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PROSPECTIVE EFFECT OF RED ALGAE, *ACTINOTRICHIA FRAGILIS*, AGAINST SOME OSTEOARTHRITIS AETIOLOGY

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Abstract

Background: Osteoarthritis (OA) is a progressive disease characterized by joints pain and articular cartilage destruction. Most of the current treatment strategies for OA are effective for symptoms relief but are accompanied with adverse side effect. Thus, the present investigation aims to evaluate the potential influence of red algae, *Actinotrichia fragilis*, in the dry powder form (AFP) or gel form (AFG) on some relevant factors of OA progression as well as assess its safety through *in vitro* and *in vivo* experiments.
Materials and Methods: *In vitro*, AFP was analyzed for its chemical constituents screening, amino acid, proteinase inhibitory activity, HRBC membrane stabilization activity, free radical scavenging activity, total antioxidant potency, nitric oxide radical scavenging power. *In vivo*, Organization for Economic Co-operation and Development (OECD) toxicity test was performed to test the safety of AFP on rats.
Results: The present findings revealed that AFP and AFG can be considered as inflammatory-proteinase-oxidant inhibitor and considered to be safe according to the OECD guideline.
Conclusion: AFP and AFG may have the potency to become the therapeutic candidate for OA disease as it prevents the key causes of OA initiation.

Key words: Osteoarthritis, Red algae**,** *Actinotrichia fragilis*, Inflammatory-Proteinase-Oxidant inhibitor.

Introduction

Osteoarthritis (OA) is a degenerative disease of joints results from structural changes of articular cartilage, subchondral bone and synovium over time which consequently ends with joint pain and damage (Murphy et al., 2014). An OA hallmark is its progression which involves the corrosion of articular cartilage, synovial membrane inflammation, and resorption of the subchondral bone (Hayami et al., 2006). These pathological alterations are accompanied with an overproduction of proinflammatory molecules such as interleukin 1β (IL-1β), nitric oxide (NO), proteinases, and reactive oxygen species (ROS) (Schuerwegh et al., 2003; Dalle-Donne et al., 2003; Kapoor et al., 2011; Narayani et al., 2014). These molecules disrupt homeostasis of the cartilage matrix favor degradation of matrix components which eventually ends with progressive damage of the joint tissue (van der Kraan and van den Berg, 2000).

OA therapy encompasses non-pharmacological with pharmacological managements. Nonpharmacological management generally includes lifestyle improvement such as physical therapy, exercises and weight reduction (Murphy et al., 2014). Pharmacological management is symptomatic treatment as it targets symptoms of the disease rather than its causes (Altman et al., 2009). Despite pharmacological treatments providing some relief from OA symptoms, they are suboptimal and accompanied with side effects on long-term use (Patrignani et al., 2011; Cheng and Visco, 2012). This means that till now, there are no effective pharmacotherapies to repair the structure and functionality of impaired articular cartilage (Walzer et al., 2015). Thereby, there is an urgent demand to find out novel safe and effective alternative remedy to alleviate the OA severity and preserve the structural integrity of joint tissues.

Marine biotechnology is an emerging field encompassing marine biomedicine aims to develop new pharmaceutical products derived from marine source (Murti and Agrawal, 2010). Marine that biotechnology including marine sources may be used as pharmaceutical drugs as it is enriched with active ingredients that can contribute to design and develop new potential pharmaceutical agents (Montaser and Luesch, 2011). Marine algae are considered as a source of bioactive agents that have a variety of biological activities (Cox et al., 2010). As the mechanisms of cartilage degradation in OA are multifactorial and some nutraceutical compounds usually containing multiple active constituents that target multiple pathways, the present study selects red algae (*Actinotrichia fragilis*) as natural marine source rather than mono-modal action of pharmacological drugs. The discovery of newly drugs, even if from naturally sources, necessitates toxicity studies to approve its safety. Thus, the present study performs the acute toxicity study using Organization for Economic Co-operation and Development (OECD) on the *Actinotrichia fragilis* to determine its safety/toxicity.

