup>th</sup> group was high significance ( $85.67 \pm 6.78$ ) in comparison with the control group furthermore, Day 15 after oral challenge significance was for 3<sup>rd</sup> ( $76.56 \pm 5.23$ ) group and high significance for 4<sup>th</sup> ( $79.25 \pm 6.21$ ) group table 3.

**Table 4: Results of lysozymal concentrations in the serum of mice after oral challenge.**

groups	Day 2 after oral challenge	Day 10 after oral challenge	Day 15 after oral challenge
gp1 (SM+5gGA)	298.45±34.40	276.15 ±35.12	272.67±56.23
gp2 (SM+10gGA)	313.43±56.34*	312.34 ±54.45	309.46±63.56
gp3 (SM+Bb12)	356.24±34.12**	335.35±68.67*	315.34±27.23*
gp4 (SM+5 g GA+ Bb12)	345.31±45.34**	365.23 ±34.98**	333.23±67.45**
gp5 (SM control)	304.24±45.34	307.33±43.32	287.13±67.24

mean ± S.E; \*significance; \*\*high significance

#### Concentrations of AST, ALT, uric acid, and creatinine:

Present study revealed that AST significantly decreased in 1<sup>st</sup> group (SM+5gGA) (40.45 µg/l) but 2<sup>nd</sup> group (SM+10gGA) and 3<sup>rd</sup> group (SM+Bb12) were higher (42.5 µg/l) and (41.15 µg/l) respectively than 1<sup>st</sup> and 4<sup>th</sup> group (SM + 5 g GA + Bb12) (39.33 µg/l) while ALT significantly decreased in 2<sup>nd</sup> group (SM+10gGA) (29.77µg/l) furthermore, 3<sup>rd</sup> group (SM+Bb12) and 4<sup>th</sup> group (SM+5 g GA+ Bb12) recorded high significance in reduction of ALT (27.56 µg/l) and (25.23 µg/l) respectively in comparison with the control group (35.34 µg/l). uric acid was not significant decreased in 1<sup>st</sup> group (SM+5gGA) (3.64

mg/dl) in comparison with the control group (3.94 mg/dl) while 2<sup>nd</sup> (SM+10gGA) was high significant and 3<sup>rd</sup> (SM+Bb12) group significantly decreased (2.16 mg/dl) and (2.45 mg/dl) respectively on the other hand 4<sup>th</sup> group (SM+5 g GA+ Bb12) was not significant (2.86 mg/dl) decreased in comparison with the control group (3.94 mg/dl). concerning, creatinine 1<sup>st</sup> (SM+5gGA) did not significantly decreased (0.64 mg/dl) in comparison with the control group (0.76 mg/dl) in contrast 3<sup>rd</sup> (SM+Bb12) and 4<sup>th</sup> group (SM+5 g GA+ Bb12) were significantly (0.46 mg/dl) and (0.61 mg/dl) decreased while 2<sup>nd</sup> (SM+10gGA) group was high significant (0.38 mg/dl) decreased in comparison with the control group (0.76 mg/dl) table 5.

**Table 5: Results of AST, ALT, uric acid, and creatinine in the serum of mice 10 days post oral challenge**

groups	AST (µg/l)	ALT (µg/l)	Uric acid (mg/dl)	Creatinine (mg/dl)
gp1 (SM+5gGA)	40.45±6.45*	31.34±4.34*	3.64±0.50	0.64±0.05
gp2 (SM+10gGA)	42.50±7.12*	29.77±5.23*	2.16±3.43**	0.38±0.04**
gp3 (SM+Bb12)	41.15±5.65*	27.56±7.23**	2.45±2.23*	0.46±0.06*
gp4 (SM+5 g GA+ Bb12)	39.33±5.23**	25.23±5.45**	2.86±3.12	0.61±0.09*
gp5 (SM control)	49.53±6.45	35.34±4.45	3.94±4.11	0.76±0.13

