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Toxicity study of bioactive water soluble glycoprotein isolated from blue green alga *spirulina platensis*

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The oral acute, subacute and chronic toxicities, as well as genotoxicity of bioactive *Spirulina platensis* water soluble polysaccharide characterized previously as glycoprotein, were examined in order to determine the possibility of the using them clinically and for human consumption. Different hematological and clinical analysis was measured. Beside, histopathological investigation was carried out on liver and kidney architectures to examine its safety. Acute toxicity study revealed treatment of both male and female mice with 1 g kg-1 and 1.5 g kg-1 of *Spirulina platensis* glycoprotein did not show any toxicity sign and normalization in liver and kidney architectures within 14 days post oral administration. However, significant increase in AST, ALT and ALP enzyme activities at dose 2000 mg kg-1 *Spirulina* polysaccharides in male and female mice were observed as compared to control mice. Regards to subacute and chronic toxicities, the hematological and biochemical parameters of treated groups with doses 325 and 750 mg kg^{-1}/day of glycoprotein in rats for two weeks as well as 20 and 200 mg kg^{-1}/day of glycoproteins in mice for one and two months did not significantly differ from those of the control. Furthermore, chromosomal aberrations results indicated that the glycoprotein has no significant effect in general on both somatic (bone- marrow) and germ (spermotocytes) cells in different doses and time of the treatment as compared to the corresponding normal control. Finally we can conclude that *Spirulina platensis* glycoprotein was safe less than 2000 mg/kg body weight.

Keywords: *Spirulina platensis* glycoprotein, Liver function enzymes, Kidney function, Chromosomal aberrations, Blood picture

INTRODUCTION

Spirulina platensis (*Arthrospira platensis*), is a blue-green microalga (cyanobacterium) belonging to family *Oscillatoriaceae,* has been paid more attention because of their wide range of applications by diverse functions, such as food, cosmetics and pharmaceutical industries (Finamore et al., 2017). Studies conducted on *Spirulina platensis* proved that it has hypolipidemic, hypoglycemic, and

antihypertensive activities. It contained functional bioactive constituents such as phenolics, phycocyanins, provitamin, minerals, proteins, polyunsaturated fatty acids such as gamma linolenic and polysaccharides with antiviral, antioxidant, anti-inflammatory, anticancer and immunostimulating properties (Matloub et al., 2017; Sharma and Sharma, 2017; Finamore et al., 2017; Andrade et al., 2018).

Several clinical studies emphasized that the Spirulina consumption could lead to the reduction of cholesterol, protection against certain types of cancers, enhance immune response, increase of intestinal lactobacilli, protection against sun radiation and alternative treatment for obesity (Andrade et al., 2018). Furthermore, American Food and Drug Administration and the European Food Safety Authority were announced the safety of food products based on spirulina for human consumption (Andrade et al., 2018). The polysaccharides isolated from natural sources like as mushrooms, bacterial, algae and plants as well as their derivatives, have gained interesting and wide applications in the biomedical and pharmaceutical industries due to their broad biological activities, such as immune modulatory, antibacterial, antiviral, anti-mutagenic, radio protective, anti-oxidative, anti-ulcer, antidepressant, anti-septicaemic or anti-inflammatory activities (Ramawat, 2015; Ahmadi et al., 2015). Polysaccharides are non-toxic, biodegradable, biocompatible, and less expensive compared to their synthetic counterparts (Liu et al., 2015). Nowadays, polysaccharides contribute to traditional disease control and healthcare the oral administration of marine polysaccharides such as chitin/chitosan has been developed for lowering serum cholesterol (Khan and Ahmad, 2013). Also in drug delivery, where chitin/chitosan was included in ophthalmic preparations to hold and to ameliorate bio distribution of drugs (Petal and Petal, 2005). In the tissue-engineering and cell immobilization fields, the use of alginate for cartilage regeneration emerged. In the last decade applications in wound dressing/healing aroused. From our previous study, polysaccharide bounded protein (glycoprotein) with Mwt 182 kDa was isolated from cold aqueous extract of *Spirulina platensis,* has been proven in vitro for its anti-hepatitis C virus and hypolipidemic activities. It constituted from sugar (67.29%), protein (44.63%), sulfur (1.22%) and the carbohydrate content composed mainly from glucose and galactose while the protein constituted mainly from aspartic and glutamic acids (Matloub et al., 2017). Such results have given rise to determine the toxicity of glycoprotein which is a crucial step in the drug discovery process for clinical trial. Hence, the aim of the present work is to evaluate the oral acute, sub-acute and chronic toxicities as well as genetoxicity of *Spirulina platensis* glycoprotein measuring different parameters; liver function enzyme activities, kidney function, blood glucose level as well as blood picture. Beside, histopathological and genotoxicity investigations are carried out to examine its safety.

MATERIALS AND METHODS

Collection of Spirulina platensis samples

The algal materials were grown in the algal biotechnology unit, National Research Centre (NRC), Dokki- Cairo, Egypt as described by Matloub et al., (2017).

Extraction of glycoproteins from Spirulina platensis

The method of isolation of glycoprotein from blue green alga *Spirulina platensis* was described in Matloub et al., (2017).

Animals

Male mice with an average weight of 20-30 g as well as male Wistar albino rats weighing 120-130 g were obtained from animal house lab, National Research Centre, Dokki, Giza and were used in this study. Animals were housed under normal laboratory condition for one week before the initiation of biological experiments (adaptation period), housed in a well-ventilated box (22 ± 20 °C) on a twelve hours light and dark cycle. Animals were fed with natural basal diet. Diets and water were supplied *ad libitum* and had free access of water. Also, they were cared for according to the guidelines for animal experiments which were approved by the Ethical Committee of Medical Research at National Research Centre, Giza, Egypt (No. 16047).

Animals for acute toxicity

Forty male and female mice were obtained; twenty mice for each sex were administered orally 1, 1.5 and 2 g kg^{-1}b.wt. of *Spirulina platensis* glycoproteins and were divided to five mice each group as follows:

Groups 1and 2:

Control male and female mice were administered orally saline.

