Phytochemical Characterization and Immune Modulation Activities of Diatom *Amphora Coffeaeformis* in Immunosuppressive Rats

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ABSTRACT

Introduction: Microalgae are photosynthetic microorganisms that represent an excellent source of new natural products. The extracts of microalgae possess multiple therapeutic effects in humans and animals, including anticancer, antiviral, antioxidant, anti-inflammatory, and immunomodulatory activities. **Aim:** This study evaluated the impact of the microalgae *Amphora coffeaeformis* (*A. coffeaeformis*) on growth performance, hematological parameters, and immune function in immunosuppressive rats. Besides, the chemical composition, fatty acids (FA), phenolics, antioxidants, and polysaccharides constituents of *A. coffeaeformis* was identified. **Methods:** Gas chromatography-mass spectrophotometry was used to evaluate the chemical components (macro and micronutrients). The FA, total antioxidants, and total flavonoids were also assayed. The biological experiment was conducted on 50 male rats of which ten rats served as the control group. While the other 40 rats were immunosuppressed by injecting a single dose of sheep red blood cells. The immunosuppressed rats were divided into 4 groups (n=10), group 2 non treated immunosuppressive, and group 3, 4, and 5 *A. coffeaeformis* treated immunosuppressive (1, 2 and 3%, respectively). **Results:** The results of this study showed that *A. coffeaeformis* contains a high % of carbohydrates, proteins, metals, vitamins, phenols, antioxidants, polysaccharides, and polyunsaturated fatty acids (PUFA). Feeding the rats with *A. coffeaeformis* dose-dependently improved body weight gain (BWG%), feed intake (FI), feed efficiency ratio (FER), complete blood counts with, especially (RBCs, Hb, WBCs, LYMPH, and NEUT), and serum immunoglobulins (IgG, and IgM) compared to the immunosuppressed rats. **Conclusion:** Both the improved growth performance and immunity could be attributed to the bioactive ingredients of *A. coffeaeformis*.

Keywords: *Amphora coffeaeformis, immunity, PUFA, polysaccharides, antioxidants, polyphenols, blood picture.*

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INTRODUCTION

Nature has provided a complete storehouse of remedies to cure all ailments of humankind [1]. The immune system is composed of a wide and very sophisticated system of cells, tissues, and organs that act together continuously to save our bodies from injuries [2]. Despite developments in knowledge and technological advances in the biomedical field, even today we are not able to provide valid and effective tools to defend ourselves against viral or bacterial infections [3]. In the case of deteriorated immunity, the body's natural safeguard could readily be suppressed, causing serious infection, illness, and even death [4]. It comprises three levels of defense: anatomical and physiological barriers, innate and adaptive immune response. The barriers include intact skin, ciliary clearance in the respiratory tract, mucosal membranes, and lysozyme in tears, saliva, stomach acid, and the commensal microbiota in skin, mouth, gastrointestinal tract, and genitourinary tract. The innate immune reaction is the first guard in the body, and it is seriously essential for prohibiting the entrance of microbes to our bodies and in

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case they navigate it eradicates them quickly. The innate immune system composed of soluble factors and cells (macrophages, neutrophils, natural killer cells, eosinophils, and basophils). Acquired immunity is highly specialized compared to innate immunity; it complements and boosts the guard offered by innate immunity [5]. The fast growth and acceptance of novel immune modulators, with the vast extraction of new substances from microalgae, could guide the discovery of much potential natural immunotherapy [6].

Microalgae are photosynthetic microorganisms acclimatized to grow in various conditions and displayed an immense biochemical and genetic variety. They exemplified a superior origin of novel natural compounds with probable implementations in sundry of biological industry fields [7]. Microalgae have several bioactive compounds such as lipopeptides, amino acids, fatty acids, macrolides, and amides. Several studies showed that extracts of raw microalgae and its fractions have several therapeutic activities in human and animals such as cytotoxic, antitumor, anticancer, antiviral, antibiotics, antioxidant, antimalarial, antimycotics, antimicrobial, anti-epilepsy, anti-inflammatory, and immunomodulatory effects [7-16].

Amphora coffeaeformis (*A. coffeaeformis*) is microalgae [17]. It is a good source of several bioactive compounds like carotenoids, phenolic, polyphenols, polyunsaturated fatty acids, sulfated polysaccharides, amino acids, β-glucans, α-tocopherol, vitamin C and E [18]. *A. coffeaeformis* extract exhibits substantial antioxidant activities in the 2,2 diphenyl 1 picrylhydrazyl (DPPH) test. *A. coffeaeformis* showed a significantly higher antioxidant activity than that of α- tocopherol [19]. *A. coffeaeformis* extract exhibited antagonistic effect against the hepatic injury and the deleterious effects induced by paracetamol in rats [18]. Few biological studies were conducted on *A. coffeaeformis*. Besides, there were no studies that assessed the immune-modulating activity of *A. coffeaeformis*. Depending on the proven immunomodulatory effects of microalgae, it can be assumed that *A. coffeaeformis* may exert the same immune effects.

The present study will evaluate the effect of *A. coffeaeformis* on growth performance, hematological parameters, and immune functions in immunosuppressive rats. Besides, the chemical composition (macro-and micro-nutrients) and the active constituents of the freeze-dried of *A. coffeaeformis* powder will be identified.

