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Effect of Microencapsulated *Spirulina platensis* and *Bifidobacterium Longum* on Metabolic Syndrome of Female Rat Model

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Abstract

Metabolic syndrome is a multifactorial disorder characterized by obesity, dyslipidemia, hypertension and 2nd type diabetes. Three of which can set the diagnosis and may be complicated by cardiovascular disease and disturbed fertility. *Spirulina platensis* (*SP*) and *Bifidobacterium longum* (*BL*) have beneficial effect to control these disorders. Aim of study was to prepare edible microencapsulated *SP* and *BL* to study its possible role to control metabolic syndrome and associated disorders in female rat model. Twenty eight female rats were divided into 4 groups, 7 each. The first group (G_1) was fed on high fat diet (HFD), G_2 received HFD plus encapsulated *BL* (10^8 CFU/ml), G_3 fed on HFD plus both encapsulated *SP* and *BL.* Animals of G_4 received basic diet only. Blood glucose and body weight were monitored weekly. After 8 weeks, animals were sacrificed and blood samples were collected for analysis of glucose, cholesterol, LDL, HDL, triglycerides, leptin, oestrogen, Malondialdhyde (MDA), catalase, Zinc and phosphrous. Histological examination was done for liver, kidneys, heart and ovaries. Abdominal and body fat were collected for adiposity index estimation. Results revealed that animals fed on HFD (G1) showed the clinical signs of metabolic syndrome like hyperglycaemia, obesity, and dyslipidaemia compared to control (G4). The examined sections of liver, kidney, heart and ovaries showed abnormal histopathological changes. Administration of the prepared microcapsules took all the changes back towards normal with improvement of histopathological findings. It was concluded that *Sp* has synergistic effect with *BL* in possible controlling of metabolic syndrome and its associated disorders in female rat model.

***Keywords*:** Metabolic syndrome; *Spirulina platensis*; *Bifidobacterium longum;* diabetes; obesity; female fertility.

1. Introduction

Metabolic syndrome (MeS) is defined as the presence of three or more of the following metabolic disorders: central obesity, dyslipidemia, hypertension and hyperglycemia with increased related morbidity of diabetes, hypertension, fatty liver, cardiovascular disease and sometimes affected fertility [1,2]. Nutrients have biologically active compounds which can improve the body metabolic status, control obesity, diabetes and associated disorders [3-4].*Spirulina platensis* (*Sp)* is blue green algae found worldwide in fresh and marine waters. It is rich in protein (up to 70%), vitamins, β-carotenes, minerals, phenolic acids, tocopherols and γ-linolenic acid [5]. Many toxicological studies have proven its biosafety [6].It has the ability to lower the Cholesterol, LDL, triglycerides and blood glucose levels [5-8]. Spirulina has shown positive influence on the female reproductive system [9]. The inflammatory disorders due to obesity and diabetes cause hypogonadism due to hypothalamic Kiss/Kisspeptin suppression which control gonadotrophines levels. The adjusted estrogen levels in females are essential to spare deposition of fat in vital organs hence decreasing the incidence of CVD in females. Females affected by MeS will not have this gift [9]. *Bifidobacterium longum* (*BL*) has many health benefits [10-11]. It reduces mutagenic enzymes [12], lowers lipid and sugar profile in patients with type 2 diabetes and improve metabolic syndrome cases [13]. Recently it has been reported that the disturbed gut microbiota is important factor in development of some reproductive disorders such as polycystic ovary and abnormal estrogen level [15]. *Spirulina platensis*

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releases extracellular carbohydrates and other growth substances which may enhance the growth of probiotic bacteria and acting together as symbiotic [16].

Microencapsulation is a process in which a substance is coated by a wall material that can mask the unpalatable aroma of spirulina and keep the bacteria viable for longer time [17]. The goal of this study was to produce microencapsulated *SP* and *BL*, to explore their possible beneficial effect to control hyperlipidemia, weight gain and hyperglycemia and possible cardiovascular or reproductive disorders associated with metabolic syndrome in rat model.

2. Experimental (MATERIAL & METHODS)

Spirulina plantesis extraction: *SP* and BL were kindly supplemented by National Research Center, Cairo Egypt. Fifty grams of SP powder was added to 200 ml of methanol in Soxhlet extractor. The extracts centrifuged at 10,000 rpm for 15 min and filtered through Whatman #1 filter paper (Whatman International Ltd., England) and the solvents were evaporated [18]. Extracts were stored in airtight glass bottles in refrigerator until use.

