



Role of Commiphora Mol Mol and Doxycycline in Prophylaxis of Spontaneous Bacterial Peritonitis in Egyptians Cirrhotic Patients

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Abstract

Aims: this study aimed to evaluate the role of commiphora mol mol and doxycycline in the prophylaxis of spontaneous bacterial peritonitis in cirrhotic patients. **Methodology:** sixty ascetic cirrhotic patients from National Liver Institute, Menofiya University (Shebeen El-Kom- Egypt) with a history of at least one previous episode of SBP were included in blinded randomized clinical study. Patients were divided into four groups in a fashion; each group consists of 15 patients. Group one received ciprofloxacin (control group), group two received doxycycline, group three received commiphora mol mol and group four received a combination of commiphora mol mol and doxycycline for six months. Serum interleukin 6 (IL 6) and C Reactive Protein (CRP) level were the primary inflammatory markers of the study to evaluate the effect of the studied medications. **Results:** both IL-6 and CRP levels showed significant decrease three and six months after treatment in all studied groups ($P < 0.05$). Group four showed a significant decrease in IL 6 and CRP level compared with other three groups ($P < 0.05$). **Conclusion:** the results of the current study strongly support the efficacy of doxycycline and Commiphora molmol as a primary prophylactic therapy in patients with SBP. Furthermore, commiphora mol mol/doxycycline combination was the most effective and showed synergistic effect which may be useful in decrease emergence of resistant strains.

Keywords: Spontaneous bacterial peritonitis; Commiphora mol mol; Doxycycline; IL 6; CRP

1. Introduction

Spontaneous bacterial peritonitis (SBP) is a bacterial infection of ascitic fluid which arises in the absence of any other source of sepsis within the peritoneum or adjacent tissues [1]. The Gram-negative bacteria are largely responsible for SBP episodes [2]. Percentage of gram positive bacteria causing SBP represent about 48 % which leading to increase resistance towards quinolones prophylactic therapy [2].

In liver cirrhosis, three mechanisms are proposed for the pathogenesis of SBP [3]. Patients with ascetic fluid PMN counts ≥ 250 cells/mm³ in a clinical setting, compatible with ascitic fluid infection, should receive empiric antibiotic therapy such as cefotaxime 2 g intravenously every 8 hours for 5 days or ciprofloxacin 200 mg intravenously every 12 hours for 7 days [4].

After the resolution of an episode of SBP, the recurrence is frequent. Therefore, intestinal decontamination is required by antibiotic prophylaxis which could not only improve short term survival but also could reduce the overall risk of infections including SBP [5]. According to European Association for the Study of the Liver guidelines the administration of prophylactic antibiotics reduces the risk of recurrent SBP, Oral norfloxacin at a dose of 400 mg daily is the prophylactic therapy of choice. Alternative prophylactic antibiotics include oral ciprofloxacin 750 mg once weekly [6]. Doxycycline exerts a broad range of antimicrobial activity against both gram-positive and gram-negative bacteria that considered predisposing agents for SBP such as Shigella, Klebsiella, Escheria coli, Enterobacter aerogenosa, Uresinia pestis [7] and Brucella [8]. Commiphora molmol, an herbal medicine that has antiseptic and antimicrobial activities was formerly approved in Egypt for management of both Fasciola hepatica and Schistosoma infections [9].

The aim of our study was directed to evaluate the role of commiphora mol mol and doxycycline in the prophylaxis of spontaneous bacterial peritonitis in Egyptian cirrhotic patients.

2. Materials and Methods

This study design was blinded randomized clinical study that was conducted in the National Liver Institute, Menofiya University (Shebeen El-Kom- Egypt) between October 2012 and November 2013. The protocol for this study was approved by the National Research Ethics Committee of Menoufiya University, Shebin El-kom, Egypt with Institutional Review Board (IRB) protocol number 0064/2012. The diagnosis of SBP was confirmed if the ascetic fluid polymorph nuclear cell (PMN) count was greater than 250 mm³ with or without positive culture and by absence of an intra- abdominal source of infection. Ascetic fluid cultures were performed using the conventional culture method and via inoculating 10 ml of fluid in aerobic and anaerobic blood culture bottles at the bedside.

A total number of 100 adult Middle-Eastern Egyptian cirrhotic patients of both sexes were enrolled in this study. 40 patients were excluded from the study (25 patients had hepatocellular carcinoma and 15 patients had severe gastrointestinal bleeding who subsequently died). The remaining 60 patients have ascetic fluid polymorph nuclear cell (PMN) count was lower than 250 mm³, show no signs or symptoms of peritoneal infection such as severe abdominal pain, fever and chills. They were randomized using stratification method into four groups: Group one or control group (15 patients) who received ciprofloxacin 750 mg/week orally for six months (CiprobayTM 750 mg tablet, Hikma pharma S.A.E under license of Bayer-Schering pharma, Germany), group two (15 patients) who received doxycycline 100 mg tablet (VibramycinTM 100 mg tablet, Pfizer, Egypt) twice daily for ten days and then twice weekly for 6 months, group three (15 patients) who were received commiphora mol mol capsule (MirazidTM S.G. capsule, Pharco pharmaceuticals company, Egypt) 10 mg/kg once daily for six days and then 10 mg/kg twice weekly for six months and group four (15 patients) who received commiphora mol mol and doxycycline combination by the same doses scheduled above for six months.