Methodology

Chemicals

Diatom *A. coffeaeformis* lyophilized powder (500 g) was obtained from Biotechnology Unit, National Research Centre (NRC), Dokki, Egypt. The identification was done by Prof. Elsayed AB, Algae Biotechnology Unit, NRC, Dokki, Egypt. Sheep red blood cells (SRBCs) (dry powder, glutaraldehyde treated) were obtained from Sigma-Aldrich Co, USA. All other chemicals were of high grade.

Analysis of *A. coffeaeformis* macro and micronutrients contents

The macro and micronutrient contents of *A. coffeaeformis* were determined according to the method described in the analytical chemists [20].

Gas chromatography-mass spectroscopy (GC-MS) analysis of *A. coffeaeformis* active constituents

The assay was performed utilizing a GC-MS (Agilent Technologies 7890A) connected to a mass-specific detector (MSD, Agilent 7000). Helium was the carrier gas. The recognition of constituents was carried out by comparing their mass spectra and retention time with the library of authentic compounds (NIST and WILEY) [21].

Gas chromatography (GC) analysis of *A. coffeaeformis* total fatty acids

Total fatty acids contents of *A. coffeaeformis* was determined by using methyl esters boron trifluoride method of [20]. *A. coffeaeformis* powder was boiled with hydrochloric acid and then subjected to petroleum ether for fatty acid extraction. The oil is saponified with sodium hydroxide in methanol. The fatty acids are methylated with boron trifluoride in methanol, extracted with heptane, and determined on an autosampler GC with FID detector (PE Auto System XL) and Ezchrom integration system. Carrier gas (He); Ca. 25 Psi–air 450 ml/min– Hydrogen 45 ml-split 100 ml/min.

Analysis of *A. coffeaeformis* antioxidant contents

A. coffeaeformis total antioxidant substances were assessed by the phosphomolybdenum method [22], total phenols content was evaluated by the Folin–Ciocalteu method [23], and total flavonoids was assessed by Dowd method using aluminum chloride colorimetric method as adapted by [24].

Animals

Fifteen adult male rats Sprague Dawley weighing 125-135 g were purchased from Helwan Experimental Animals Farm, Giza, Egypt.

Experimental design

Rats were housed in well-aerated cages under hygienic conditions and fed on a standard diet for one week for adaptation in the animal house of Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt. The standard diet was composed of 14% casein (> 85 % protein), 4% corn oil, 0.25% choline chloride, 1% vitamin

mixture, 3.5% mineral mixture, 5% cellulose, 10% sucrose, 0.3% DL-methionine and corn starch up to 100 g according to [25].

The rats were divided into two main groups. The first main group, control negative (n=10): rats were fed a standard diet, and intraperitoneal (i.p.) injected with a single dose of phosphate-buffered saline (PBS). The second main group (n = 40): rats were i.p. injected with a single dose of SRBCs (0.2 ml of a 10 % suspension of washed SRBCs in PBS) to induced immune suppression [26]. The rats of the second main group were divided into 4 subgroups (10 rats each) as follows: immunosuppressive group: rats were fed the standard diet, *A. coffeaeformis* 1% group: rats were fed the standard diet to which 1% lyophilized *A. coffeaeformis* was added, *A. coffeaeformis* 2% group: rats were fed the standard diet to which 2% lyophilized *A. coffeaeformis* was added, and *A. coffeaeformis* 3% group: rats were fed the standard diet to which 3% lyophilized *A. coffeaeformis* was added. *A. coffeaeformis* doses were adopted based on [27]. After eight weeks, the rats were fasted overnight before scarifying. Blood samples were collected from retro-orbital plexus of each rat in plain tubes, centrifuged at 3000 rpm for 15 min. to obtain serum, then stored at –80 °C to be utilized for the biochemical analysis. Another whole blood samples were withdrawn in EDTA tubes for measurements of hematological parameters. Spleen specimens were dissected from each rat and preserved in 10% buffered formalin solution for the histopathological examination.

Biological markers evaluation

Over eight weeks, the daily diets consumed, and the initial and final body weight (IBW and FBW) were recorded. Biological evaluations, including body weight gain % (BWG %) and feed intake (FI), and feed efficiency ratio (FER) for each group was calculated.

Determination of complete blood cells (CBC)

Red blood cell (RBCs) count, hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), white blood cell count (WBCs), eosinophils (EO), monocyte (MONO), lymphocyte (LYMPH), and neutrophils (NEUT) were estimated using the standard hematological technique (Sysmex, XS-500I, Germany).

Determination of serum immunoglobulins (Igs)

Serum IgM and IgG were measured using the Sandwich-ELISA assay kits according to the manufacture instructions.

Histopathological examination

The formalin-fixed spleen samples were processed, stained with hematoxylin and eosin (H & E), and examined under light microscopy.

Statistical analysis

The obtained data were analyzed using the SPSS program, version 24. Data were figured as mean ± SE (for data of the biological study n=10, where for data of the *in vitro* study 3 replicates were used). ANOVA test was used to compare results among groups and $P < 0.05$ was significant.