Determination of antimicrobial activity

Methanol extracts of *Spirulina plantesis* was mixed with Dimethyl sulfoxide (DMSO) 0.5% and several concentrations of 1, 10 and 100 mg/mL were prepared. The antimicrobial assay was to estimate the degree of commensalism or synergism between *Spirulina plantesis* and probiotic bacteria. Three different species of probiotic bacteria have been supplied by Department of Dairy Microbiology, National Research Center. The bacterial species were *Lactobacillus plantarum* ATCC 8014, *Lactobacillus rhamnosus* ATCC 7469, Leuconostocmesenteroides ATCC 8293, and *Bifidobacterium longum* ATCC 15697). Each strain was activated in Tryptone soy broth or MRS broth by incubation at 37 ºC for 24 h [19, 20] then cultivated against 50µL of *Spirulina plantesis* extract in agar well diffusion method [21].The zone diameter of wells cut in Mueller-Hinton agar was 5.0 mm and the diameter of inhibition zone (DIZ) of negative control for each bacterium was also 5.0 mm. If the DIZ value is 5.0 mm (*), that means the sample has no inhibitory activity against that bacterium. The plates were incubated at 37°C for 24 h. Zones of inhibition were measured at the end of the incubation period [22]. One species of the tested probiotic bacteria was chosen to be encapsulated and administered to animals.

Microencapsulation procedure by Freeze drying method:

The culture of *Bifidobacterium longum* was sub-cultured in MRS broth supplemented with 2 gm/l sodium propionate and 3 gm. /l lithium chloride and incubated for 48h at 37 ºC. The cells were harvested and washed by centrifugation at 5000 rpm for 15 min at 4 ºC. The cell suspension (25 g) was mixed with a 100 ml of wall material sodium alginate solution (3.0% w/ v). After that, 3% of spirulina powder was added and stirred using magnetic stirrer. The mixture was kept for 24 h at -8ºC then subjected to Freeze dryer for 8h. The obtained microcapsules or freeze-dried powder was stored at 4º C. The same encapsulation process was done for bacterial suspension by alginate only as coating material without adding the spirulina powder [23].The two types of microcapsules were stored at 4 °C. To determine the microencapsulation efficiency (EE), 1gm of the *BL* microcapsules was dissolved in 9 ml of sterile tri sodium citrate solution 2% (w/v) and stirred to release the bacterial cells, and then serial dilution with physiological saline was done. Cultivation on plates containing MRS agar supplemented with 2gm/l sodium propionate and 3gm/l lithium chloride, anaerobically incubated at 37ºC for 48h. The encapsulation efficiency (EE) was determined using the following equation as described by [24].

$$EE = Log_{10}N / Log_{10}No \times 100$$

Where

N = the number of the *B. longum* loaded inside the microcapsules.

No = the number of the free *B. longum* added to the sodium alginate mixture during the process of microencapsulation.

Diet formulation:

Normal balanced diet and high fat diet were formulated to be given to animals [25].

Animal experiment

Animal experiment was designed and done under the regulation of ethical committee of NRC (ethical approval NO. 19179), Twenty eight Sprague - dawely adult female rats were purchased from animal house of NRC, divided into 4 groups, seven each. G1 was control positive group received high fat diet, G2, G3 rats received either HFD and encapsulated bacteria (8^{10} CFU/ml) or HFD plus encapsulated bacteria and spirulina (0.37gm/kg) respectively. Animals in G4 received basic diet only (control normal) for 8weeks. Blood glucose and body weight were monitored weekly, at the end of the experiment animals were fasted and sacrificed. Blood samples were collected into two tubes. The first, contained sodium citrate, for rapid analysis of blood glucose and the other contained EDTA for analysis of serum cholesterol , LDL, HDL, TG, MDA, catalase, GGT, urea, creatinine, zinc, phosphorus, leptin and estrogen using kits from Biodiagnostic Egypt. Weights of

whole body, organs and body fat were estimated. The adiposity index (ADI) was calculated (ADI = BF (BW-BF) /100) where BF means body fat and BW means body weight [26]. Organs were preserved in 10% formalin and sent for histopathological examination.