Inclusion criteria were patients with cirrhosis and ascites who had history of at least one previous episode of SBP, age >18 and <80 years old and both sexes. Exclusion criteria included active gastrointestinal bleeding, encephalopathy > grade 2, hepatocarcinoma or other malignancies, patients with any other inflammatory disease, patients on any medications that can affect inflammatory markers assessment and patients allergic to study medications. All patients of the study took propranolol, spironolactone and furosemide for hypertension and ascites respectively. All patients were classified as Child C using Child-Pugh Classification.

Eligible patients gave their written informed consent. After signing a consent form, all individuals included in the study were submitted to physical examination, medical history (past or current illness), demography (age, sex, smoking habits) and measurement of weight. Body weight was measured using Detecto scale (Detecto Company, 203 East Daugherty Street, USA). All patients were submitted to blood sample collection at enrollment (baseline), three and six months after treatment for assessment of liver function, renal function, sodium level, red and white blood cells count, platelet count, hemoglobin value, serum IL-6 level and C-RP concentration.

Patients were followed up closely every month with careful assessment to rule out any complications such as fever, abdominal pain or other symptoms or signs of infection. Study medication was discontinued in the case of recurrent SBP that represents end point of the trial. The drugs used in the study were withdrawn in patients suffering from other complications such as gastrointestinal bleeding or encephalopathy and receiving the standard treatment in each case.

2.1 Biochemical assays

2.1.1 Measurement of inflammatory markers

Serum interleukin-6 (IL-6) was assayed using Enzyme-Linked Immunosorbent Assay kit (AviBion Human IL-6 ELISA Kit, Orgenium

Laboratories, Finland) [10]. Serum CRP was assayed turbidimetrically using fixed-time measurement (Elicta 1.2, Vita Chem Co., Italy) [11].

2.1.2 Measurement of liver function parameters:

Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were measured spectrophotometrically using kinetic method [12, 13], serum bilirubin level (total and direct) was measured spectrophotometrically using colorimetric (Diazo) method [14], measurement of serum albumin concentration was determined spectrophotometrically using modified bromocresol green colorimetric method [15], prothrombin time was determined by coagulation method [16].

2.1.3 Measurement of kidney function parameters:

Blood urea nitrogen was determined spectrophotometrically using enzymatic (fixed rate) UV method with urease and glutamate dehydrogenase [17], serum creatinine concentration was determined spectrophotometrically using buffered kinetic Jaffé reaction without deproteinization method [18], sodium level was determined colorimetrically [19].

2.1.4 Measurement of hematological parameters:

The Sysmex[®] Automated Hematology Analyzer KX-21N is used for the assay of haemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs). (Sysmex Corporation, Kobe 651-0073, Japan).

3. Statistical analysis

Minitab release 15, Pine Hall Road, State College, PA, USA was used for statistical analysis of obtained data. Paired t-test was used to assess any significant difference between each group before and after treatment course. One-way analysis of variance test and two-sample t-test were used to assess any significant

difference between groups after treatment. Data were presented as the mean \pm S.D and the level of significance was set at $P<0.05$.

4. Results

Demographic data for all patients in the four studied groups are shown in Table 1 that showed non significant variation between them. Table 2 showed also base line data obtained for measuring parameters for all studied groups and

did not show any significant difference; therefore any changes happened after treatment was attributed to the used medications and not due to the individual variations. Laboratory data for patients in the four studied groups three and six months after treatment were demonstrated in Table 3 and Table 4 respectively .The data obtained for all studied groups showed statistically significant decrease ($p<0.05$) in IL-6 and CRP levels three and six months after treatment as compared to their baseline data .

Table 1: Demographic data of the participants

Parameters	Group 1 (Control)	Group 2 Doxycycline	Group 3 Commiphora	Group 4 Combination	P value ANOVA test
Age (years)	61 \pm 7.63	59.4 \pm 6.75	58.4 \pm 7.51	60.2 \pm 7.31	0.791
Sex (male)	10 (66.67 %)	13 (86.67%)	11 (73.33%)	9 (60 %)	0.428
Weight (kg)	78.47 \pm 5.88	76.43 \pm 7.357	77.57 \pm 8.27	77.97 \pm 7.31	0.887
Smoking (%)	3 (20 %)	2 (13.33 %)	2 (13.33 %)	1 (6.67 %)	0.778
Diabetes (%)	1(6.67 %)	2 (13.33 %)	3 (20%)	2 (13.33 %)	0.778

$n = 15$ for all groups.