RESULTS

Chemical composition, macro, and micronutrients contents of *A. coffeaeformis*

The results of this study revealed that lyophilized *A. coffeaeformis* powder is rich in many chemical compounds, macro, and micronutrients (Table 1). Concerning the chemical composition, it contained 7.3 %. moisture, high carbohydrate (27.96%), high protein (21.40 %), moderate fiber (11.93 %), and low fats (5.91 %). The polysaccharides' contents were glucan (86.8 g/k) and mannan (69.4 g/k).

Table 1: Chemical composition, macro and micronutrients contents of *A. coffeaeformis*

Components	Mean ± SE
Moisture (%)	7.30 ± 0.75
Protein (%)	21.40 ± 1.15
Fats (%)	5.91 ± 0.81
Ash (%)	25.50 ± 0.87
Fiber (%)	11.93 ± 0.58
Carbohydrate (%)	27.96 ± 2.28
Polysaccharides	
Glucan (g/k)	86.81 ± 5.8
Mannan (g/k)	69.36 ± 5.8
Minerals	
Calcium (%)	3.68 ± 0.29
Potassium (%)	0.94 ± 0.12
Iron (%)	0.63 ± 0.08
Manganese (%)	0.20 ± 0.06
Zinc (%)	1.18 ± 0.05
Sodium (%)	0.21 ± 0.03
Copper (%)	0.01 ± 0.002
Selenium (%)	0.0 ± 0.0
Vitamins	
Ascorbic acid (Vit C) (g/k)	0.15 ± 0.06
Thiamine (Vit B1) (g/k)	0.045 ± 0.005
Riboflavin (Vit B2) (g/k)	0.072 ± 0.12

Values were presented as the mean of three replicates ± SE.

Concerning the macro, and micronutrients contents, *A. coffeaeformis* possessed high content of calcium, potassium, iron, manganese, and zinc (3.68 %, 0.94 %, 0.63 %, 0.20 %, and 1.18 % respectively), low content of

sodium and copper (0.21 % and 0.01 % respectively). Furthermore, it contained ascorbic acid (Vit C), thiamine (Vit B1), and riboflavin (Vit B2) (0.15 %, 0.045 %, and 0.072 % respectively).

The GC-MS analysis of *A. coffeaeformis* was presented in Fig. 1 and Table 2. The results showed that there were 3 main compounds present in *A. coffeaeformis,* geranyl isovalerate (33.98 %), hexahydro-farnesyl (15.1%), and isomyristic acid (10.93%).

Active constituents of *A. coffeaeformis* (GC-MS)

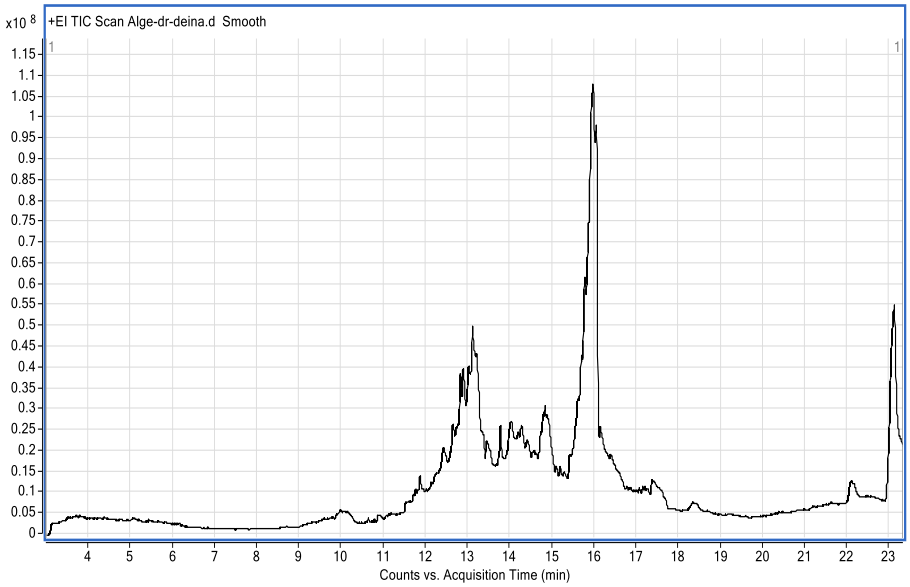

Figure 1: Gas chromatography-mass spectroscopy (GC-MS) spectra of the *A. coffeaeformis* active constituents

Table 2: Active constituents of *A. coffeaeformis* (gas chromatography-mass spectroscopy, GC-MS)

Constituents	RT (min)	Concentrations (%)
6,2',3'-Trimethoxyflavone	5.072	0.46
D- (-)-Ribose	8.701	0.64
2-Undecanol	10.095	1.76
d-Mannose	10.903	0.54
Pentadecanoic acid, 14-methyl	11.867	1.29
D-Xylose	12.432	2.07
Heptacosane	12.646	0.99
Phytol	12.818	0.92
L-Arabinitol	12.879	1.11
Isomyristic acid	13.109	10.93
Octacosanoic acid, methyl ester	13.478	1.41
Hexadecanoic acid, ethyl ester	13.769	2.05
19(20)-EpDPE	14.028	3.14
11,14-Eicosadienoic acid, methyl ester	14.803	5.54
Geranyl isovalerate	15.701	33.98
17,18-DiHETE	16.017	6.55
Heptadecanoic acid	16.41	6.00
Tetracosanoic acid, methyl ester	17.37	2.78
Pentadecanoic acid	18.375	1.25
Eicosanoic acid	22.103	1.48
Hexahydro-farnesyl	23.07	15.1
Non-identified compounds	> 23.07	0.01

*RT: Retention time.