Groups 3, 4:

Male and female mice were administered orally 1g kg^{-1}b.wt. once a day of *Spirulina platensis* glycoproteins for 24 hrs.

Groups 5, 6:

Male and female mice were administered orally 1.5 g kg^{-1}b.wt. once a day of *Spirulina platensis* glycoproteins for 24 hrs.

Groups 7, 8:

Male and female mice were administered orally 2 g kg^{-1}b.wt. once a day of *Spirulina platensis* glycoproteins for 24 hrs.

All mice were sacrificed after 14 days post administration of 1, 1.5 and 2 g kg^{-1}b.wt. of *Spirulina platensis* glycoproteins, and fasting blood samples were collected and centrifuged at 3000 rpm for 15 minutes to separate serum. For histopathological examination, the liver and kidney were removed immediately (parts of them were fixed in 10% formalin).

Animal for sub-acute toxicity

Thirty male Wister rats were divided into three main groups of ten rats each.

Group 1:

Served as control rats were administered orally saline.

Group 2:

Rats were administered orally 325 mg kg^{-1}b.wt. of *Spirulina platensis* glycoproteins once daily at 24 hrs intervals for two weeks.

Group 3:

Rats were administered orally 750 mg kg^{-1}b.wt. of *Spirulina platensis* glycoproteins once daily at 24 hrs intervals for one month (one week).

Blood sampling collected after one week post administration of 325 and 750 mg kg^{-1}b.wt. of glycoproteins, fasting blood samples were withdrawn by cutting the subtingual vein, centrifuge at 3000 rpm for 15 minutes to separate serum. Total blood was used for hematology analyses. The separated serum was used for biochemical analysis of AST, ALT, ALP, bilirubin, creatinine and urea. After blood collection, the rats of each group were sacrificed after diethyl ether anesthesia. For histopathological examination, the liver and kidney were removed immediately (parts of them were fixed in 10% formalin). The animals were observed daily for signs and behavioral changes. Food and water intakes were also measured daily.

Animal for chronic toxicity

Sixty male mice were divided randomly into 6 main groups of ten mice each to study the hepatorenal bioactivity as follow:

Groups 1, 2:

Negative control male mice administered orally saline for one and two months, respectively.

Groups 3, 4:

Male mice were administered orally 20 mg kg^{-1}b.wt. once a day of glycoprotein for one and two months, respectively.

Groups 5, 6:

Male mice were administered orally 200 mg kg^{-1}b.wt. once a day of glycoprotein for one and two months, respectively.

Blood sampling collected after one month and two months post administration of 20 and 200 mg kg^{-1}b.wt. of glycoprotein, fasting blood samples were withdrawn by cutting the subtingual vein, centrifuge at 3000 rpm for 15 minutes to separate serum. After blood collection, mice of each group were sacrificed by decapitation, the liver and kidney were removed immediately (a part was fixed in 10% formalin for histopathological examination). The animals were observed daily for signs and behavioral changes. Food and water intakes were also measured daily.

Clinical chemistry analysis

Biochemical parameters were determined in serum using Bio diagnostic kits (Bio diagnostics Co., Egypt).

Methods

Liver function enzyme activities, alanine and a spartate amino tranferases (AST and ALT) as well as alkaline phosphatase (ALP) were determined in mice and rat sera according to the methods of Reitman and Frankel (1957) and Belfield and Goldberg (1971), respectively. Total urea and creatinine were determined according to the methods of (Bartles et al., 1972) and Fawcett and Soctt (1960). Bilirubin was determined according to the method of Walter and Gerade (1970). Glucose level was measured using colorimetric kits according to the method of Trinder (1969). The hematology was evaluated according to Adeyemo-Salami and Makinde, 2013).

Doses and experimental design for cytogenetic analysis

For genotoxic evaluation a total of 45 male mice divided into five groups of five animals in each group were used. Doses at 1500 and 2000 mg kg $^{-1}$ b.wt. of glycoprotein isolated from *Spirulina platensis*a were administered to mice as follow:

Group 1:

Negative control male mice were orally administered saline.

Groups 2, 3:

Mice were orally administered 1500 mg kg $^{-1}$ b.wt. of glycoprotein as once dose.

Groups 4, 5:

Mice were orally administered 2000 mg kg $^{-1}$ b.wt. of glycoprotein as once dose.

Groups 6, 7:

Mice were orally administered 20 mg kg $^{-1}$ b.wt. of glycoprotein once a day for 1and 2 months, respectively.

Groups 8, 9:

Mice were orally administered 200 mg kg $^{-1}$ b.wt. of glycoprotein once a day for 1and 2 months, respectively.

The samples were collected after 24hs of the last treatment.

Cytogenetic analysis

Chromosomal aberrations in somatic cells

Chromosome preparations from bone marrow (somatic cells) were carried out according to the method of Fahmy et al., (2017). One hundred well-spread metaphases were analyzed per mouse. Metaphases with different kinds of aberrations (gaps, breaks and fragments) were recorded under 2500X magnification with a light microscope (Olympus, Saitama, Japan).

Chromosomal abnormalities in germ cells

Chromosome preparations from spermatocytes (germ cells) were made according to the technique of Fahmy et al., (2017). One hundred well-spread diakinase-metaphase I cells were analysed per animal for chromosomal aberrations. Metaphases with X-Y and auto somal univalents were recorded under light microscope with 2500X magnification.