Table 2: Laboratory baseline data for SBP patients included in the four studied groups

Parameters	Group 1 (Control)	Group 2 Doxycycline	Group 3 Commiphora	Group 4 Combination	P value ANOVA test
AST (IU/L)	77.60 \pm 22.5	76.07 \pm 20.81	75.60 \pm 19.51	74.47 \pm 18.24	0.980
ALT (IU/L)	67.4 \pm 14.37	64.8 \pm 16.18	64.4 \pm 17.09	59.87 \pm 12.72	0.617
BIL-T (mg/dl)	2.23 \pm 0.93	1.97 \pm 0.57	2.41 \pm 0.74	2.32 \pm 0.58	0.569
BIL-D (mg/dl)	1.26 \pm 0.74	1.22 \pm 0.59	1.57 \pm 0.64	1.59 \pm 0.60	0.245
Albumin(g/dl)	2.12 \pm 0.38	2.05 \pm 0.44	2.21 \pm 0.47	2.20 \pm 0.53	0.756
PT (Sec.)	26.73 \pm 4.95	26.89 \pm 4.28	26.67 \pm 4.77	26.79 \pm 4.54	0.999
BUN (mg/dl)	56.93 \pm 17.06	53.27 \pm 16.06	56.07 \pm 21.27	59.4 \pm 17.8	0.827
S.Cr (mg/dl)	1.19 \pm 0.59	1.13 \pm 0.46	1.05 \pm 0.51	1.22 \pm 0.74	0.856
Sodium (mEq/L)	127.47 \pm 5.49	126.8 \pm 4.31	127.47 \pm 4.76	127.73 \pm 5.44	0.963
Hb (g/dl)	10.31 \pm 1.44	10.49 \pm 1.63	10.28 \pm 1.48	10.01 \pm 1.05	0.826
RBCs(103/ μ l)	3.36 \pm 0.76	3.38 \pm 0.66	2.99 \pm 0.59	2.94 \pm 0.44	0.101
WBCs(103/ μ l)	6.58 \pm 1.62	6.17 \pm 1.3	6.04 \pm 1.3	6.21 \pm 1.54	0.771
Platelets (103/ μ l)	72.5 \pm 11.76	78.13 \pm 16.24	79.8 \pm 5.8	79.33 \pm 9.90	0.295
IL-6 (pg/ml)	189.36 \pm 25.76	187.30 \pm 22.28	185.02 \pm 19.99	185.76 \pm 23.41	0.993
C-RP (mg/l)	60.73 \pm 8.69	52.70 \pm 8.71	55.16 \pm 8.95	58.47 \pm 8.74	0.073

Data are presented as mean \pm SD , $P < 0.05$ is considered significant(ANOVA test)

AST (Aspartate transaminase); ALT (Alanine-aminotransferase); BIL-T (Total bilirubin); BIL-D (Direct bilirubin); PT (Prothrombin time); BUN (Blood Urea Nitrogen); S.Cr (Serum creatinine); Hb (Hemoglobin); RBCs (Red blood cells); WBCs (White blood cells); IL-6 (interleukin-6); C-RP (C-Reactive Protein).

$n = 15$ for all groups.

Furthermore, the levels of both IL-6 and CRP six month after treatment were significantly lower than their detected levels three months after treatment ($p<0.05$)

There is significant decrease in IL-6 and CRP levels three and six months after treatment as compared to their baseline data (paired t- test) ($p<0.05$) The change in serum IL-6 and CRP levels within the four studied groups through the

study period was demonstrated in Figure 1 and Figure 2 respectively.

There was significant decrease in ALT and AST in group 3 &4 when compared to group 1 & 2 in follow up after 3 & 6 months($p<0.05$) . As regarding hematological parameters, group 3 showed statistically significant increase in WBCs both three and six months after treatment in comparing to its base line data (paired t- test) ($p<0.05$) as shown in Figure 3.

Table 3: Laboratory data for SBP patients included in the four studied groups 3 months after prophylactic therapy