Histopathological examination

After collection of Liver, kidneys, heart and ovary specimens, the organs were fixed in neutral buffered formalin 10%, washed, dehydrated, cleared and embedded in paraffin blocks which were sectioned at 5 micron thickness and stained with Haematoxylin and Eosin for light microscopic examination (Olympus BX50, Tokyo, Japan) [27]. Histopathological alterations were graded as (0) indicated no changes, 1, 2 and 3 indicated mild, moderate and severe changes, respectively, while the grading was determined by percentage as follows: (<30%) showed mild changes, (<30% – 50%) indicated moderate changes and changes more than 50% (>50%) indicated severe changes[28].

The number of different ovarian follicles (total number of normal follicles as well as atretic follicles) from five sections of each ovary per rat (n=7) were counted by light microscope (Olympus BX50, Japan) using TS View version 6.2.4.5 software [29].

RESULTS & DISCUSSION

The current study was done to spotlight the possible effect of microencapsulated *spirulina platensis* if given in combination to encapsulated probiotic bacteria like *Bifidobacterium longum*. Aiming to control the experimentally induced metabolic syndrome in albino female rats. And if this can be implemented to help metabolic syndrome patients in further study.

Incubation of *spirulina platensis extract* with the tested bacteria showed enhanced growth of BL (Table 2). *Bifidobacterium Longum* has been chosen to be used combined with *spirulina platensis* to be administered to laboratory animals. *spirulina platensis* was categorized as prebiotic to enhances the growth of beneficial bacteria in the gut via secretion of fructooligosccharide which is consumed by bacteria as a nutrient [16]. Also, earlier studies reported that some lactic acid bacteria and bifidobacteria have resistance genes to antimicrobial agents [30].

The encapsulation can prevent the reaction between nutrients and reduce the astringency of the added bioactive compounds in functional foods. It also improves their bioavailability by protecting them against oxidation and processing conditions for better delivery and to control their release [18]. In the present situation the encapsulation efficiency of *Spirulina platensis* and BL recorded 93.4%, previous studies indicated that extrusion technique is very gentle on probiotic and yield great encapsulation efficiency [24, 31].

The weekly estimation of the body weight (Fig 1) showed different rates of elevation of BW by time. Rats given HFD in G1 showed the highest, while those in G4 showed the lowest rates. Animals fed the microcapsules showed less increment in body weight (G2, G3). Similar results were reported in human fed on probiotic bacterial combination [32, 33]. *Spirulina platensis* contains C-phycocyanin which exhibits anti-diabetic, antioxidant, hypolipidemic effect which consequently controls the increased body weight [34].

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The current Table (3) showed significant elevation in serum total cholesterol, triglyceride and LDL in rats fed on HFD (G1) versus control normal (G4). Treatment with either bacteria in G2 or *Spirulina platensis* combined with *BL* in G3 took the values of lipids back towards normal in both treatment groups. Earlier study showed that probiotics down regulate cholesterol absorption by genetic expression of NPC1L1 and PPAR [35]. Also, *BL* secreats enzyme called ferulic acid esterase which works on the dietary fibers to release antioxidant and hypolipidemic compounds. So that *Bifidobacterium longum* was reported as an ideal candidate bacteria to delay metabolic syndrome Symptoms [36]. *Spirulina* contains B-sitosterol, stigma sterol and C-phycocyanin which lowers cholesterol level [34].

The current study showed normal values γ glutamy-ltraferase, alkaline phosphatase, urea and creatinine in all groups indicating the biosafety of the used microcapsules. Previous study reported that administration of spirulina platensis did not show any adverse effect or toxicity symptoms when given daily at high dose of 30 and 10 g/kg body weight for 12 weeks [37]. *Spirulina platensis* contains C-phycocyanin which exhibits hepato-protective and reno-protective effect [34].

Blood glucose level in the current results showed significant elevation in G1 fed on HFD versus all other groups. Animals given the microcapsules (G2 and G3) showed lower levels. This coincide with earlier studies which reported the effectiveness of probiotics in treatment of patients with type 2 diabetes[38] and enhancement of insulin secretion

[39]. Also, spirulina platensis was previously used in controlling 2nd type of diabetes where it contains C-phycocyanin which exhibits anti-diabetic effect [34]. The present study showed significant elevation in lipid peroxidation marker (MDA) and lowered the catalase level in G1 which fed on HFD versus control group fed on basal diet. Feeding the microcapsules controlled the oxidative stress, lowered MDA and elevated serum catalase values in G2 and G3 [39]. It is well known that T2DM with insulin-resistance have higher metabolic endotoxemia than normal ones [40] where the increased levels of circulating insulin changes intestinal permeability, and allow gut endotoxins to leak in the circulation causing inflammatory reactions triggered by immune pathway [41]. The probiotics supplementation controls the endotoxin influx by improving the intestinal barrier permeability and consequently decreasing the proinflammatory mediators [42].