Statistical analysis

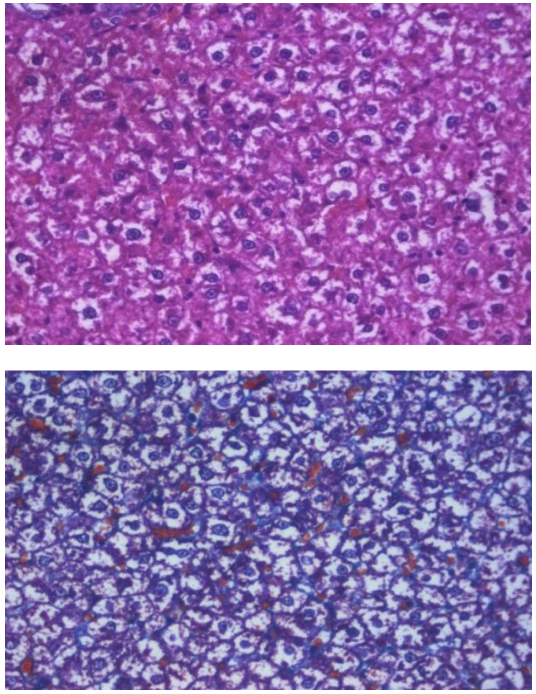
For cytogenetic statistical analysis, the difference between treated groups and controls were tested with the t-test. Beside, statistical analysis for biochemical parts is carried out using SPSS computer program (version 8) combined with *co state* computer program, where unshared letters are significant at *P≤0.05.*

RESULTS

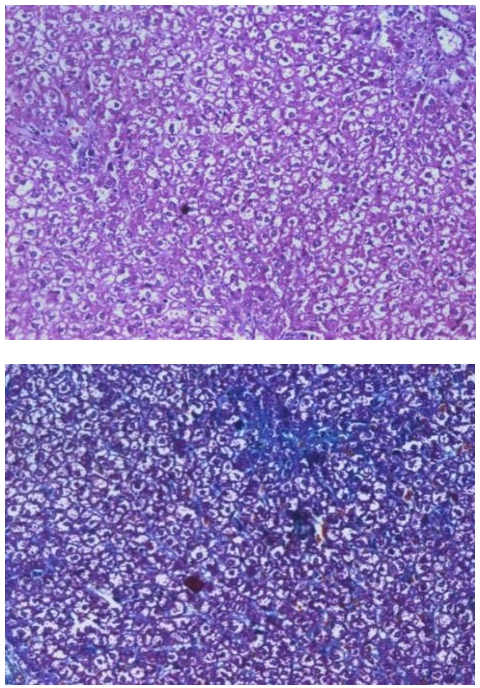
Acute, sub-acute and chronic toxicity of water soluble glycoprotein isolated from Spirulina platensis

Acute toxicity in mice

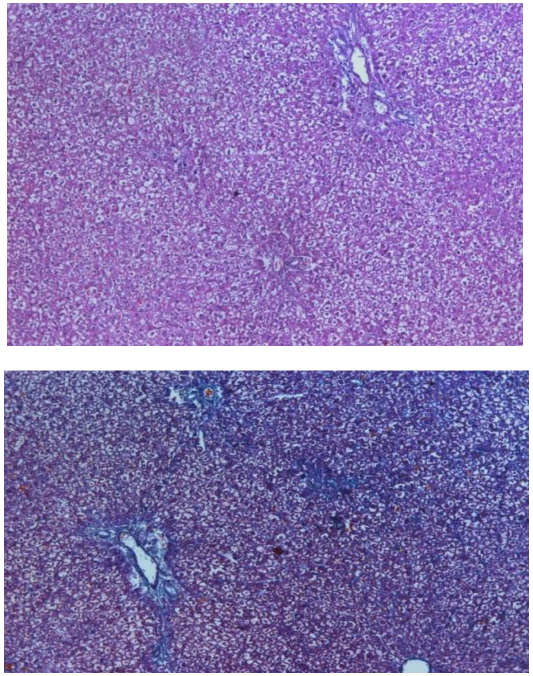
From Table (1), the experimental group, treated with 1000 mg and 1500 mg kg-1 of *Spirulina platensis* glycoprotein showed no toxic symptoms or death in addition no abnormal appearances or clinical signs in both sex mice after 14 days oral administration. While the highest dose 2000 mg kg-1 of glycoprotein exhibited 10% mortality rates in both male and female mice. On the other hand, the clinical chemistry results showed significant increase in AST and ALT enzyme activities at 2000 mg kg-1 *Spirulina platensis* glycoprotein in male and female mice as compared to the relative control group. While, an insignificant change was observed in AST and ALT enzyme activities at the dose 1000 and 1500 mg kg^{-1} of glycoprotein in both male and female mice. However, ALP enzyme activity showed a significant increase in male and female mice serum at dose 1500 and 2000 mg/kg a.b.w. *Spirulina platensis* glycoprotein as compared to the corresponding control mice while an insignificant change was detected at dose 1000 mg kg^{-1} glycoprotein in male and female mice. With respect to kidney function tests, an insignificant change was detected in total urea and creatinine levels at different doses of glycoprotein as compared to the corresponding normal control level. Also, histopathological investigation (Photomicrographs 1- 6) showed normalization in hepatic and renal architectures**.**



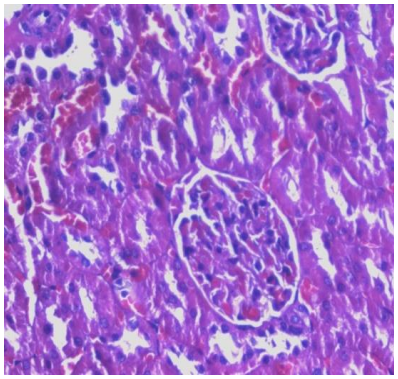
Photomicrograph 1: Liver sections from Control group showed preserved (intact) lobular hepatic architecture, with normal hepatocyte (H&E, x400) and Masson's trichome stain

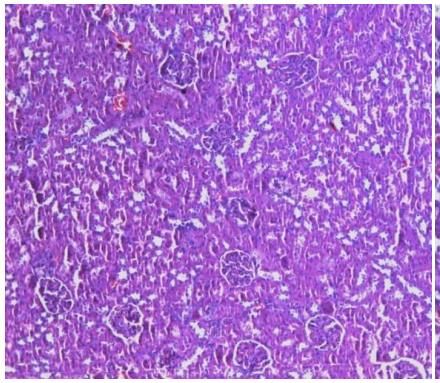


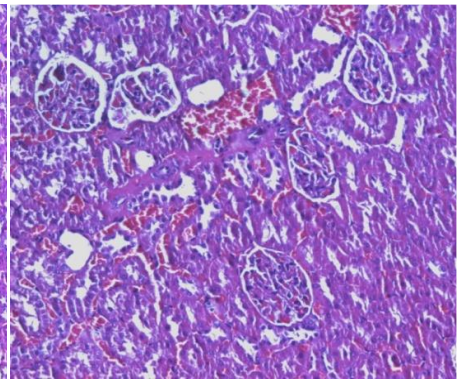
Photomicrograph 2: Liver section from 2000 mg group showed non-significant change in lobular hepatic architecture, hepatocyte almost normal arranged (H&E, x200) and Masson's trichome stain.