Parameters	Group 1 (Control)	Group 2 Doxycycline	Group 3 Commiphora	Group 4 Combination	P value ANOVA test
AST (IU/L)	85.7±14.89	83.8±10.41	70.47±13.39	72.60±14.1	0.005
ALT (IU/L)	71.67±10.81	69.60±11.81	59.60±9.05	57.60±10.09	0.001
BIL-T (mg/dl)	2.41±0.69	2.18±0.62	2.22±0.75	2.44±0.58	0.569
BIL-D (mg/dl)	1.54±0.46	1.37±0.40	1.45±0.59	1.65±0.57	0.476
Albumin(g/dl)	1.99±0.50	1.98±0.40	2.28±0.45	2.26±0.44	0.114
PT (Sec.)	27.15±3.85	27.79±3.78	27.89±3.75	27.69±3.22	0.926
BUN (mg/dl)	59.13±16.15	54.4±16.32	56.93±18.85	61.93±15.5	0.652
S.Cr (mg/dl)	1.33±0.39	1.22±0.32	1.10±0.41	1.46±0.42	0.08
Sodium (mEq/L)	126.2±5.47	126.13±4.26	127.67±4.50	127.53±4.91	0.720
Hb (g/dl)	10.14±1.37	10.22±1.54	10.71±1.15	10.3±0.94	0.617
RBCs(103/ μ l)	3.25±0.65	3.22±0.60	3.06±0.51	3.01±0.3	0.521
WBCs(103/ μ l)	6.41±1.67	5.91±1.35	6.47±1.13	6.37±1.44	0.722
Platelets (103/ μ l)	68.3±16.77	73.67±17.62	78.27±8.05	76.6±12.92	0.257
IL-6 (pg/ml)	151.68± 20.54	149 ± 23.18	134.71± 27.01	89.73± 13.77	0.000
C-RP (mg/l)	37.71±6.05	35.26 ±4.37	34..37 ±8.10	24.78 ±6.38	0.000

Data are presented as mean \pm SD ,P <0.05 is considered significant(ANOVA test)

AST (Aspartate transaminase); ALT (Alanine-aminotransferase); BIL-T (Total bilirubin); BIL-D (Direct bilirubin); PT (Prothrombin time); BUN (Blood Urea Nitrogen); S.Cr (Serum creatinine); Hb (Hemoglobin); RBCs (Red blood cells); WBCs (White blood cells); IL-6 (interleukin-6); C-RP (C-Reactive Protein).

n = 15 for all groups.

Table 4: Laboratory data for SBP patients included in the four studied groups 6 months after prophylactic therapy

Parameters	Group 1 (Control)	Group 2 Doxycycline	Group 3 Commiphora	Group 4 Combination	P value ANOVA test
AST (IU/L)	86.87±16.81	85.33±11.56	65.33±16.4	69.2±10.9	0.000
ALT (IU/L)	75.33±16.29	71.2±12.36	52.67±14.80	55.73±10.51	0.000
BIL-T (mg/dl)	2.56±0.74	2.21±0.62	1.99±0.89	2.54±0.49	0.087
BIL-D (mg/dl)	1.74±0.45	1.55±0.55	1.24±0.65	1.75±0.53	0.210
Albumin(g/dl)	1.96±0.50	1.95±0.46	2.43±0.63	2.33±0.46	0.060
PT (Sec.)	28.46±4.38	28.33±3.72	28.75±3.70	28.76±3.16	0.986
BUN (mg/dl)	63.33±18.2	56.67±11.2	59.13±20.25	64.13±14.28	0.557
S.Cr (mg/dl)	1.52±0.29	1.41±0.42	1.16±0.4	1.60±0.36	0.362
Sodium (mEq/L)	125.03±5.02	125.8±4.54	127.93±4.61	127.13±4.05	0.530
Hb (g/dl)	9.96±1.26	10.15±1.56	10.81±0.79	10.39±1.18	0.267
RBCs(103/μl)	3.16±0.59	3.17±0.7	3.19±0.4	3.12±0.44	0.985
WBCs(103/μl)	5.81±1.8	5.55±0.9	6.87±1.25	6.33±1.32	0.114
Platelets (103/μl)	64.4±20.19	72±21.26	77.13±12.82	75.8±14.79	0.205
IL-6 (pg/ml)	102.82± 13.37	99.32± 13.00	96.41± 18.91	65.95± 11.86	0.000
C-RP (mg/l)	25.33±7.39	22.07 ±2.11	21.46 ±4.62	14.65 ±0.68	0.000

Data are presented as mean ± SD, P <0.05 is considered significant(ANOVA test)

AST (Aspartate transaminase); ALT (Alanine-aminotransferase); BIL-T (Total bilirubin); BIL-D (Direct bilirubin); PT (Prothrombin time); BUN (Blood Urea Nitrogen); S.Cr (Serum creatinine); Hb (Hemoglobin); RBCs (Red blood cells); WBCs (White blood cells); IL-6 (interleukin-6); C-RP (C-Reactive Protein).

n = 15 for all groups.