Total fatty acids contents of *A. coffeaformis* (GC)

Total fatty acids (FA) substances of *A. coffeaformis* (GC) were presented in Fig. 2 and Table 3. The *A. coffeaformis* contains 55.82% monounsaturated fatty acids (MUFA) (oleic acid 44.30%, stearic acid 9.24 %, and vaccenic acid 2.28%); 35.05% saturated fatty acids (SFA) (palmitic acid 30.18%, palmitoleic acid 3.86%, and undecanoic acid 1.01%); and polyunsaturated fatty acids (PUFA) (linoleic acid 7.28 % and linolenic acid 1.84 %).

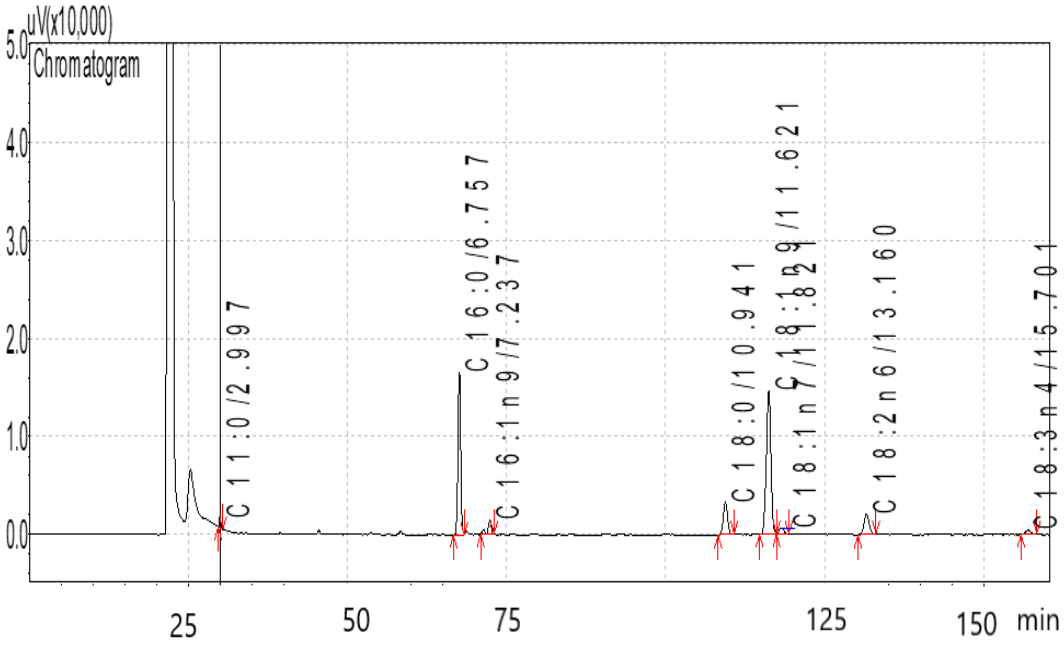

Figure 2: Fatty acids contents of *A. Coffeaformis* (gas chromatography, GC)

Table 3: Fatty acids contents of *A. Coffeaformis* (GC)

Fatty acids	Name	Relative distribution %
SFA		
C11:0	Undecanoic acid	1.01
C16:0	Palmitic acid	30.18
C16:1 ω7	Palmitoleic acid	3.86
MUFA		
C18:0	Stearic acid	9.24
C18:1 ω7	Vaccenic acid	2.28
C18:1 ω9	Oleic acid	44.30
PUFA		
C18:2ω6	Linoleic acid	7.28
C18:3ω3	Linolenic acid	1.84
Non identified fatty acids		0.01

GC: Gas chromatography; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

Total antioxidants, flavonoids and phenols contents of *A. coffeaeformis*

A. coffeaformis contained 1001.70 ± 29.44 (mg/ 100 g ascorbic acid) total antioxidants, 34.88 ± 2.31 (mg/ 100 g quercetin) total flavonoids, and 372.10 ± 6.93 (mg/ 100 g gallic acid) total phenols (Table 4).

Table 4: Total antioxidants, flavonoids, and phenols of *A. coffeaformis*

Antioxidant constituents	Mean ± SE
Total antioxidants (mg/ 100 g ascorbic acid)	1001.70 ± 29.44
Total flavonoids (mg/ 100 g quercetin)	34.88 ± 2.31
Total phenols (mg/ 100 g gallic acid)	372.10 ± 6.93

Values were presented as the mean of three replicates ± SE.