mean ± S.E; \*significance; \*\*high significance

#### Discussion:

The symbiotic relationship between the host and intestinal microbiota has been extensively studied, in part because of its implications in intestinal health. To promote the development of beneficial microbiota in the intestine. Prebiotics and probiotics can restore the balance of bacteria in the digestive tract. Probiotics are beneficial bacteria that can be found in various foods, or in the form of dietary supplements. When you consume probiotics, you add these healthy bacteria to your intestinal tract **Gourbeyre et al.(2001)**. Common strains include *Lactobacillus* and *Bifidobacterium* families of bacteria. Prebiotics are non-digestible foods that make their way through our digestive system and help good bacteria grow and flourish. Prebiotics help feed and keep beneficial bacteria healthy. on its impact towards human health since the history of mankind. This exclusive Natural Prebiotic is exudates from Acacia senegal and Acacia seyal solely obtained from Sudan. Sudanese used it traditionally as food to maintain health and longevity (**Roller et al., 2003; Calame,2008; Kamada et al.(2013)**).

The finest quality of Gum arabic maintains more than 85% soluble dietary fiber (oligosaccharide) and high combination of minerals (calcium, potassium, magnesium and iron) from the resin (part of plant used) based on size reduction method (invented and patented) with no introduction to high temperature and no chemical extraction (**Williams and phillip,2000; Calame et al.,2008**).

Present study aimed to investigate the effect of gum Arabic as natural prebiotics in comparison with *Bifidobacterium animalis* subsp. *lactis* (Bb12) as probiotic when administrated as food additive for experimentally infected albino mice with *salmonella typhimurium*.

Concerning the Antagonistic activity of Gum Arabic and Bb12 against *Salmonella typhimurium* in vitro; present study revealed that the antagonistic activity of Gum Arabic (prebiotic) and Bb12 (probiotic) against *Salmonella typhimurium* in vitro was recorded as the 1<sup>st</sup> group (5gGA) recorded 0.31 mm inhibition zone while 2<sup>nd</sup> group recorded 0.42 mm and 3<sup>rd</sup> group recorded 9.65 mm and 4<sup>th</sup> group recorded 9.77 mm in

contrast the control group revealed 0 mm inhibition zone it was shown that gum Arabic in 2 doses recorded very small inhibition zone while probiotic bacteria recorded 9.65mm and in association with gum Arabic recorded 9.77mm inhibition zone this maybe due to that Bb12 bacteria secrete lactic acid inhibit the growth of *Salmonella typhimurium* the results confirmed by **Vandenbergh (1993); Jakee et al. (2010)**.

Regarding *Salmonella typhimurium* colony count from fecal samples of mice, it was noticed that in 2<sup>nd</sup> day after challenge the lowest bacterial colony count was recorded in the 4<sup>th</sup> group (SM + 5 g GA+ Bb12)  $9 \times 10^4$  followed by 3<sup>rd</sup> group (Bb12)  $12 \times 10^4$  followed by 2<sup>nd</sup> group (SM+10g GA)  $17 \times 10^4$  while the highest bacterial colony count was recorded in the 1<sup>st</sup> group (SM+5g GA)  $23 \times 10^4$  in comparison with the control group. With the time after 5 days and 10 days, bacterial colony count decreased and *Salmonella typhimurium* bacteria completely diminished after 10 days in 4<sup>th</sup> group (SM +5 g GA+ Bb12) and 2<sup>nd</sup> group (SM+10 gGA) it was clear that with time (after 10 day) the activity of probiotic Bb12 due to the activation of natural prebiotic gum arabic with the large dose 10 g the results confirmed by **Silva et al.(2004)** who observed that there was improved survival of mice pretreated with *Bifidobacterium longum* during challenge with salmonella sp. furthermore **Kamada et al.(2013)** who reported that the interaction of the microbiota with pathogens and the host might provide new insights into the pathogenesis of disease, as well as novel avenues for preventing and treating intestinal and systemic disorders. Various carbohydrate sources including gum Arabic are known to support the growth and activity of Bb12 inhibiting growth of *salmonella typhimurium* reducing its colony count in feces.

Regarding daily weight gain of the examined mice, present study revealed that, in all examined groups there is no significant increase of daily weight gain 2,5 day post administration while there is significant increase after 10 and 20 day particularly in 4<sup>th</sup> group, 3<sup>rd</sup> group and 2<sup>nd</sup> group the results indicated that there is great role of combination between probiotic as Bb12 and prebiotic as Gum Arabic in association of time for significance increase of daily weight gain in experimental animals, the results nearly agree with **Isk et al.(2004) and Osman et al (2010)**.