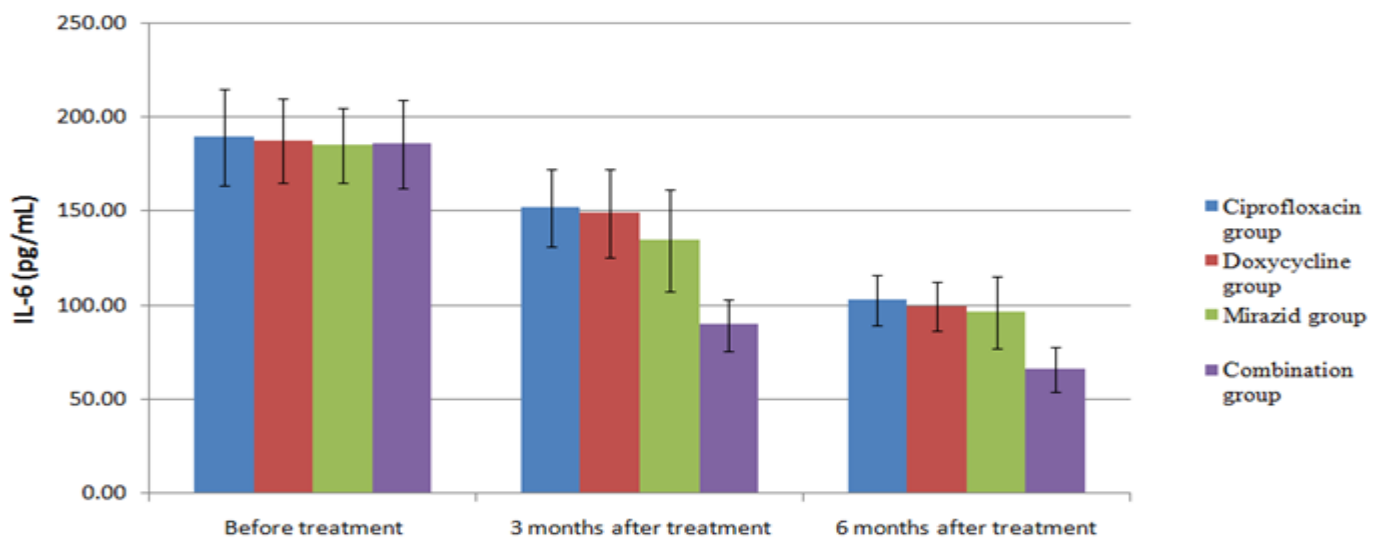


Figure 1. Changes in the level of IL- 6 (pg/ml) in the four studied groups before, three and six months after treatment. Data presented by mean ± SD. IL- 6 level in the four groups decreases significantly ($P < 0.05$) three and six months after treatment in comparison with its baseline level (paired t-test),n=15.