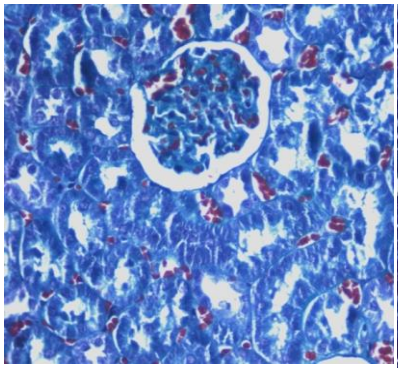


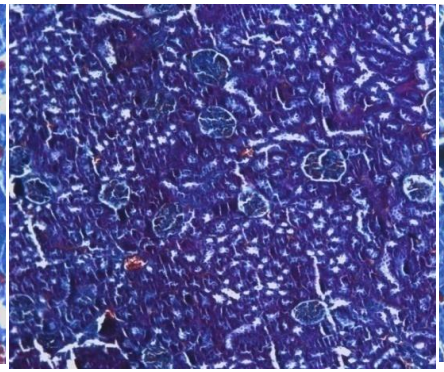
Photomicrograph 3: Liver section from 1500 mg group showed preserved (intact) lobular hepatic architecture, mild sinusoidal dilatation and hepatocyte appeared normal arranged (H&E, x100) Masson's trichome stain.

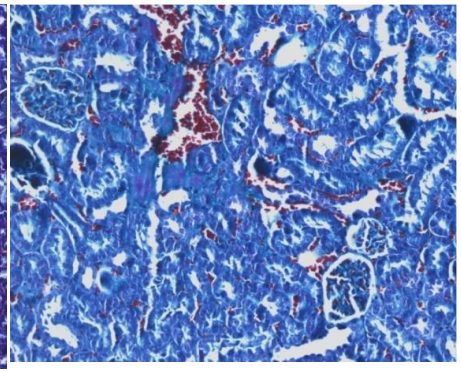












Photomicrograph 4: Kidney section from control group showed renal cortex of renal corpuscle with normal glomerulus (H & E, x400) and Masson's trichome stain.

Photomicrograph 5: Kidney section from 2000 mg group showed normal renal cortex with normal glomerulus (H & E, x400) and Masson's trichome stain.

Photomicrograph 6: Kidney section from 1500 mg group showed non-significant change kidney cells in renal cortex of renal corpuscle with normal glomerulus (H & E, x200) and Masson's trichome stain.

Table (1): Liver function enzyme activities, total urea and creatinine levels in male and female mice post administration of 1000,1500 and 2000 mg of glycoprotein isolated from *Spirulinaplatensis*

Groups	ALT(U/l)	AST(U/l)	ALP(U/l)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control male	32.00±2.44[a]	70.00± 2.78[a]	43.65±2.12[a]	24.22±1.89[a]	0.17±0.02[a]
Normal control female	32.11±1.54[a]	72.00± 1.58[a]	44.90±3.10[a]	24.96±1.22[a]	0.18±0.03[a]
1000mg male	31.33±1.77[a]	74.00± 3.78[a]	40.00±2.87[a]	21.66±1.90[a]	0.17±0.01[a]
1000mg female	29.67±1.36[a]	81.00±2.89[a]	47.00±3.12[b]	24.33±2.10[a]	0.18±0.02[a]
1500mg male	29.00±1.21[a]	79.00±3.33[a]	56.66± 5.06[b]	24.5±1.52[a]	0.18±0.04[a]
1500 mg female	31.33±1.33[a]	81.5±2.63[a]	60.5±3.85[b]	21.00±1.44[a]	0.18±0.02[a]
2000 mg male	46.66±2.06[b]	118.00±9.20[b]	67.00±5.22[b]	24.00±1.10[a]	0.17±0.03[a]
2000 mg female	48.00±2. 51[b]	102.5±7.86[b]	63.00±6.12[b]	21.00±0.13[a]	0.18±0.02[a]

Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at $P \leq 0.05$.

Sub-acute toxicity in rat

Liver enzyme activities, kidney function and blood glucose level in serum rat as well as blood profile of both doses 325 and 750 mg kg^{-1} *Spirulina platensis* polysaccharide were recorded in Tables (2 & 3).

The biochemical results clearly demonstrated no consistent significant difference in liver enzyme activities and kidney function, blood glucose level as well as blood profile. Also, histopathological investigation (Photomicrographs 7- 12) showed normalization in liver and kidney architectures**.**

Chronic toxicity in mice

The male mice administered cold polysaccharide extract of *Spirulina platensis* at doses 20 and 200 mg kg^{-1}a.b.w. for one and two months (Table 4). After one month, the clinical chemistry results showed no significant difference in liver function enzyme activities AST, ALT and ALP and total bilirubin levels. Also, kidney function

biomarkers recorded insignificant change in total urea, and creatinine levels in male mice administered 20 and 200 mg glycoprotein as compared to control group**.** Furthermore, the mice administered 200 mg/ kg b.w. of glycoprotein showed insignificant change in liver function enzyme activities AST, ALT and ALP and total bilirubin level after two months. Also, kidney function biomarkers recorded insignificant change in total urea, and creatinine levels in male mice 200 mg / kg b.w of glycoprotein as compared to control group**.** On the other hand, decrease of body weight gain and increase in feces was observed in mice administrated 200 mg/ kg b.w of glycoprotein during the second month's examination.