Regarding the immunomodulatory effect of Probiotics (PRO) modulate systemic immunity in animals and humans. In contrast, the effects of prebiotics (PRE) on systemic and intestinal immunity have not been investigated. Whether the combined application of PRO and PRE [synbiotics (SYN)] has synergistic or additive effects. The phagocytic activity in present study revealed that, day 2 after oral challenge was significant record for group 3 and 4

only in comparison with the control group, while at day 10 after challenge was significant only for 2 groups 2<sup>nd</sup> and 3<sup>rd</sup> groups while 4<sup>th</sup> group was high significance in comparison with the control group furthermore, Day 15 after oral challenge significance was for 3<sup>rd</sup> group and high significance for 4<sup>th</sup> group the results nearly agree with. **Sheil et al.(2006)** and **Calame et al. (2008)** who reported that gum Arabic improve all physiological functions for human. The colonic microbiota affects mucosal and systemic immunity in the host (Famularo et al.,1997). Intestinal epithelial cells, blood leucocytes, B and T lymphocytes, and accessory cells of the immune system are all implicated (Schiffirin et al.,1997). Bacterial products with immunomodulatory properties include endotoxin lipopolysaccharide, peptidoglycans, and lipoteichoic acids (Standiford et al.,1994) Lipoteichoic acids of Gram positive bacteria such as bifidobacteria possess high binding affinity for epithelial cell membranes and can also serve as carriers for other antigens, binding them to target tissues, where they provoke an immune reaction (Op et al.,1984) while lactobacilli which adhere to human intestinal epithelial cells are capable of activating macrophages (Kleeman and Klaenhammet,1982; Perdigon et al.,1986) There are as yet no experimental data to support the immunostimulatory properties of non digestible oligosaccharides in humans. However, probiotic organisms interact with the immune system at many levels, including cytokine production, mononuclear cell proliferation, macrophage phagocytosis and killing, modulation of autoimmunity, and immunity to bacterial and protozoan pathogens (Famularo et al.,1997; Schiffirin et al.,1997; Matsumara et al.,1992).

The lysozyme concentration, present study displayed that lysozymal concentrations in the serum, day 2 after oral challenge was significant recorded for group 3 and 4 only in comparison with the control group, while at day 10 after challenge was significant only for 2 groups and 3<sup>rd</sup> groups while 4<sup>th</sup> group was high significance in comparison with the control group furthermore, Day 15 after oral challenge significance was for 3<sup>rd</sup> group and high significance for 4<sup>th</sup> group the results nearly agree with **Roller et al., (2003)** and **Gourbeyre et al.(2001)** who reported that Functions of immune cells isolated from peripheral blood mononuclear cells (PBMC), spleen, mesenteric lymph nodes and Peyer's patches were investigated. The SYN supplement increased secretory immunoglobulin A (sIgA) production in the ileum compared with control, and decreased the oxidative burst activity of blood neutrophils compared with rats fed PRO. The PRE supplement enhanced the production of interleukin-10 in PP as well as the production of sIgA in the cecum, compared with controls. The PRO

supplement modestly affected immune functions, whereas systemic immunomodulatory effects were observed in rats fed SYN.

Concerning the concentrations of AST, ALT, uric acid, and creatinine, Present study revealed that AST significantly decreased in 1<sup>st</sup> group (SM+5gGA) but 2<sup>nd</sup> group (SM+10gGA) and 3<sup>rd</sup> group (SM+Bb12) were higher than 1<sup>st</sup> and 4<sup>th</sup> group (SM + 5 g GA + Bb12) while ALT significantly decreased in 2<sup>nd</sup> group (SM+10gGA) furthermore, 3<sup>rd</sup> group (SM+Bb12) and 4<sup>th</sup> group (SM+5 g GA+ Bb12) recorded high significance in reduction of ALT in comparison with the control group. the results disagree with **Antunovic et al.(2005)** who reported that the probiotic pioneer PDFM significantly reduced serum glucose and urea levels and activates AST and ALT in lambs and agree with **El-Jakee et al.(2010)** who recorded that AST and ALT were reduced in mice supplemented probiotics with skimmed milk.