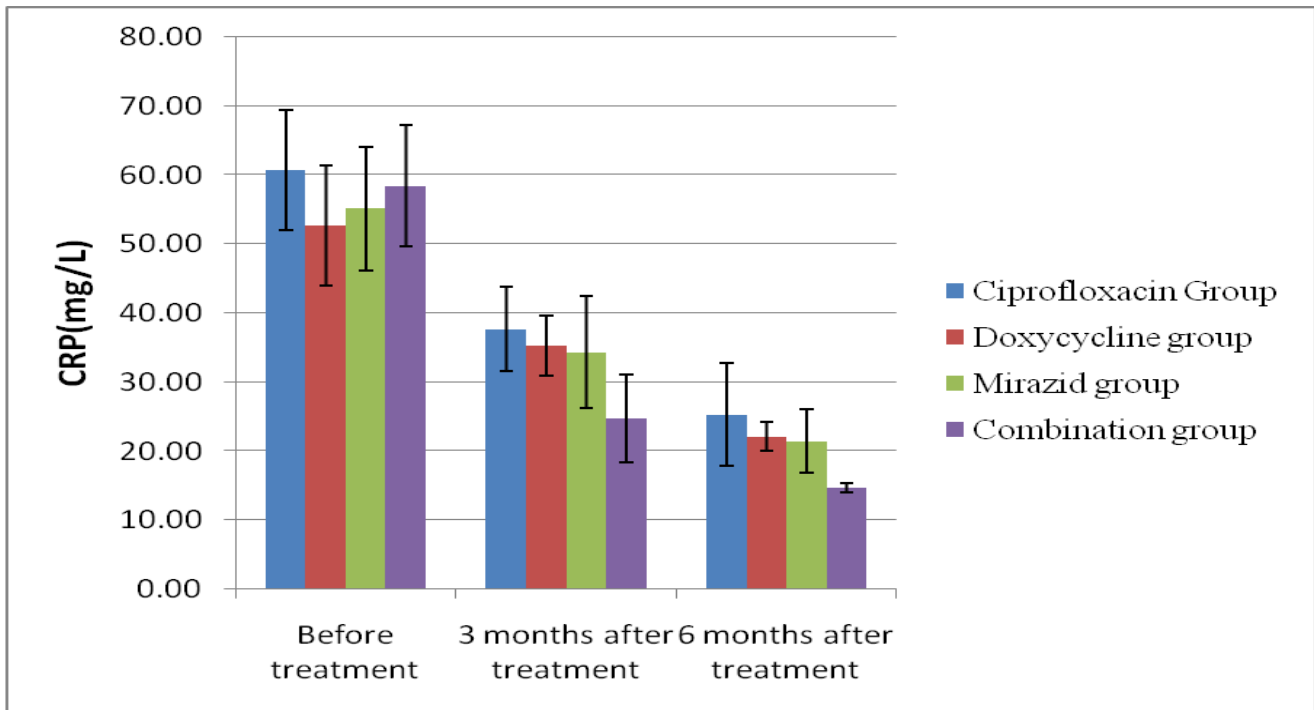


Figure 2. Changes in the level of CRP (mg/L) in the four studied groups before, three and six months after treatment. Data presented by mean \pm SD. CRP level in the four groups decreases significantly ($P < 0.05$) three and six months after treatment in comparison with its baseline level (paired t test), $n=15$.

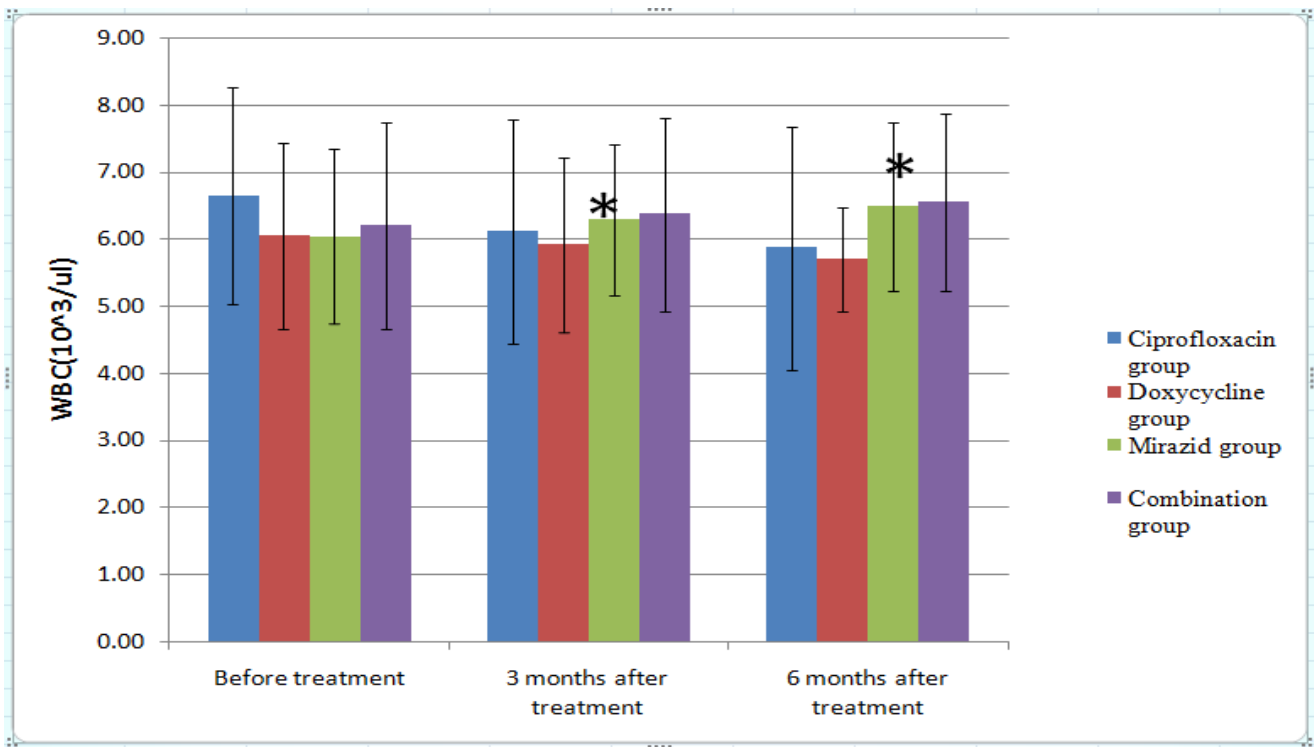


Figure 3. Changes in the level of WBC ($10^3/\text{ul}$) in the four studied groups before, three and six months after treatment. Data presented by mean \pm SD. WBC count in the group three decreases significantly ($P < 0.05$) three and six months after treatment in comparison with its baseline level (paired t test), $n=15$.

5. Discussion

The results of the current study are promising and demonstrate the efficacy of doxycycline and Commiphora molmol as primary prophylactic therapy against SBP. Diagnostic paracentesis remains the gold standard for rapid diagnosis of SBP, its associated risks [20] makes efforts seek for alternative safe tests for diagnosing SBP. The measurement of inflammatory markers such as serum IL-6 and CRP seems to be helpful whereas IL-6 plasma level was found to be the most sensitive and specific tool for the diagnosis of bacterial infection in decompensate cirrhotic patients [21].