However, the histopathological investigation of liver from group, administered 200 mg/ kg b.w. of glycoprotein for two months, showed preserved (intact) lobular hepatic architecture and with minor lobular inflammation, minor hydropic degeneration and thin fibrous tissue bands and congested blood vessels (Photomicrograph 13). While, the kidney section from group, administered 200 mg/ kg b.w. of glycoprotein for two months, showed renal cortex with most of the corpuscles with high cellularity and obliterated capsular space. Proximal convoluted tubules show destructed epithelial lining, destructed epithelial lining of distal convoluted tubules and conges (Photomicrograph 14).

Cytogenetic analysis

Furthermore, the results of chromosomal aberrations in both somatic and germ cells of glycoprotein isolated from *Spirulina platensis* at doses 1500 and 2000 mg/kg a.b.w as once dose as well as 20 and 200 mg/kg a.b.w for one and two months are compiled in Tables (5 and 6). The obtained results indicated that the glycoprotein did not exhibit significant abnormality in general in both somatic (bone-marrow) and germ (spermotocytes) in both doses as compared to the corresponding normal control.

Table (2): Liver and Kidney Function in rats treated with glycoprotein isolated from *Spirulina platensis* at doses 325 and 750 mg/kg b.w/day for a week

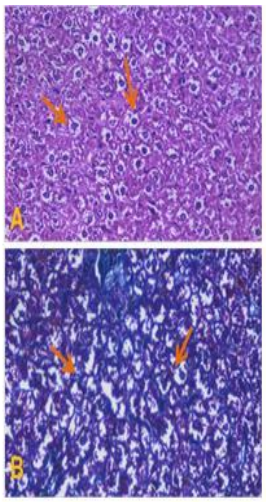
Biomarkers	Control	325 mg/kg	750 mg/kg
Prothrombin Time (Sec)	16.00 ± 1.50	16.120 ± 1.11	16.65 ±1.90
Creatinine (mg)	0.61 ±0.01	0.60 ± 0.02 ^{ns}	0.58 ± 0.01 ^{ns}
Urea (mg%)	46.00 ± 3.10	51.75 ± 3.90 ^{ns}	44.90 ± 5.20 ^{ns}
Bilirubin (mg%)	0.88 ± 0.02	0.81 ± 0.02 ^{ns}	0.833 ± 0.01^{ns}
AST (U/L)	20.67 ± 1.90	21.55 ± 1.28 ^{ns}	22.50 ± 1.92
ALT (U/L)	68.33 ± 9.23ns	67.50 ± 5.90 ^{ns}	69.66 ± 9.10^{ns}
ALP (U/L)	288.33 ± 23.10	300.00 ± 20.10 ^{ns}	320.00 ±15.90^{ns}
Glucose (mg/dl)	100.50 ± 3.90	100.83 ± 5.90	107.10 ± 8.20

Statistical analysis is carried out using Paired T –test, where P≤0.0001 is considered significant

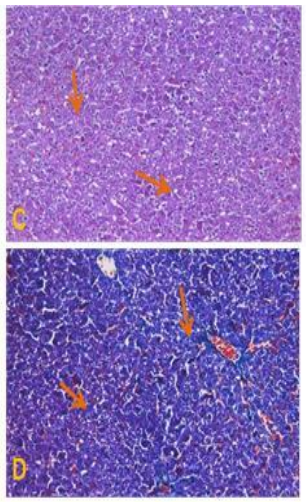
Table (3): Blood profile of rats treated with glycoprotein isolated from *Spirulinaplatensis at* doses 325 and 750 mg/kg b.w/day for a week

Biomarkers	Control	325 mg/kg	750 mg/kg
HB (g/L)	12.00 ± 1.10	12.98 ± 1.29 ^{ns}	12.20 ± 1.90 ^{ns}
RBCs(million cells/ul)	6.30 ± 0.91	6.86 ± 0.98 ^{ns}	6.37 ± 0.88 ^{ns}
PCV (%)	35.80 ± 5.10	34.95 ± 3.97^{ns}	35.75 ± 3.50 ^{ns}
WBCs(× 10^9/L)	9.9666± 110.50	10.067 ±113.59 ^{ns}	10.600 ± 100.50 ^{ns}
Neutrophils (×10^9/l)	20.66 ± 1.15	19.50 ± 1.23^{ns}	19.93 ± 2.10 ^{ns}
Eosinophil (X100 cells /mcL).	1.66 ± 0.05	1.35 ± 0.01^{ns}	1.43 ± 0.04 ^{ns}
Lymphocyte(10^9/l)	77.33 ± 9.23	75.67 ± 10.11^{ns}	76.90 ± 9.80 ^{ns}
Monocyte(10^9/l)	1.66 ±0.03	1.50 ± 0.05^{ns}	1.66 ± 0.02 ^{ns}

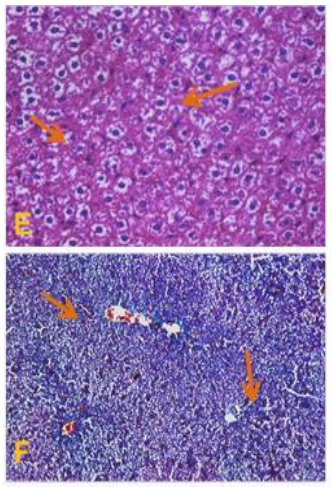
Statistical analysis is carried out using Paired T –test, where P≤0.0001 is considered significant Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter issignificant at P ≤ 0.05.



Photomicrograph 7: (A) Liver section from control group showed normal hepatic cell (H & E, x200). (B) Liver section from control group showed normal distribution of collagen hepatic cells (massons' trichome, x 200).