Uric acid was not significant decreased in 1<sup>st</sup> group (SM+5gGA) in comparison with the control group while 2<sup>nd</sup> (SM+10gGA) was high significant and 3<sup>rd</sup> (SM+Bb12) group significantly decreased on the other hand 4<sup>th</sup> group (SM+5 g GA+ Bb12) was not significant decreased in comparison with the control group. concerning, creatinine 1<sup>st</sup> (SM+5gGA) did not significantly decreased in comparison with the control group in contrast 3<sup>rd</sup> (SM+Bb12) and 4<sup>th</sup> group (SM+5 g GA+ Bb12) were significantly decreased while 2<sup>nd</sup> (SM+10gGA) group was high significant (0.38 mg/dl) decreased. the results nearly agree with **Aled et al.(2011)** who reported that in vivo and in vitro with gum arabic, which show its compatibility in the diet of patients suffering with diabetes mellitus and reduction in systolic blood pressure, which may translate into improved cardiovascular outcome and a reduction in the progression of renal diseases. Nasir (2007) reported that GA treatment was associated with an increased 24 h-creatinine clearance in healthy mice. The exact mechanism for this remains unclear, since it represents a remote effect of GA on the kidney, which requires one or more humoral factors. It is well known that GA is fermented by intestinal bacteria leading to formation of various degradation products, such as short chain fatty acids (Bliss et al., 1996). In a recent study, serum butyrate concentrations were increased following treatment with GA in healthy subjects (Matsumoto et al., 2006) and this may have a role in the claimed salutatory effect on creatinine clearance. Recently, a report from Sudan assessed the effect of GA on the concentration of certain metabolites in the sera of patients with CRF on a low-protein diet. GA was given at an oral dose of 50 g/day

for 3 months, with or without supplementing the diet with ferrous sulfate (200 mg/day) and folic acid (5 mg/day). Serum creatinine, urea, phosphate and uric acid concentrations were reported to be significantly reduced by GA, (Ali et al.,2008).

From present study it was concluded that there are symbiosis between prebiotics and probiotics, the results indicated that gum Arabic is good for health as natural prebiotic especially in the larger dose, *Bifidobacterium lactis* Bb12 also good for health and immune status while the combination between gum Arabic as prebiotic and *Bifidobacterium* as probiotic (5 g GA+ Bb12) was better whether the combined application of PRO and PRE (synbiotics) has synergistic or additive effects significantly, they give very good results than each one alone and improve the health conditions, physiological and immune response; cellular and humeral for challenged mice with *salmonella typhimuarium*.

#### References:

1. Aled O. Phillips, Glyn O. (2011): Phillips Biofunctional behaviour and health benefits of a specific gum arabic Food Hydrocolloids, Volume 25, Issue 2, March 2011, Pages 165-169.
2. Ali, A.A., Ali, K.E., Fadlalla, A., Khalid, K.E., 2008. The effects of G.A. oral treatment on the metabolic profile of chronic renal failure patients under regular haemodialysis in Central Sudan. Nat. Prod. Res. 22, 12–21.
3. Belline Paula, Patrícia da Silva Melo, Marcela Haun, Fernanda Boucault Palhares, Patricia Aline
4. Bliss, D.Z., Stein, T.P., Schleifer, C.R., Settle, R.G., 1996. Supplementation with G.A. fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet. Am. J. Clin. Nutr. 63, 392–398.
5. Boer, José Antônio Rocha Gontijo José Francisco Figueiredo (2004): Effect of Angiotensin II and Losartan on the Phagocytic Activity of Peritoneal Macrophages from Balb/C mice Mem Inst Oswaldo Cruz, Rio de Janeiro, 99 (2): 167-172.
6. Calame Wim, Antje R. Weseler, Christer Viebke, Cal Flynn and André D. Siemensma (2008):Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner British Journal of Nutrition, 100, 1269–1275.
7. Chloé Butstraen, Fabien Salaün (2014): Preparation of microcapsules by complex coacervation of gum Arabic and chitosan Carbohydrate Polymers, Volume 99, Pages 608-616.