After scarification of animals, the organs weights were recorded. Evaluation of the weight of total body fat was to calculate the adiposity index (ADI) [26]. Results showed that ADI was significantly higher in G1 fed on HFD versus control normal diet (G4) indicating increment of fat around internal organs as one of metabolic syndrome Signs. This was confirmed by elevated heart and liver weight in G1 compared to G4, Table (4).

The histopathological examination of liver, kidney and heart obtained from G1 exhibited marked cytoplasmic vacuolization of epithelial lining of hepatocytes, renal tubules, endothelial lining of glomerular tuft and cardiac myocytes (Fig. 2a,3a,4a), Kupffer cells activation, focal hepatocellular necrosis associated with mononuclear cell infiltration (Fig. 2b),congestion of central hepatic veins and myocardial vessels associated with oedema and focal hemorrhage (Fig. 2c, 4b,4c).This indicates deleterious inflammatory effect of HFD on level of cells, blood vessels and tissues hence affecting organ function[43]. Earlier studies reported hepatic steatosis and focal necrosis accompanied with mononuclear cell infiltration in rats fed on high fat diet and referred the condition to the lipid peroxidation and liberation of ROS [39, 44].

Supplementation of the microcapsules in G2 and G3 lowered the ADI back toward control values with significant loss of fat around internal organs (Table4). Also the weight of heart and liver of rats decreased in animals fed the microcapsules (G2, G3) versus G1.Marked restoration of the normal histological picture of hepatic, cardiacc and renal tissue was recorded in G2 and G3. Examined sections revealed no changes except slight cytoplasmic vacuolization of some hepatocytes and cardiac myocytes (Fig. 2d, 2e, 3c, and 4c) and congestion of central hepatic vein. The effect of the supplements showed hard evidence of improving inflammatory stage in all organs examined and consequently better function. This was comparable with the normal histological architecture of hepatic lobule, renal parenchyma and cardiac myocytes seen in normal controls G 4 (Fig.2f, 3d, 4d) [43].

The current study registered lesions regression in G3 when spirulina was co-administered with HFD and bacteria. This protective role of spirulina in recovering tissue damages may be attributed to its high content of nutrients likeprotein, amino acid, iron, β-carotene, phycocyanin, γ-lenolenic acid, vitamin B1, B2, B3, B6, B12 and essential fatty acid which are very much helpful to maintain the normal health [45, 46]. Probiotics enhance secretion of active enzymes which regulates lipid deposition and formation in liver the production of another microbial protein, cinnamoyl esterase, have shown significant levels of antioxidant activity and prevent lipid peroxidation [47, 48].

Histopathological alterations of liver, kidneys and heart were graded and scored according to their severity as shown in Table (5).

The current study showed increased number of follicles in ovaries collected from G3 which were treated with spirulina. Similar results were reported in earlier study [49]. Morphometric examination of sections of ovarian tissue showed that the HFD group G1, showed significant decrease in the number of ovarian follicles and significant increase in atretic follicles ($P < 0.01$) in comparison with control group. Co-treatment either with probiotic or combination of probiotic and spirulina significantly increased the number of ovarian follicles ($P < 0.01$) compared to group 1 and non-significant increase in atretic follicles when compared to the control rats (table 6).

Microscopically, ovaries of rats from G1 (fed HFD) showed great reduction in number of growing follicles and increased number of atretic follicles in comparison with control group. Additionally, examined sections revealed congestion of blood vessels in ovarian medulla and multiple atritic follicles (Fig. 5a) as well as vacuolar degeneration and apoptosis of lutein cells of corpus luteum indicating complication affecting ovarian function. Meanwhile, ovaries of rats from groups 2 & 3 revealed improved histopathological picture, examined sections exhibited multiple large numbers of follicles in various stages of development (Figs. 5 b & c). Moreover, ovaries of rats from group 4 (normal control) appeared histological normal and the ovarian cortex containing follicles in various stages of development (Fig.5d).