Photomicrograph 8: (C) Liver section from *Spirulina* polysaccharide group (325 mg/kg) showed no observed change in hepatic cell (H&E, x200). (D) Liver section from *Spirulina* polysaccharide group (325 mg/kg) showed normal hepatic collagen (massons' trichome, x 200).

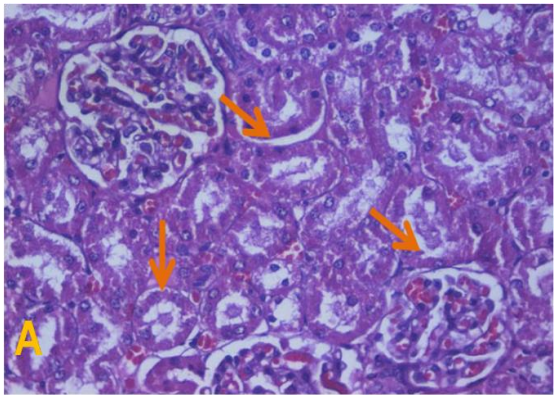


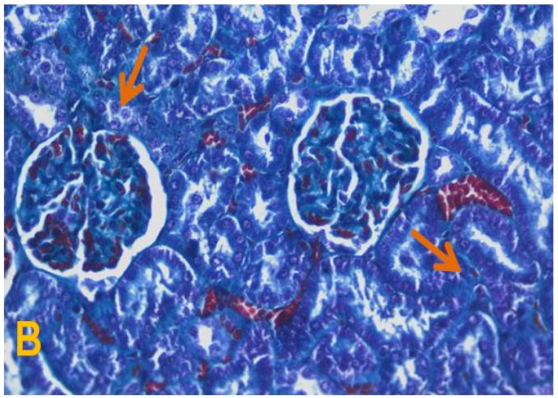
Photomicrograph 9: (E) Liver section from *Spirulina* polysaccharide group (750 mg/kg) showed preserved (intact) lobular hepatic architecture (H & E, x200). (F) Liver section from *Spirulina* group (750 mg/kg) showed hepatocyte almost normal arranged in thin plates with normal collagen (massons' trichome, x 200).

Table (4): Liver function enzyme activities, bilirubin , total urea and creatinine levels in male mice post administration of 20 and 200 mg /kg b.w of glycoprotein isolated from *Spirulinaplatensis*

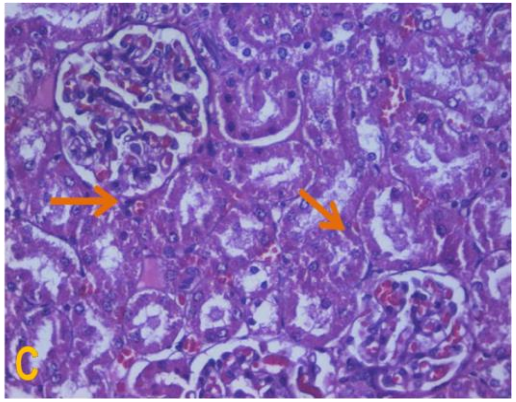
Groups / parameters	Duration of treatment	ALT (U/l)	AST (U/l)	ALP (U/l)	Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control male	1&2 month	32.00±2.78[a]	70.00±3.20[a]	43.65±2.12[a]	0.75±0.02[a]	24.22±3.20[a]	0.17±0.04[a]
20 mg /kg b.w	1 month	26.75±3.57[a]	71.88±5.34[a]	46.00±4.77 [a]	0.84±0.03[a]	27.00±3.10 [a]	0.17±0.02[a]
200 mg/kg b.w	1 month	28.66±2.11[a]	74.09±6.72[a]	48.00±5.88 [a]	0.81±0.03[a]	30.00±3.80 [a]	0.18±0.03 [a]
200 mg/kg b.w	2 months	33.66±3.65[a]	67.3±5.80[a]	46.00±3.32 [a]	0.71±0.03[a]	28.56±2.76 [a]	0.20±0.01 [a]

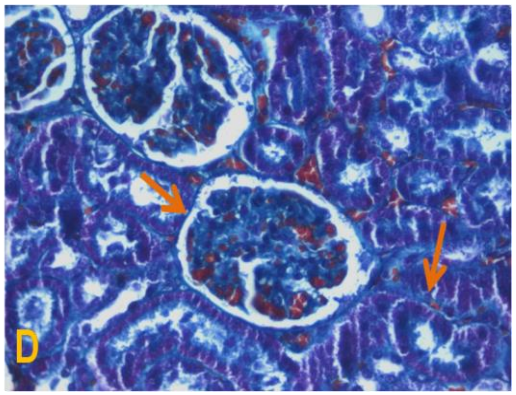
Statistical analysis is carried out using Paired T –test, where P≤0.0001 is considered significant Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter issignificant at P ≤ 0.05.



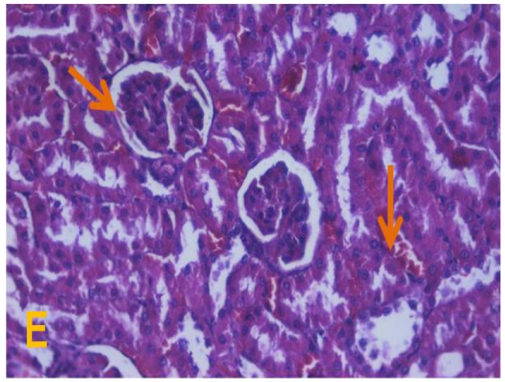


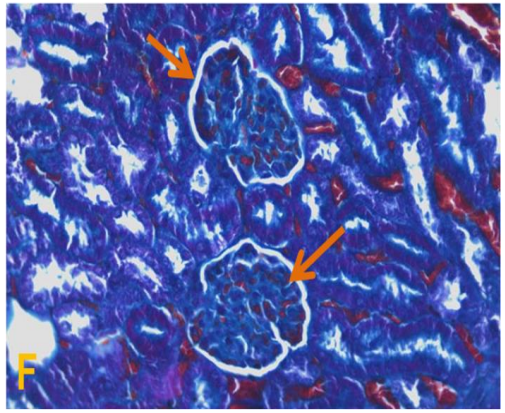
Photomicrograph 10: (A) Kidney section from control group showed normal hepatic cell (H & E, x200). (B) Kidney section from control group showed normal distribution of collagen hepatic cells (massons' trichome, x 200).