8. Collins M, Gibson G (1999): Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *Am J Clin Nutr* 69: 1052S–1057S.
9. Da Silva Sandra Tavares, Carolina Araújo dos Santos and Josefina (2013): intestinal microbiota relevance to obesity and modulation by prebiotics and probiotics *Nutr Hosp.*28(4):1039-1048.
10. El-Jakee, I.M. Moussa, S.A. Nada, Kh. F. Mohamed, M. H. Ashgan and M.L. Mohamed (2010): Influence of Probiotics Mixture on *Salmonella typhimurium* in mice, *International Journal of Microbiological Research* 1(2):50-61.
11. Famularo G, Moretti S, Marcellini S, De Simone C. Stimulation of immunity by probiotics. In: Fuller R, ed. *Probiotics: therapeutic and other beneficial effects*. London: Chapman and Hall, 1997:133-61.
12. Fletcher CM, Coyne MJ, Villa OF, Chatzidakis-Livanis M, Comstock LE. (2009): A general O-glycosylation system important to the physiology of a major human intestinal symbiont. *Cell*; 137 (2): 321-31.
13. Garrett WS, Gallini CA, Yatsunenkov T, Michaud M, DuBois A, Delaney ML, (2010). Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell*; 8 (3); 292-300.
14. Gerstenzang, James and Sanders, Edmund (2007): "Impact of Bush's Sudan sanctions doubted". *Los Angeles Times*. Retrieved 1 June 2007.
15. Gibson G, Beatty E, Wang X, Cummings J (1995): Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 108: 975–982.
16. Gourbeyre Pascal, Sandra Denery, and Marie Bodinier (2011): Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions Volume 89, *Journal of Leukocyte Biology* 685-695.
17. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. (2011): Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. *Trends Microbiol*; 19 (7): 349-59.
18. Isk, M., F. Ekimler, N. Ozen and M.Z. Frat, (2004): Effect of using probiotics on growth performance and health of dairy calves. *Truk-Veterinrlık Ve Hayvanclık-Dergisi*.28: 63-69.
19. Kamada Nobuhiko, Sang-Uk Seo, Grace Y. Chen & Gabriel Núñez (2013): Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews Immunology* 13, 321-335
20. Kaplan, L.A. and A.J. Pesce, (1996). *Clinical Chemistry*., Moby Ed.
21. Kassinen A, Krogius-Kurikka L, Mäkiyuokko H, Rinttilä T, Paulin L, Corander J (2007). The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology*; 133 (1): 24-33.
22. Kleeman EG, Klaenhammet TR. (1982): Adherence of *Lactobacillus* species to human fetal intestinal cells *J Dairy Sci*;65:2063-9.
23. Matsumara K, Kitazawa H, Itoh T, Yamaguchi T. (1992) Interferon induction by murine peritoneal macrophage stimulated with *Lactobacillus gasserii* *Animal Sci Technol (Jpn)*;63:1157-9.
24. Matsumoto, N., Riley, S., Fraser, D., Al-Assaf, S., Ishimura, E., Wolever, T., Phillips, G.O., Phillips, A.O., 2006. Butyrate modulates TGF-beta1 generation and function: potential renal benefit for *Acacia (sen) SUPERGUM (G.A.)?* *Kidney Int.* 69, 257–265.
25. Nasir, O.D.S., (2007). Physiological effects of kinases, pioglitazone and G.A. on renal function. Doctor of Philosophy in Zoology. University of Khartoum.
26. Nyman M (2002): Fermentation and bulking capacity of indigestible carbohydrates: the case of inulin and oligofructose. *Br J Nutr* 87 Suppl 2: S163–168.
27. Op den Camp HJM, Oosterhof A, Veerkamp JH. (1984): Interaction of bifidobacterial lipoteichoic acid with human intestinal epithelial cells. *Infect Immun*;47:332-4.
28. Osman H.A.M., Taghreed B. E. Ibrahim, W.E. Soliman and Maather M. Monier (2010): Influence of dietary commercial brewer's yeast, *Saccharomyces cerevisiae* on growth performance, survival and immunostimulation of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. *Journal of Nature and Science*; 8 (3), 96-103.
29. Patterson J, Yasuda K, Welch R, Miller D, Lei X (2010): Supplemental dietary inulin of variable chain lengths alters intestinal bacterial populations in young pigs. *J Nutr* 140: 2158–2161.
30. Perdigon G, Nader De Macios, ME, Alvarez S, Oliver G, Pesce AA, Ruiz Holgado AAP. (1986): Effect of perorally administered lactobacilli on macrophage activation in mice. *Infect Immun*;53:404-10.
31. Quigley E. (2010): Prebiotics and probiotics; modifying and mining the microbiota. *Pharmacol Res* 61: 213–218.