Table (1): formulation of control balanced and high fat diet

Ingredients	Balanced diet (gm)	High fat diet (gm)
Casein	10	10
Cellulose	10	-
Corn oil	10	-
Slat mixture	4	4
Vitamin mixture	1	1
L-Cystine	0.018	0.018
Choline chloride	0.025	0.025
Corn starch	64.957	43.545
Cow fat	-	20
cholesterol	-	2
Bile salt	-	0.025

Table (2) Antimicrobial activity of *Spirulina platensis* extracts against some probiotic bacteria

Probiotic bacteria	Methanolic extract of *spirulina platensis*
Leuconostocmesenteroides	Nil
Lactobacillus plantarum,	Nil
Bifidobacterium longum	Nil

Nil; not detected

Table 3: Plasma biochemistry of rats administered the microcapsules.

	No of follicle Rovary	No of folliclesL. ovary	Genital tract	Liver	Heart	Kidney	Fat	**ADI**
G1	3.33± 0.21^{a}	3.90±0.36^{a}	93.33±3.29 ^{ab}	10.20 ± 0.48^{a}	1.12 ± 0.06^{a}	1.86 ±0.29^{a}	7.92 ± 1.74^{a}	7.5±0.37^{a}
G2	5.33± 0.42^{b}	4.33±0.21^{a}	91.0± 3.52^{a}	9.71 ± 0.81^{a}	1.02 ± 0.08^{a}	1.68 ±0.11^{a}	8.11 ± 2.18^{a}	5.75±0.5^{b}
G3	6.66± 0.21^{c}	6.00±0.2^{b}	107.3±6.10^{b}	9.29 ± 0.66^{a}	0.90 ± 0.04^{ab}	1.55 ±0.08^{a}	4.29 ± 0.87^{a}	4.1±0.42^{c}
G4	4.33±0.51^{d}	4.33±0.22^{a}	99.66± 6.5^{ab}	6.57 ± 1.16^{b}	0.78 ± 0.07^{b}	1.68 ± 0.15^{a}	5.45 ± 1.30^{a}	4.1±0.46^{c}