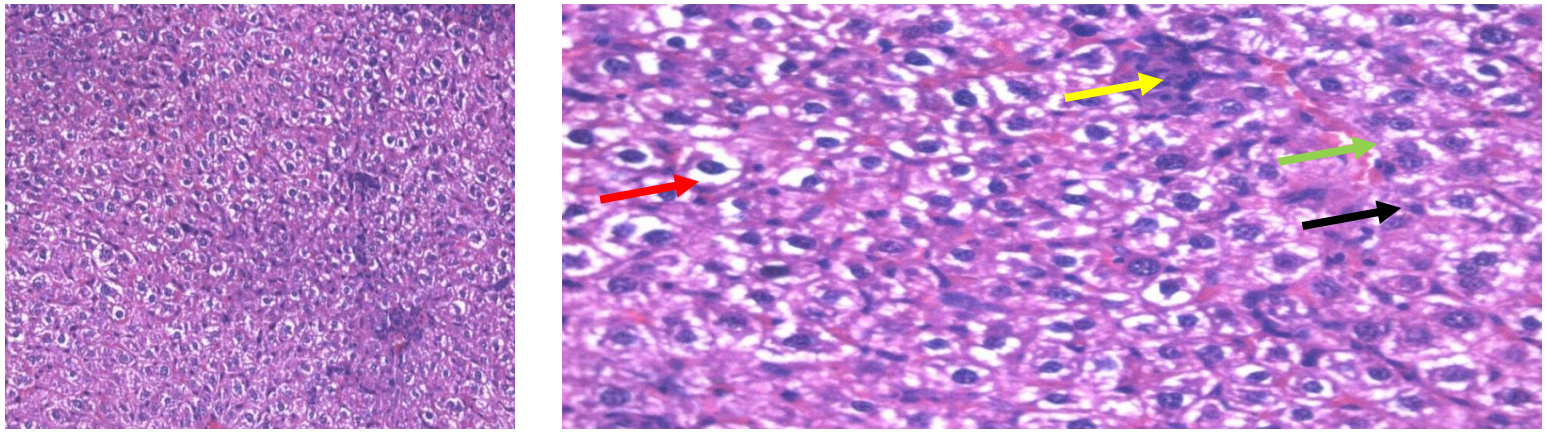


Photomicrograph 11: (C) Kidney sectio from *Spirulina* polysaccharide group (325 mg/kg) showed renal cortex of renal corpuscle with normal glomerulus in kidney cells (H & E, x200). (D) Kidney section from *Spirulina* polysaccharide group (325 mg/kg) showed normal distribution collagen (massons' trichome, x 200).

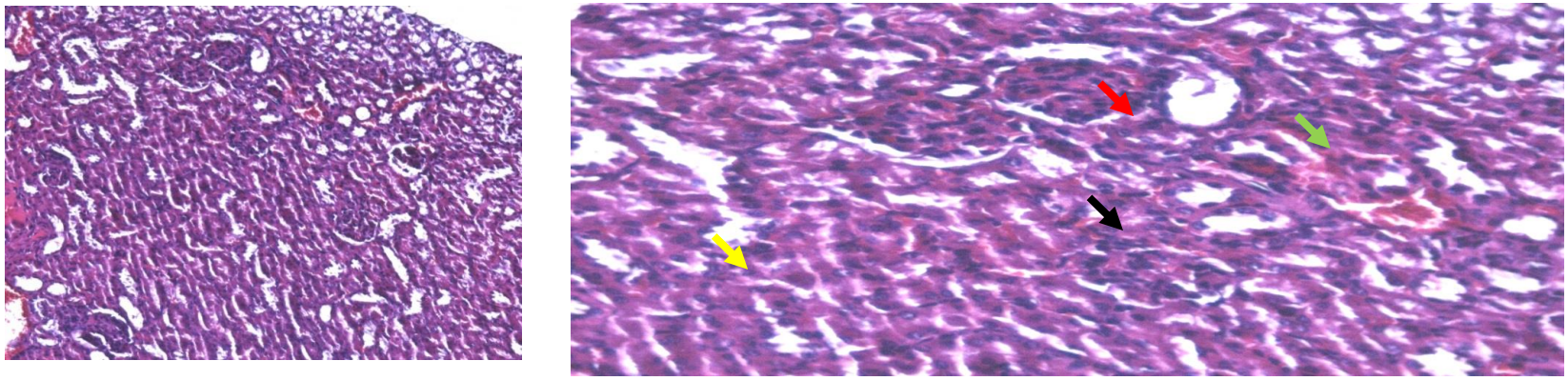




Photomicrograph 12: (E) Kidney section from *Spirulina* polysaccharide group (750 mg/kg) showed renal cortex of renal corpuscle with normal glomerulus nearly normal kidney cell (H & E, x200). (F) Kidney section from *Spirulina* polysaccharide group (750 mg/kg) showed no observed changes collagen (massons' trichome, x 200).



Photomicrograph 13: Liver section from group administrated 200 mg/kg a.b.w for two months showed preserved (intact) lobular hepatic architecture and with minor lobular inflammation (yellow arrows), minorhydropic degeneration (red arrow) and thin fibrous tissue bands(black arrow) and congested blood vessels(green arrow)(H&E, x200).



Photomicrograph14: Kidney section from group administrated 200 mg/kg a.b.w for two months showed renal cortex with most of the corpuscles with high cellularity and obliterated capsular space (black arrow). Proximal convoluted tubules show destructed epithelial lining (red arrow), destructed epithelial lining of distal convoluted tubules (yellow arrow) and conges.