Effect of *A. coffeaformis* on biological markers (FBW, FI, BWG %, and FER) determined in immunosuppressive rats

Injection of SRBCs significantly decreased FBW, FI, BWG %, and FER compared to the negative control group. Treatment with *A. coffeaeformis* (1, 2, and 3 %) induced noticeable improvement in all biological markers compared to the immunosuppressive group. Immunosuppressive rats treated with 3 % *A. coffeaformis* revealed a significant increase in BWG% as compared to immunosuppressive rats treated with 1 % *A. coffeaformis* (Fig. 3 and Table 5).

Table 5: Effect of *A. coffeaformis* on biological parameters determined in the immunosuppressive rats

Experimental groups	IBW (g)	FBW (g)	FI (g/day/rat)
Control negative	130.54 ±1.87	162.22 ± 1.53	13.36 ± 0.42
Immunosuppressive	130.02 ± 0.85	115.46 ± 2.23 [a]	9.70 ± 0.41 [a]
A. coffeaeformis 1%	130.12 ± 1.22	163.10 ± 0.82 [b]	12.18 ± 0.43 [b]
A. coffeaeformis 2%	129.10 ± 0.56	165.12 ± 0.72 [b]	12.78 ± 0.26 [b]
A. coffeaeformis 3%	128.20 ± 1.09	166.90 ± 0.98 [b]	13.02 ± 0.25 [b]

Values were presented as mean ± SE (n=10). [a] Significant difference from control negative, [b] significant difference from control positive group, [c] significant difference from *A. coffeaeformis* 1% group, [d] significant difference from *A. coffeaeformis* 2% group (P< 0.05 was statistically significant). IBW: Initial body weight; FBW: Final body weight; FI: Feed intake

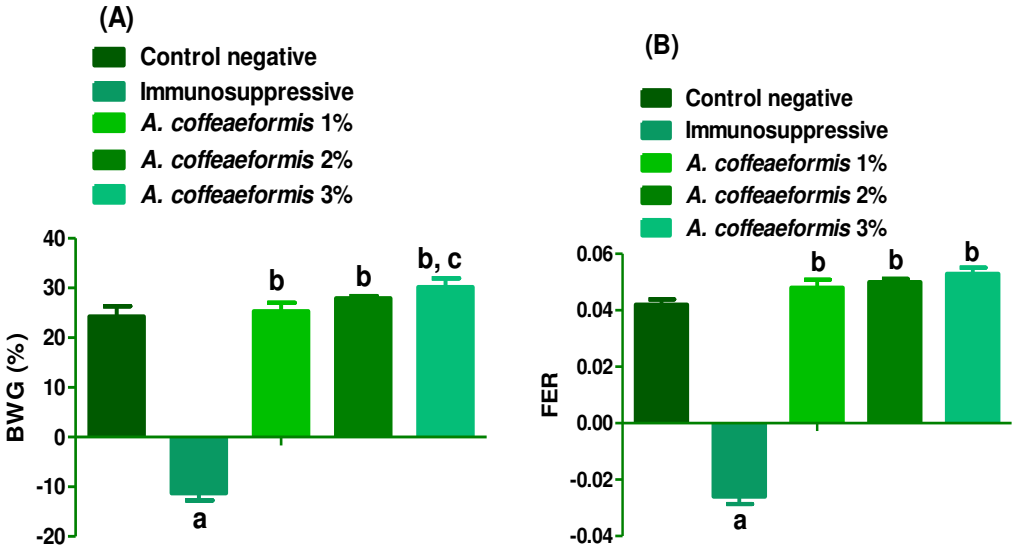

Figure 3: Effect of *A. Coffeaformis* on A: BWG (%) and B: FER determined in immunosuppressive.

Values were presented as mean ± SE (n=10). [a] Significant difference from control negative, [b] significant difference from control positive group, [c] significant difference from *A. coffeaeformis* 1% group. P < 0.05 was statistically significant.
BWG%: Body weight gain percent; FER: Feed efficiency ratio.

Effect of *A. coffeaformis* on the complete blood cells (CBC)

Injection of SRBC significantly induced hematological disorders as evidence by a significant decrease in RBCs, Hb, HCT, MCV, MCH, MCHC, PLT, WBCs, MONO, LYMPH, and NEUT compared the negative control group. On the other hand, the treatment of rats with *A. coffeaformis* (1, 2, and 3 %) significantly improved all these parameters compared to the immunosuppressive group in a dose-dependent fashion. There was significant increases in RBCs, Hb, and HCT in *A. coffeaformis* 3% group compared to *A. coffeaformis* 2% group. Concerning the WBCs and LYMPH, they were significantly increased in *A. coffeaformis* 2% and 3% groups compared to *A. coffeaformis* 1% group (Table 6).

Effect of *A. coffeaeformis* on serum immunoglobulins

Injection of SRBCs significantly decreased serum IgM and IgG levels in the immunosuppressive group compared to the negative control group. On the other hand, the treatment of rats with *A. coffeaformis* (1, 2, and 3 %) produce a significant dose-dependent increase in the serum IgM and IgG levels compared to the immunosuppressive group. The highest values of serum IgM and IgG levels were seen in the group treated with 3 % *A. coffeaformis* (Fig. 4).