IL-6 is a multifunctional cytokine that mediates several acute phase inflammations. IL-6 is produced by some hepatic cells such as kupffer cells and sinusoidal endothelial cells (SEC) which mediate hepatic fibrogenesis [22]. It was found an initial IL-6 level was significantly higher in infected cirrhotics than non-infected cirrhotic patients [23]. In addition, hepatocytes produce CRP in large amounts upon stimulation by the cytokines like IL-6 and TNF- α during the acute-phase response [24]. It was therefore hypothesized that the use of CRP could be useful in the detection of SBP. It has been suggested that CRP and TNF- α serum levels were significantly higher in patients with SBP comparing to patients with sterile ascites [25]. The serum CRP concentration was found to separate patients into infected peritonitis and non-infected categories better than the ascitic fluid CRP concentrations [26]. The previous data justifies our selection of both serum IL-6 and CRP as main parameter to test the efficacy of studied medications. Our study duration was six months which seems acceptable and matches with other findings suggested that six months follow-up period is the required duration to investigate the prophylactic activity of antibiotics against SBP [27].

Group one patients (control group) were received ciprofloxacin 750 mg/week depending on the report of previous studies reported high fecal concentration of ciprofloxacin up to 6 days after a single oral dose and suggested its ability to

provide long term prevention of SBP in cirrhotic patients [27]. Group one showed significant decrease in IL-6 level three and six months after ciprofloxacin administration which agreed with reported findings that serum TNF- α and IL-6 levels were reduced significantly 24 and 48 h after ciprofloxacin treatment [28].

In addition, group one showed significant decrease in CRP level three and six months following ciprofloxacin administration. The overall results indicate that ciprofloxacin may have anti-inflammatory and immunomodulatory effect on SBP patients by attenuating the proinflammatory response independently on its antibacterial effect.

Unfortunately, it has been suggested that the microbial etiology of SBP have been changed with subsequent increased chance of quinolone prophylaxis failure [29]. Thus, we aimed to test another antibiotic (doxycycline) which exerts a broad range of antimicrobial activity against both Gram-positive and Gram-negative bacteria [7,8]. Doxycycline was absorbed very rapidly from the peritoneal cavity and due to its slow excretion, it is used for peritoneal lavage for patients suffering from diffuse peritonitis due to perforated appendicitis [30] suggesting its use as prophylactic agent [31]. Dosing schedule for group two was based its long elimination half-life which (12 - 25 h) which may be prolonged in patients with liver cirrhosis [32]. Secondly, doxycycline 200 mg/week was formerly used for prophylaxis against leptospirosis [33].

Group two patients showed significant decrease in IL-6 levels three and six months a result compatible with previously study demonstrated that treatment with doxycycline resulted in a significant decline in cytokine levels (IL-6 and TNF- α) in patients with scrub typhus [34]. Furthermore, Group two showed significant decrease in CRP level which seems parallel with other studies investigated that sub antimicrobial doses of doxycycline could significantly reduce serum CRP and serum IL-6 levels in patients with coronary artery disease and in postmenopausal women with chronic periodontitis [35].

An alternative therapy to guard against antibiotic resistance is the implication of medicinal plants that provide many advantages

such as fewer side effects, good patient tolerance and less cost. Group three dosing schedule based on its approved dose as anti schistosomal agent in Egypt [36]. This approved dose appears to be safe, effective, non hepatotoxic and non-carcinogenic dose [37]. Furthermore, we aimed to test the use of Commiphora molmol as prophylactic therapy depending on the reports of previous studies demonstrated its antifungal and antibacterial activities against some microbes that implicated in SBP episodes [38]. In addition it has antibacterial and antifungal activity against standard pathogenic strains of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans that considered predisposing agent for SBP due to sesquiterpenes derived from myrrh or Commiphora molmol extract (furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one) [37, 39]. The petroleum ether extract of myrrh was also found effective as anti-inflammatory activity [40]. Also, it was found that the crude resin of C. molmol has anti-inflammatory activity [41, 42].

Group 3 patients showed significant decrease in IL-6 and CRP levels three and six months after using Commiphora mol mol. Our findings are in accordance with results from other studies where there was a significant reduction of IL-1 β -stimulated IL-6 and IL-8 production by fibroblasts treated with myrrh [43]. Moreover, IL-6 and CRP levels were decreased in hyperlipidemic subjects on myrrh [44]. Additionally, it has been demonstrated that, Commiphora species have anti-inflammatory effects through suppression of many cytokines (IL-12, IL-6, TNF- α and IL-1 β) in serum, liver and peritoneal fluids [45]. It was found that, pretreatment of myrrh inhibited LPS-induced production of proinflammatory cytokines, NO, and PGE2 in peritoneal macrophages. In addition, myrrh inhibited CLP-induced production of IL-1 β , IL-6, and TNF- α in mice serum and liver. These results suggest that myrrh may have anti-inflammatory properties [46].