Table (5): Type and percentage of metaphases with chromosomal aberrations induced in mouse bone - marrow cells after treatment with *Spirulinaplatensis*glycoproteinwith different doses *in vivo*.

Treatment (mg/kgb.wt.)	Time of treatment	Abnormal metaphases		Type of aberrations		
		No.	Mean (%) ± SE	Gap	Break	Fragment
Control	**-**	**20**	4.00±0.55	10	4	6
Acute treatment 1500	**After 24 hrs**	**15**	3.00± 0.48	6	2	7
2000	**After 15 days**	**19**	3.80± 0.58	6	3	10
Chronic treatment 20	**One month**	**22**	4.40 ± 0.40	11	6	5
200	**One month**	**28**	5.60 ± 0.60	13	5	10
200	**Two month**	**25**	5.00±0.62	8	5	12

Total number of examined metaphases = 500 (5 animals/ group).

Table (6): Types and percentage of metaphases with chromosomal aberrations induced in mouse spermatocyte cells after treatment with *Spirulina platensis* glycoprotein with different doses *in vivo*.

Treatment (mg/kgb.wt.)	Time of treatment	Abnormal metaphases		Type of aberrations	
		No.	Mean (%) ± SE	X-Y univalent	Autosomal univalent
Control	**-**	**15**	3.00±0.44	10	5
Acute treatment 1500	**After 24 hrs**	**18**	3.60±0.62	14	4
2000	**After 15 days**	**16**	3.20±0.40	10	6
Chronic treatment 20	**One month**	**18**	3.60±0.65	13	5
200	**One month**	**24**	4.80 ± 0.48	16	8
200	**Two month**	**25**	5.00±0.55	18	7

Total number of examined metaphases = 500 (5 animals/ group)

DISCUSSION

To the best of our knowledge, this is the first study that has investigated the possible toxicity and genetoxicity effect of *Spirulina platensis* glycoprotein extract. Our results in both mice and rat clearly demonstrated the safety of glycoprotein isolated from cold water extract of *Spirulina platensis*. At a single dose up to 1500 mg kg^{-1} b.w., it did not cause any toxicity. Numerous animal toxicological studies on Spirulina include acute, subchronic, chronic, mutagenic, teratogenic/ developmental toxicity, carcinogenic, and multiple generational/ reproduction studies were done (Pyne et al., 2017). Such studies are pivotal in the determination that a water extract of Spirulina is safe. It is worth to mention that the cold aqueous extract of *Spirulina platensis* constituted of 4.45% glycoprotein (Matloub et al., 2017). Furthermore, (Chen et al., 2016) found that cold water extract of Spirulina has low cellular toxicity; in addition it is well-tolerated in animal models at one dose 5,000 mg/kg for acute toxicity and 3,000 mg/kg/day for 14 successive days for sub-acute toxicity.

Several study conducted to hepatoprotective of polysaccharide which reduced the hepatocellular degeneration and necrosis, as well as inflammatory infiltration on acute hepatic injury induced by CCl4 in mice and might be related to its activation of ethanol dehydrogenase, elimination of free radicals and/or inhibition of lipid peroxidation capacities (Huang et al., 2016, Zhou et al., 2017).

Bioactive water soluble polysaccharide of Spirulina reduced allergic inflammation and enhanced defense activity against infectious diseases through supporting the functions of the mucosal immune system as well as cause immune modulation via increased proliferation of erythrocytes, granulocyte–monocyte, and fibroblast line age cells derived from bone marrow cells of mice (Hayashi et al., 2004). Furthermore, Immulina (high molecular weight polysaccharide) from Spirulina exhibited a potent activator of NF-kappa B and induced both IL-1 β and TNF-α mRNAs in THP-1 human monocytes (Pugh et al., 2001).

Suppression of the increase of body weight in mice administrated 200 mg/ kg b.w of glycoprotein during the second month's trial may be due to increase in feces. This effect was observed in previous studies either in mice fed *Arthrospira maxima* for 13 weeks or in rats fed *A. platensis* for 12 weeks (Hutadilok-Towatana et al., 2010).

Concerning to genetoxicity effect of *Spirulina platensis* glycoprotein, our results showed that administration of glycoprotein at doses 1500 and 2000 mg kg^{-1} b.wt. for 1 and 2 months did not induced a significant percentage of mutations in somatic and germ cells. Our results supported by many reports proved that polysaccharide administrations was save (El Souda et al., 2014, Zhang et al., 2016). It is worth to mention that polysaccharide extract not only save on DNA but also has antimutagenic activity. Zhang et al., (2016) reported that polysaccharide isolated from *Dioscorea opposita* could be a potential candidate of the natural antimutagen. El Souda et al., (2014) reported that administration of polysaccharide extract from *P. albicans* in bone marrow cells of mice *in vivo* for 7 days not only save on DNA but also prohibit the DNA damage in bone marrow cells induced by the anticancer drug cyclophosphamide(CP). Also, Chen et al., (2005) observed that the polysaccharide extracted from *Gracilaria lemaneiformis* had antioxidant activity and inhibitory effect on mouse bone marrow micronucleus and abnormal sperm induced by CP. In addition, Zhou et al., (2013) reported that a novel salt-soluble polysaccharide from *Auricularia polytricha* had antimutagenic activity against DNA-damage induced by CP.

CONCLUSION

The toxicity study of *Spirulina platensis* glycoprotein was proved its safety. Alongside to our previous study of its antiviral activity against to HCV, further studies including clinical trials to exploit the antiviral activity against viral infections in human is pivotal needed for the development new antiviral drugs.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

AAM , SSME ,AEBE designed the experiment , performed the chemical analyses such as isolation and characterization of spirulina glycoprotein and share in writing and manuscript revision . HFA,SAA,MH,NSE performed animals treatment , biochemical evaluations and histopathological examination ,data analyses and writing and reviewed the manuscript .MAF ,AAF and ZMH performed cytogenetic analysis , and reviewed the manuscript .All authors read and approved the final version

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