*different superscripts means significance P≤ 0.05

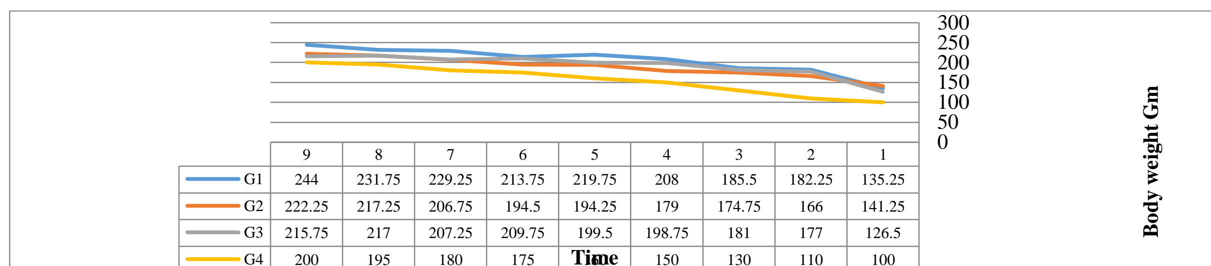

Fig (1) Changes of BW in different groups

Table 4: weight of internal organs and adiposity index

	G1	G2	G3	G4
T. cholesterol (mg/dl)	184±8.24 ^{a}	116±3.37 ^{b}	131.7±6.74 ^{b}	75.28±0.18 ^{c}
LDL (mg/dl)	140.2± 6.4 ^{a}	95.1±9.5 ^{b}	105.69±9.4 ^{b}	57.49±8.4 ^{c}
HDL mg/dl	13.12±0.92 ^{a}	49.34±0.08 ^{b}	17.73±0.75 ^{a}	18.78±0.34 ^{c}
TG (mg/dl)	200.95 ±6.4 ^{a}	138 ±8.8 ^{b}	110± 3.96 ^{c}	91.6 ± 5.56 ^{d}
Glucose (mg/dl)	152.66±30.17 ^{a}	96.66±11.23 ^{b}	124.5±8.76 ^{c}	62.66±74.04 ^{d}
MDA(nmol/ml)	4.41±0.48^{a}	3.09±0.83 ^{b}	1.99±0.15 ^{c}	1.19±0.42 ^{c}
Catalase (IU/L)	223.2±23.59 ^{a}	505.3±68.90^{b}	267±35.35 ^{a}	261.3±43.70 ^{a}
Zinc(µg/dl)	166±27.70 ^{a}	138.5±8.03 ^{a}	127.9±7.12 ^{a}	122.1±2.35 ^{a}
GGT (U/L)	4.94±0.72 ^{a}	13.64±2.19 ^{a}	10.15±2.78 ^{a}	7.94±0.79 ^{a}
Albumin (mg/dl)	1.16±0.53 ^{a}	1.24±0.60 ^{a}	1.17±0.60 ^{a}	0.74±0.30 ^{a}
Pi (mg/dl)	9.62±0.70 ^{a}	9.87±0.54 ^{a}	10.5±0.91 ^{a}	11.49±1.52 ^{a}
Alk.phosph (IU/l)	20.08±3.05 ^{a}	25.14±3.70 ^{a}	10.28±3.83 ^{a}	17.68±3.40 ^{a}
Urea(mg/dl)	17.08±4.78 ^{a}	24.58±4.63 ^{a}	20.13±3.95 ^{a}	21.35±4.23 ^{a}
Creatinine (mg/dl)	0.70±0.17 ^{a}	0.95±0.12 ^{a}	0.63±0.14 ^{a}	0.98±0.13 ^{a}
Leptin (ng/dl)	1.54±0.38^{a}	1.49±0.34 ^{a}	1.54± 0.39 ^{a}	1.24±.33^{b}
Estrogen (pg/ml)	125.16± 8.6^{a}	128.16±8.9^{a}	181.30± 8.9^{b}	127.29± 5.26^{a}

*calculated adiposity index ADI = BF (BW-BF) /100) where BF means body fat and BW means body weight.

Table (5): Histopathological lesion score in organs of different groups

Organs	Lesions	G 1	G 2	G 3	G 4
Liver	Kupffer cell activation	3	1	1	0
	Hepatocellular steatosis	3	2	1	0
	Hepatocellular necrosis	2	1	0	0
	Inflammatory cells infiltration	2	1	0	0
Kidneys	Cytoplasmic vacuolization of epithelial lining renal tubules Congestion of renal blood vessels and glomerular tufts Focal renal hemorrhage - Interstitial mononuclear Interstitial inflammatory cells infiltration	3 3 2 2	2 2 0 0	1 1 0 0	0 0 0 0
Heart	Cytoplasmic vacuolization of the cardiac myocytes Congestion of myocardial blood vessels Intermyocardialedema	3 2 2	2 1 1	1 1 1	0 0 0

Table (6): Histomorphometric analysis of ovarian follicle counts in all experimental groups

Group	Total number of normal follicles	Number of atretic follicles
G 1	13.0000 ± 1.00^{c}	8.4000 ± 0.55^{b}
G 2	23.0000 ± 1.00^{b}	3.6000 ± 0.55^{a}
G 3	26.6000 ± 0.89^{a}	3.0000 ± 0.71^{a}
G 4	28.0000 ± 0.70^{a}	2.8000 ± 0.84^{a}

The values are expressed as the means ± SD, where n=7. Superscript refers to significance from control group. *P*< 0.01 was considered significant.