Effect of *A. coffeaformis* on spleen histopathology

Light microscopic examination of spleen sections revealed that SRBCs produced an apparent decrease in white pulp size and activation of the germinal center with slight lymphocytic necrosis and depletion. On the other hand, treatment with *A. coffeaformis* 1%, 2%, and 3 % protect the spleen as minimal histopathological changes were seen in Fig. 5.

Table 6: **Effect of *A. coffeaformis* on the complete blood cells (CBC) determined in the immunosuppressive rats**

CBC Parameters	Experimental groups				
	Control negative	Immunosuppressive	*A. coffeaeformis* 1%	*A. coffeaeformis* 2%	*A. coffeaeformis* 3%
RBCs (x10^6/µl)	9.42 ± 0.20	7.24 ± 0.16 ^{a}	8.59 ± 0.11 ^{b}	9.19 ± 0.18 ^{b,c}	10.07 ± 0.10 ^{b,c,d}
Hb (g/dl)	15.70 ± 0.28	8.74 ± 0.24 ^{a}	14.36 ± 0.34 ^{b}	16.48 ± 0.33 ^{b,c}	18.80 ± 0.33 ^{b,c,d}
HCT (%)	42.44 ± 0.24	26.10 ± 1.19 ^{a}	40.72 ± 0.38 ^{b}	42.90 ± 0.79 ^{b,c}	50.38 ± 0.68 ^{b,c,d}
MCV (g/dl)	45.18 ± 0.82	35.86 ± 1.69 ^{a}	46.74 ± 1.05 ^{b}	47.52 ± 98 ^{b}	49.98 ± 0.80 ^{b,c}
MCH (g/dl)	16.66 ± 0.18	12.88 ± 0.42 ^{a}	16.66 ± 0.20 ^{b}	17.96 ± 0.64 ^{b,c}	18.62 ± 0.35 ^{b,c}
MCHC (g/dl)	36.96 ± 0.52	31.88 ± 1.31 ^{a}	37.30 ± 0.46 ^{b}	38.56 ± 1.12 ^{b}	37.44 ± 1.13 ^{b}
PLT (x10^3/UI)	1307.20± 80.27	730.40 ± 44.09 ^{a}	914.60 ± 26.35 ^{b}	975.40 ± 22.91 ^{b}	1018.40 ± 17.48 ^{b}
WBCs (x10^3/UI)	13.52 ± 0.60	5.29 ± 0.19 ^{a}	10.48 ± 0.14 ^{b}	11.98 ± 0.15 ^{b,c}	12.82 ± 0.13 ^{b,c}
EO (x10^3/UI)	0.24 ± 0.03	0.17 ± 0.03	0.22 ± 0.02	0.27 ± 0.05 ^{b}	0.33 ± 0.03 ^{b,c}
MONO (x10^3/UI)	1.21 ± 0.30	0.27 ± 0.06 ^{a}	0.74 ± 0.12 ^{b}	1.05 ± 0.03 ^{b}	1.18 ± 0.18 ^{b}
LYMPH (x10^3/UI)	9.65 ± 0.27	3.92 ± 0.10 ^{a}	6.76 ± 0.29 ^{b}	8.51 ± 0.28 ^{b,c}	8.55 ± 0.21 ^{b,c}
NEUT (x10^3/UI)	2.20 ± 0.41	0.93 ± 0.10 ^{a}	2.19 ± 0.15 ^{b}	2.39 ± 0.29 ^{b}	2.92 ± 0.15 ^{b,c}

Values were presented as mean ± SE (n=10). ^{a} Significant difference from control negative, ^{b} significant difference from control immunosuppressive group, ^{c} significant difference from *A. coffeaeformis* 1% group, ^{d} significant difference from *A. coffeaeformis* 2% group. P< 0.05 was statistically significant

RBC: Red blood cells count; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelets; WBCs: White blood cells count; EO: Eosinophils; MONO: Monocyte; LYMPH: Lymphocyte; NEUT: Neutrophils.