32. Renard D, Lavenant-Gourgeon L, Ralet MC and Sanchez C, *Biomacromolecules*, September (2006), volume 7, issue 9, pages 2637-2649.
33. Roller Monika, Gerhard Rechkemmer and Bernhard Watz (2003): Prebiotic Inulin Enriched with Oligofructose in Combination with the Probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* Modulates Intestinal Immune Functions in Rats *Journal of nutrition*.
34. Schiffrin EJ, Brassart D, Servin AL, Rochat F, Donnet Hughes A. (1997): Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. *Am J Clin Nutr*; 66 (suppl):15-20.
35. Schultz, L.A., (1987). Lysozyme. In: *Methods in Clinical Chemistry*. A.J. Pesce and L.A. Kaplan, (Eds). St. Louis: Mosby, pp: 742-746.
36. Sheil B, MacSharry J, O'Callaghan L, O'Riordan A, Waters A, et al. (2006) Role of interleukin (IL-10) in probiotic-mediated immune modulation: an assessment in wild-type and IL-10 knock-out mice. *Clin Exp Immunol* 144: 273–280.
37. Shinohara K, Ohashi Y, Kawasumi K, Terada A, Fujisawa T. (2010). Effect of apple intake on fecal microbiota and metabolites in humans. *Anaerobe*; 16 (5): 510-5.
38. Silva, A.M., E.A. Bmbirra, A.L. Oliveira, P.P. Souza, D.A. Gomes, E. C. Vieira, and J.R. Nicoli, (1999). Protective effect of bifidus milk on the experimental infection with *Salmonella enteritidis* sub sp. Typhimurium in conventional and gnotobiotic mice *J. Appl. Microbiol.*, 86: 331-6.
39. Solano-Aguilar G, Dawson H, Restrepo M, Andrews K, Vinyard B. (2008): Detection of *Bifidobacterium animalis* subsp. *lactis* (Bb12) in the intestine after feeding of sows and their piglets. *Appl Environ Microbiol* 74: 6338–6347.
40. Standiford TK, Arenberg DA, Danforth JM, Kunkel SL, Van Otteren GM, Strieter RM. (1994): Lipoteichoic acid induces secretion of interleukin-8 from human blood monocytes: a cellular and molecular analysis. *Infect Immun*; 62:119-25.
41. Tietz, N.W. (1995). *Clinical Guide to Laboratory Tests*, 3 ed.
42. Vandenberg, P. A., (1993). Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiol. Rev.*, 12:221-238.
43. Vernazza C, Gibson G, Rastall R (2006): Carbohydrate preference, acid tolerance and bile tolerance in five strains of *Bifidobacterium*. *J Appl Microbiol* 100: 846–853.
44. Vinderola CG, Reinheimer JA (2003): Lactic acid starter and probiotic bacteria: a comparative “*in vitro*” study of probiotic characteristics and biological barrier resistance. *Food Res. Inter.* 36: 895–904.
45. Williams P.A. & Phillips G.O. (2000): Gum arabic. In *Handbook of Hydrocolloids*, pp. 155–168 [GO Phillips and PA Williams, editors]. Boca Raton, FL: CRC Press.
46. Yasuda K, Maiorano R, Welch R, Miller D, Lei X (2007): Cecum is the major degradation site of ingested inulin in young pigs. *J Nutr* 137: 2399–2404.

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