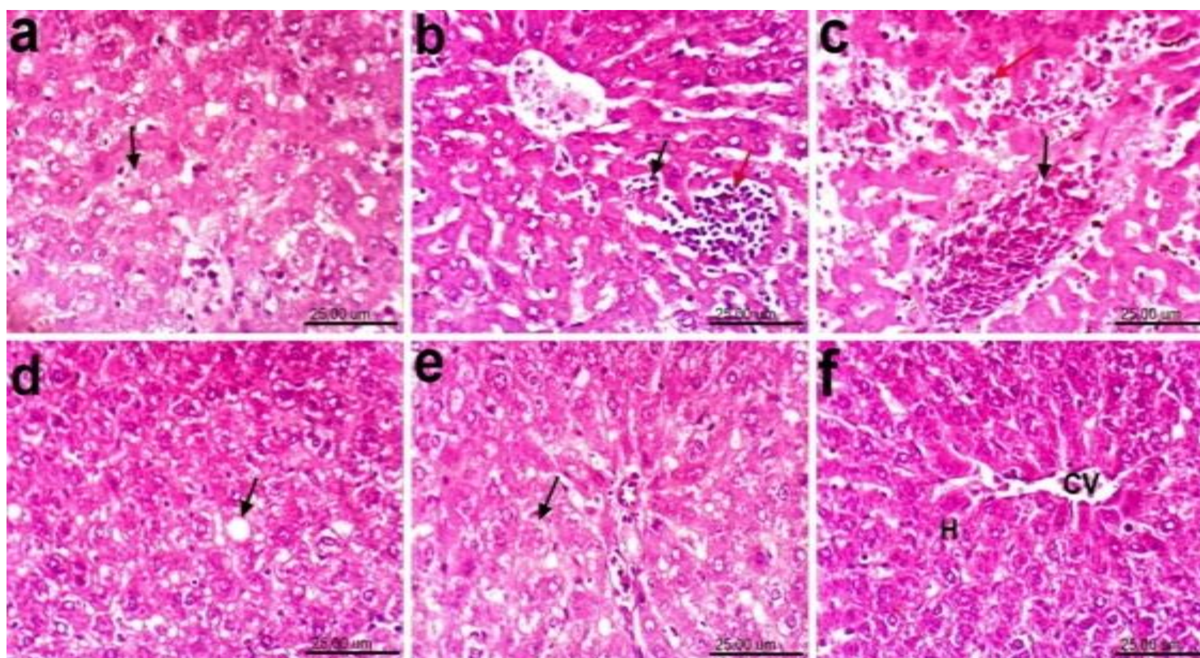

Fig.(2): Representative photomicrographs of H & E stained liver sections of rats.**a), b)** and **c)** HFD, showing marked hepatocellular vacuolization (black arrow)a), Kupffer cells activation (black arrow) and focal hepatocellular necrosis associated with mononuclear cell infiltration (red arrow) b),congestion of central vein (black arrow) and focal hepatic haemorrhage (red arrow) (c). d) HFD + capsulated bacteria, showing cytoplasmic vacuolization of some hepatocytes (black arrow). e) HFD + capsulated bacteria+ spirulina, showing slight cytoplasmic vacuolization of some hepatocytes (black arrow).f) normal control, showing the normal histological architecture of hepatic lobule, normal central vein (CV) and hepatocytes (H). (scale bar 25um).

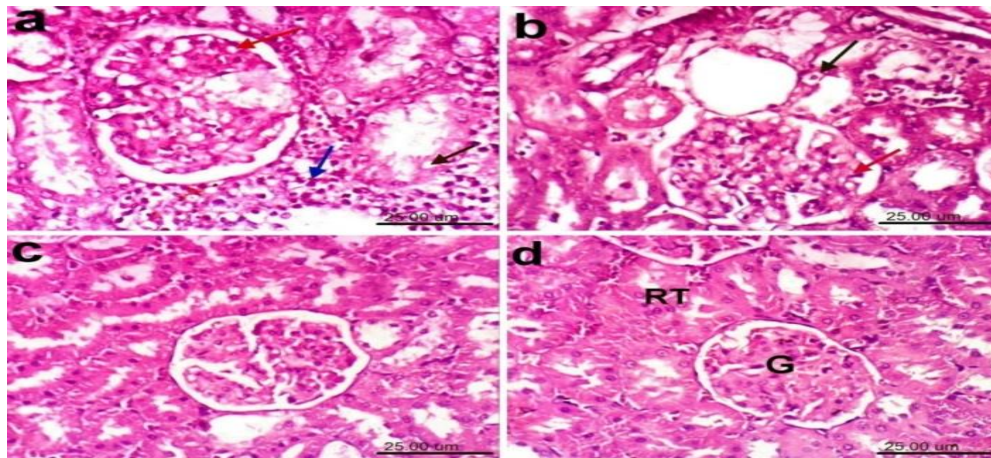

Fig. (3): Representative photomicrographs of H & E stained kidneys sections of rats,**a)** HFD, showing cytoplasmic vacuolization of epithelial lining renal tubules (black arrow) and endothelial lining glomerular tuft (red arrow) as well as interstitial inflammatory cells infiltration (blue arrow). b) HFD + capsulated bacteria, showingslight cytoplasmic vacuolization of epithelial lining some renal tubules (black arrow) and endothelial lining glomerular tuft (red arrow). c) HFD + capsulated bacteria+ spirulina, showing no histopathological alterations. d) Normal control, showing the normal histological architecture of renal parenchyma. Note normal glomerulus (G) and renal tubules (RT). (Scale bar 25um).