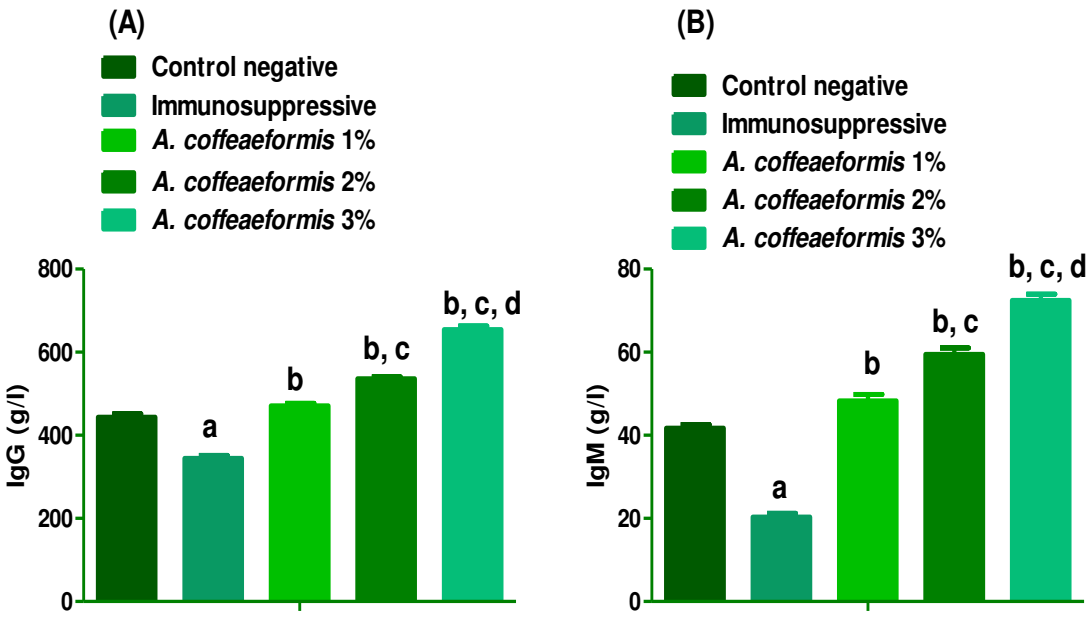

Figure 4: Effect of *A. Coffeaformis* on serum immunoglobulins determined in immunosuppressive rats. A: IgG; B: IgM. Values were presented as mean ± SE (n=10). ^{a}significant difference from control negative. ^{b}significant difference from control positive group. ^{c}significant difference from *A. coffeaeformis* 1% group. ^{d}significant difference from *A. coffeaeformis* 2% group. P < 0.05 was statistically significant. IgM: Immunoglobulin M; IgG: Immunoglobulin G

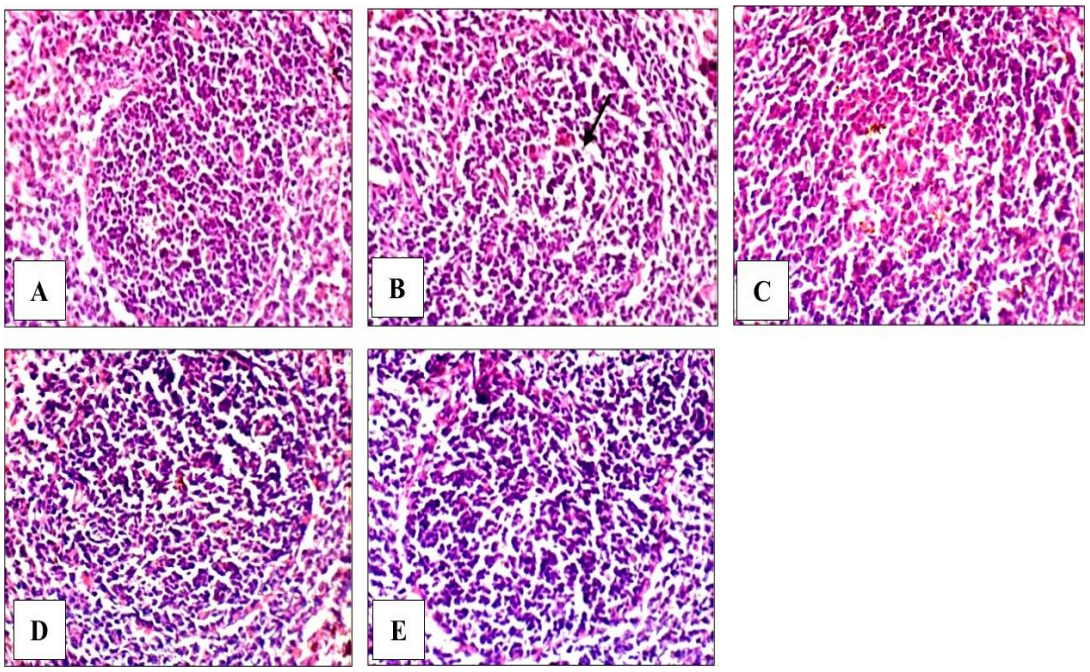

Figure 5: Effect of *A. coffeaformis* on spleen tissue histopathology determined in immunosuppressive rats (H & E x 200). Microscopically, spleen sections of rats from the negative control group revealed no histopathological changes with a normal lymphoid follicle (Photo A). However, spleen sections of rats from the immunosuppressive group showed an apparent decrease in white pulp size and activation of the germinal center with slight lymphocytic necrosis and depletion (arrow) (Photo B). Meanwhile, the spleen of rats fed on 1%, 2%, and 3% *A. Coffeaformis* revealed no histopathological changes (Photos C, D, and E, respectively).

DISCUSSION

This study revealed that carbohydrate (27.96%) constitutes the majority of the chemical composition of *A. coffeaeformis* followed by ash (25.5%), proteins (21.4%). Similarly, it was previously reported that *A. coffeaeformis* possessing high % of carbohydrates (about 56% of its overall cellular carbons) and protein [28]. In a previous conclusion, the chemical composition of the *Amphora* species reported by El-Sayed *et al.* [18] is considered taxonomic criteria for this algae. Their results showed that carbohydrates (33.6%), ash (30.4%), and protein (15.7) were the most essential chemical composition found in *A. coffeaeformis*. In agreement with this study results, other findings documented the high ash content of *A. coffeaeformis* (37.87%) [9] and 55.8-67.9% [19]. The elevated ash component of *A. coffeaeformis* dried powder probably attributed to the classic process applied for mass production [18]. The ash of *A. coffeaeformis* is of great importance in the pharmaceutical industry as it includes the entity for nanoformulations and drug carriers [29].