However, the effects and the mechanisms underlying anti-inflammatory activities of myrrh are poorly defined. I suggest some expectations for explanation of anti-inflammatory mechanism of myrrh. In inflammatory processes,

macrophages are important players providing immediate defense against foreign agents. Upon activation, macrophages produce excess inflammatory mediators, such as tumor necrosis factor- α (TNF- α), interleukin (IL), leukotrienes, nitric oxide (NO), and prostaglandin E2 (PGE2) [47, 48]. Thus, inhibition of inflammatory responses is an important target in the treatment of inflammatory diseases.

First, myrrh might inhibit the production of NO through suppression of iNOS (inducible nitric oxide synthase), expression which is an important regulatory molecule in a range of physiological and pathological processes [49]. Secondly, myrrh may inhibit the production of PGE2 (key inflammatory mediator) through the suppression of COX-2 expression [50].

Thirdly, myrrh might inhibit production of NO, PGE2, IL-6 and TNF- α through the inhibition of the JNK (c-Jun N-terminal kinases) pathway which regulate various cellular activities including signal transduction and expression of proinflammatory [51].

Therefore, it is likely that Myrrh exerted an anti-inflammatory effect in vivo by increasing bacterial clearance and inhibiting proinflammatory cytokines production such as IL-1 β , IL-6, and TNF- α . From all the above, it is clear that Commiphora molmol can be used in prophylaxis of SBP due to dual action, its antibacterial and anti-inflammatory activities.

On the other hand, both three and six months after Commiphora molmol intake, we reported a statistically significant increase in WBCs. This increase in white blood cells count may be attributed to an elevated rate of myrrh- antigen-driven leukocytes proliferation [52].

Following three and six months of treatment course, there was a statistically significant decrease in AST and ALT levels in group three and group four when compared to group one and group two ($p < 0.05$). This can be explained as group one and group two administered ciprofloxacin and doxycycline respectively cause serum enzyme elevations during therapy which considered an adverse effect [53,54].

Group three and group four administered commiphora molmol alone or in combination with doxycycline respectively provides many

advantages such as fewer side effects safe, effective and non-hepatotoxic [37]. Clinical study of using mirazid in patients with compensated hepatosplenic schistosomiasis or schistosom colitis alone showed that liver function tests exhibited a significant improvement with regard to the levels of liver enzymes [55].

Group four showed significant decrease in IL-6 and CRP levels three and six months after therapy. Furthermore, group four showed significantly lower IL-6 and CRP levels as compared to the other studied groups. This result may be attributed to the synergistic effect between Commiphora molmol and doxycycline. It has been reported that, the crude extract of the oleo-resin of Commiphora molmol displayed potentiation of tetracycline effect against against *S. aureus*, several *Salmonella enterica*, *Typhimurium* strains and two *Klebsiella pneumonia* strains [56]. Furthermore, it was reported that, the implication of Commiphora molmol with tetracycline resulted in synergetic interaction against Gram positive bacteria (*Staphylococcus aureus* and *Bacillus megaterium*) and Gram negative bacteria (*E. coli*, *Pseudomonas aeruginosa* and *K. pneumonia*) [57]. This synergistic effect may be attributed to the ability of Commiphora molmol to significantly increase the Post Antibiotic Effect (PAE) of tetracycline by extending the lifetime of fading antibiotics [57]. The above mentioned information may give logical justification for our result obtained with Commiphora molmol/doxycycline combination. The limitation of our study includes the relatively small numbers of patients investigated. In terms of a clinical application, larger controlled clinical trials are needed to establish the impact of such therapies on outcomes for patients.

6. Conclusion

To our knowledge, this study is the first clinical trial aimed to evaluate the outcome of implication of doxycycline, Commiphora molmol and their combination as prophylactic therapy against SBP. The results of the current study are promising and demonstrate the efficacy of doxycycline and Commiphora molmol as primary

prophylactic therapy against SBP. Furthermore, commiphora mol mol /doxycycline combination was the most effect and showed additive effect which may be useful in decrease emergence of resistant strains. Consequently we are recommending doxycycline and commiphora mol mol as alternative prophylactic therapy for ciprofloxacin failure in patients with SBP.

Authors' contributions

Tarek Mostafa and Eman Elberri designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Osama Ibrahim managed the analyses of the study. Gamal Badra managed the literature search. All authors read and approved the final manuscript.

Compliance with Ethical Standards

- Tarek mostafa, Osama ibrahim, Gamal badra and Eman elberri declare that they have no conflict of interest.
- Ethical approval: The protocol for this study was approved by the National Research Ethics Committee of Menoufiya University, Shebin El-kom, Egypt with Institutional Review Board (IRB) protocol number 0064/2012.
- Informed consent: Informed consent was obtained from all individual participants included in the study.

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