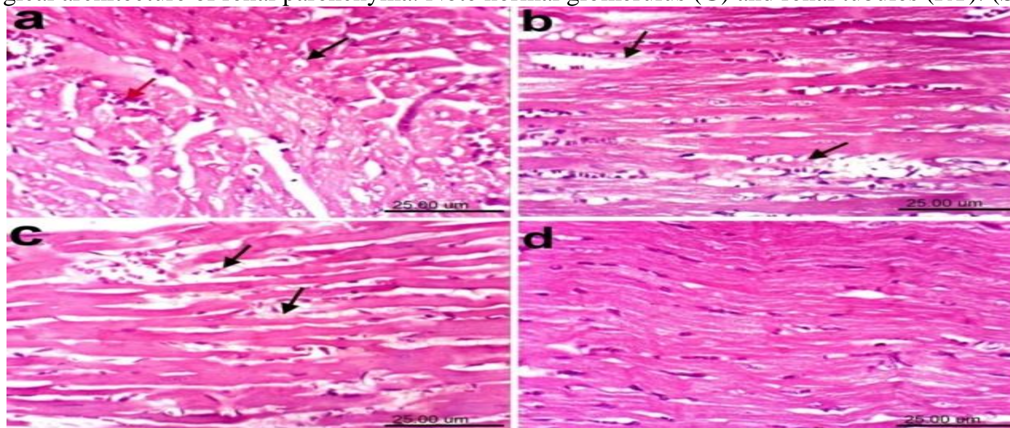

Fig. (4): Representative photomicrographs of H & E stained heart sections of rats.**a)** HFD, showing cytoplasmic vacuolization of the cardiac myocytes (black arrow) and congestion of myocardial blood vessel (red arrow).

b) HFD + capsulated bacteria, showing slight intermyocardialedema (black arrow). c) HFD + capsulated bacteria+ spirulina, showing slight intermyocardialedema (black arrow). d) Normal control, showing the normal histological structure of cardiac myocytes. (scale bar 25um).

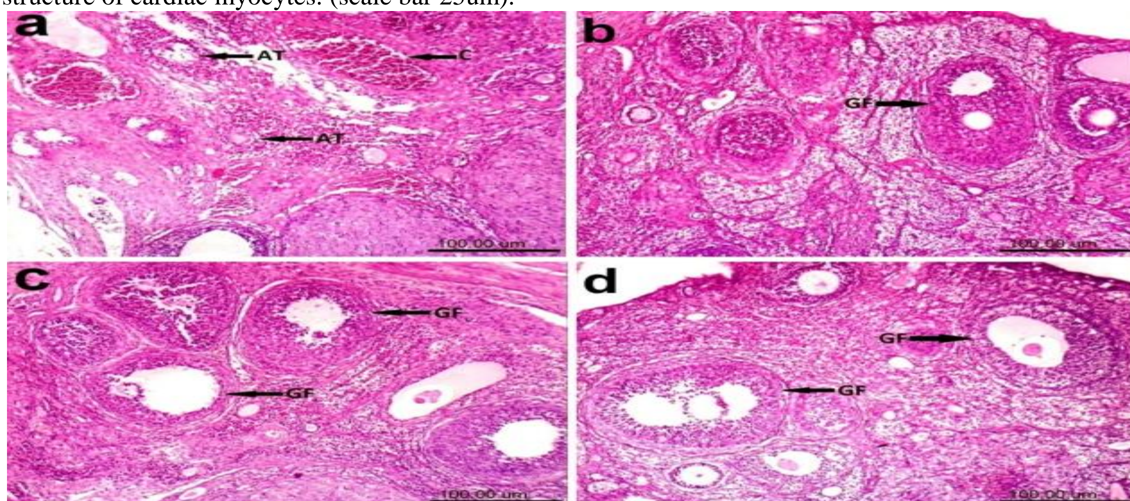

Fig. (5): Representative photomicrographs of H & E stained ovary sections of rats. **a)** HFD, showing congestion of blood vessels (C) in ovarian medulla and multiple atritic follicles (AT). b) HFD + capsulated bacteria, showingmultiple follicles in various stages of development. Note graafian follicle (GF). c) HFD + capsulated bacteria+ spirulina, showing large number of follicles in various stages of development. Note multiple graafian follicles (GF). d) normal control, showing the normal histological structure of ovarian cortex with multiple follicles in various stages of development. Note graafian follicles (GF). (scale bar 100um).

3. Conclusion

Current study reached the conclusion that micro encapsulated Sp and BL worked in synergy in possible controlling of metabolic syndrome and its associated disorders. Possible effect can be tested on large scale study on human to proof such results.

4. Conflict of interest: There is no conflict of interests

5. Acknowledgments

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