Regarding the macro and micronutrients composition of *A. coffeaeformis*, the results of the current work reported different % compared to previous research, where we reported iron (3.68%), phosphorus (0.8%), and zinc (1.18%) while their study reported 7.89%, 1.32%, and 13.52% respectively [18]. During alga mass-production, salinity levels play an essential role in controlling the chemical composition of the diatoms. As for diatoms, they were developed under varying salinity conditions, which stimulated the production of many secondary metabolites [30]. The heavy metals contents of *A. coffeaeformis* clarified the distinct metals chelating and antioxidant activity of the diatom [31].

The present study reported that the total antioxidant, flavonoids, and phenols content of *A. coffeaformis* was 1001.7 mg/100 g ascorbic acid, 34.88 mg/100 g quercetin, and 372.1 (mg/100 g gallic acid, respectively. The extract also contained a relatively high amount of ascorbic acid (0.15%). Like our results, *A. coffeaeformis* extract constituted high values of total polyphenols (0.594 mg/100 g gallic acid), reducing activity (7.98 µg/ml) and antioxidant efficiency (85.22 mg/ g gallic acid) [18]. Previous research notified that the phenolic compounds and ascorbic acid contents of *A. coffeaeformis* are mostly the most potent antioxidants that guard versus the deleterious effects of the reactive oxygen species and the other free radicals [32, 33]. Based on the findings of recent years, the roles of antioxidants as health-promoting factors are noticeable [34].

Microalgae are a leading origin of PUFA, and they are capable of producing both omega 3 and omega 6 FA, and numerous species of microalgae were described by their PUFA synthesis [7]. Also, the fusiform morphotype of the Bacillariophyceae *Phaeodactylum tricornutum* contained elevated concentrations of palmitoleic acid and other

bioactive FA [35]. The results of this study revealed that *A. coffeaformis* contained PUFA as linoleic acid (7.28 %, omega 6 FA) and linolenic acid (1.84 %, omega 3 FA). Besides, *A. coffeaformis* contained MUFA as oleic acid (44.30%), stearic acid (9.24 %), and vaccenic acid (2.28%). It also contained SFA as palmitic acid (30.18%), palmitoleic acid (3.86%), and undecanoic acid (1.01%). Notably, PUFA was recognized to offer numerous immunomodulatory activities that accomplished via the T cell-dependent pathway. Few of these immune actions related to PUFA-produced modifications of the cell membranes structure resulting in alterations of the signaling pathways. PUFA are the raw material for the synthesis of prostaglandins and leukotrienes, which modulate the activity of T cells, as well as the production of cytokines, thereby modifying the immunity [36].

The results of this study presented that, rats fed with different levels of *A. coffeaeformis* showed significantly increased BWG% and FER. This improvement may be attributed to the amended intestinal tract conditions due to the antimicrobial effects of *A. coffeaeformis* [17]. The reason may also be due to *A. coffeaeformis* high content of PUFA, nutrients, antioxidants, and phenolic compounds. These results agree with the finding of Abdelnour *et al.* [37], who supplemented rabbit diets with *Chlorella vulgaris* Microalgae. Moreover, Ayoub *et al.* [17] showed that supplemented *Nile tilapia* diets with *A. coffeaeformis* diatoms algae with three concentrations (10, 20, and 30 g/kg diet) enhanced the growth performance and feed efficiency.

One of the results of this study was that *A. coffeaformis* contained high levels of the polysaccharide compounds, glucan (86.81%) and mannan (69.36%). Polysaccharides of marine origin increased immunity in mice by enhancing bone marrow activity, thymus, and spleen growth besides enhancing WBCs production [38]. Furthermore, the % of phagocytic macrophages increased by increasing the content of the microalgae in animals' diets [39]. The results of the current study showed that feeding rats with *A. coffeaformis* improved the blood image with all its components, especially RBCs, Hb, all kinds of WBCs, especially LYMPH and NEUT. Our results agree with the finding of El-Ratel [40] who indicated that dietary microalgae *Spirulina platensis* exerted a significant modulation on all the hematology traits. The improvement of CBC observed in *A coffeaeformis* groups might be due to the high content of both polyphenol antioxidants and PUFA (especially omega 3 FA) anti-inflammatory of *A. coffeaeformis* [41, 42]. In the same direction towards increasing the immunity, our study showed an increase of both IgG and IgM in the groups treated with *A. coffeaeformis*. The histopathological examination of the spleen confirmed its tissue improvement and showed an increase in the number of lymphocytes in treatment groups. Similarly, a previous study found that mice fed with Spirulina alga showed a significant increase in splenic cells producing IgM antibody [43].

CONCLUSION

In conclusion, the results of this study showed that *A. coffeaeformis* contains a high % of carbohydrates, proteins, metals, vitamins, phenols, antioxidants, polysaccharides, and unsaturated fatty acids. Also, *A. coffeaeformis* modulates the immunity in the immunosuppression model in rats.

Conflict of interest

No conflict of interest.

Financial resources

No financial resources.

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