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OA associated with inflammation, protein degrading enzymes, ROS and NO generation which considered the causative factors for OA progression (Li et al., 2012; Meszaros et al., 2012; Moradi et al., 2013). Therefore, the present study assesses the anti-inflammatory, proteinase inhibitor, antioxidant, and NO scavenging activities of *A. fragilis*. To minimize the side effects produced by the current tablets or capsules, the present work evaluates the ameliorative efficacy of *A. fragilis* in its two forms; powder (AFP) and gel (AFG). The gel form was postulated to have fewer side effects than drugs taken as tablets or capsules.

Materials and Methods

Collection of *Actinotrichia fragilis*

Actinotrichia fragilis collected from the coastal region of Hurghada, Egypt in October 2014. After collection, the seaweeds were washed with sea water to eliminate sand, mutt, overgrowing debris and epiphytes. The red algae were authenticated by Professor Sanaa Mahmoud Metwally Shanab, Professor of Phycology, Botany Department, Faculty of Science, Cairo University.

Preparation of *Actinotrichia fragilis* powder (AFP)

The cleaned *A. fragilis* was air dried ~~to~~ in shade for 7 days; and the obtained dried sample was finely milled and stored at – 20 °C until use.

Preparation of *Actinotrichia fragilis* gel (AFG)

Preparation of AFG was carried out according to Candra et al. (2011) method. AFP (100 g) was added to 300 ml distilled water for 2 h. Then, the wetted AFP boiled at 80^{o}C until AFP converted to semisolid paste (gel) form. The latter was cooled at room temperature.

Chemical screening of AFP and AFG

The AFP and AFG were evaluated for the presence of different active constituents according to the methods adopted by Sawadogo et al. (2006).

Amino acid analysis of AFP and AFG

The amino acid composition of AFP and AFG was analyzed according to Csomos and Simon-Sarkadi (2002) using the Automatic Amino Acid Analyzer (AAA 400, INGOS Ltd.). AFP (100 mg) was hydrolyzed with 10 ml of HCl (6 N) in a sealed tube at 110ºC in an oven for 24 h. Following hydrolysis, the HCl acid was evaporated at 80ºC under vacuum with addition of distilled water until dryness. The free HCl residue was dissolved in 2 ml of loading buffer (6.2 M, pH 2.2).

In vitro experiments

As the difference between AFP and AFG almost non-existent (concerning their constituents) except ~~only~~ in their physical nature only, at least in the present experimental conditions. Thus, the current work analyzes and evaluates the following *in vitro* studies on AFP only.

Evaluation of anti-arthritic effect of AFP

Proteinase inhibitory activity assay

This assay was performed according to Oyedapo and Famurewa (1995) and Sakat et al. (2010). The reaction mixture contained 50 µl tested sample (AFP) at different concentrations (50 – 400 µg/ml), 350 µl of 25 mM Tris-HCl buffer (pH 7.4), 100 µl of trypsin (0.6 mg/ml), and the mixture was incubated at 37^{0}C for 5 min and then 500 µl of 2% (w/v) casein was added. The mixture was incubated for an extra 20 min. Two ºC of 5% trichloroacetic acid (TCA) was added to stop the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 280 nm against blank (buffer). Aspirin was used as standard drug. AFP proteinase inhibition (%) was compared with the control tube which contains all reagents except sample or standard. The percentage inhibition of proteinase inhibitory activity was determined according to the following equation:

Percent of proteinase inhibition= [O.Dsample or standard/O.Dcontrol] × 100

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Evaluation of anti-inflammatory activity of AFP

Human red blood cell (HRBC) membrane stabilization method (heat induced hemolysis)

To assess the *in vitro* anti-inflammatory activity of AFP, the present study assesses the stability of HRBC membrane by AFP. 5 ml blood was collected in heparinized tube from healthy volunteer who had not taken any NSAIDS for 2 weeks preceding to the experiment and then centrifuged at 3,000 rpm for 10 min. The packed red blood cells were washed three times with equal volume of normal saline. The volume of the packed red blood cells was measured and reconstituted as 10% v/v HRBC suspension with normal saline. To 1 ml of HRBC suspension, equal volume of each of AFP concentrations (50 – 400 µg/ml) was added followed by incubation at 56 ºC for 30 minutes and then centrifuged at 2500 rpm for 5 min. To assess the efficacy of anti-inflammatory activity of AFP, the same concentrations of Aspirin (standard anti-inflammatory drug) were prepared and tested similarly. Additionally, saline was added to HRBC suspension instead of test sample to use as control tube. The optical density (OD) of supernatant hemoglobin content was determined using spectrophotometer at 560 nm (Gandhisan et al., 1991). Then, percentages of heat induced HRBC hemolysis and membrane stabilization or protection were calculated by the following formula:

Percent of hemolysis = $[O.D_{sample\ or\ standard}/O.D_{control}] \times 100$

The percentage of protection can be hence calculated from the equation as given below:

Percent of protection = $100 - [(O.D_{sample\ or\ standard}/O.D_{control}) \times 100]$

Evaluation of free radical scavenging activity of AFP

DPPH (1,1- diphenyl-2-picrylhydrazyl) free radical scavenging test was performed on AFP to evaluate its free radical scavenging activity according to Brand et al. (1995). The following concentrations (10, 20, 30, 40, 50, 60, 70 and 80 mg/ml methanol) of AFP and ascorbic acid (standard free radical scavenger) were prepared. Each tube contained 2 ml DPPH (0.1 mM in methanol) and completed to the final volume of 4 ml with AFP (certain concentration) + methanol. Tubes were shaken and incubated at 37°C for 30 min. The control tube (DPPH only) underwent the same manners and methanol was used as a blank. The O.D was measured at λ = 517 nm. The radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (%) = $[(O.D_{control} - O.D_{sample\ or\ standard})/ O.D_{control}] \times 100$

Evaluation of total antioxidant capacity of AFP

The total antioxidant capacity (TAC) of the AFP was determined by the phosphomolybdenum (Mo) method according to Prieto et al. (1999). Briefly, the reduction of Mo (VI) to Mo (V) by the tested sample (AFP) and formation of green phosphate/Mo (V) complex indicates the TAC activity of the tested sample. A 0.1 ml of AFP (10 mg/ml DMSO) was mixed with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95ºC for 90 min. After cooling, the absorbance of the sample was measured at 695 nm using a UV-VIS spectrophotometer against blank (0.1 ml DMSO and 1 ml of reagent solution). The TAC activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared using different concentrations of ascorbic acid (1.25-10 mg/ml DMSO). The percentage of TAC of AFP is calculated as follow:

$Y = X + Factor$ (1)

$A = (CXV)/W$ (2)

Where, Y is absorbance; X is the concentration of ascorbic acid (mg/ml); A is the TAC of AFP (mg/ml); C is the concentration of ascorbic acid established from calibration curve equation (1); V is the volume of sample (ml); and W is weight of AFP (g).

Evaluation of nitric oxide radical scavenging activity of AFP

Nitric oxide (NO) scavenging activity was detected by Griess Ilosvoy reaction (Garrat 1964) with minor modification. The assay principal is the decomposition of sodium nitroprusside in aqueous solution at physiological pH (7.2) to produce NO. Under aerobic conditions, NO reacts with oxygen to produce nitrate and nitrite. The concentrations of these products can be determined using Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of nitrite ions. For the experiment, 5 mM sodium nitroprusside (in phosphate buffered saline, pH 7.2) was added to different concentrations (5– 200 µg/ml) of AFP, and incubated at 25ºC for 150 min. AFP is replaced with phosphate buffered saline in control tube. Then, 0.5 ml of Griess reagent (prepared by Biodiagnostic Company, Egypt) was added. The absorbance of the chromophore (formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride) was immediately read at 550 nm against blank (phosphate buffer saline). Inhibition of nitrite formation by the AFP and the standard antioxidant (ascorbic acid) were calculated relative to the control. The percentage nitrite radical scavenging activity of the AFP was calculated using the following formula: NO scavenging activity (%) = $[(A_{control} - A_{sample\ or\ standard})/ A_{sample\ or\ standard}] \times 100$

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***In vivo* experiment**

Animals

Female Wistar albino rats (*Rattus norvegicus*) weighing 170-190 g were purchased from National Research Center (NRC), Egypt. The animals were maintained at controlled temperature (21 ± 2°C) and on a 12 h dark–light cycle. Water and food were provided *ad libitum*.

Ethical consideration

Experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee (IACUC); Cairo University, Faculty of Science, Egypt (CUFS/F/22/14). All the experimental procedures were performed according to the international care and use of laboratory animals' guidelines.

Acute toxicity test-fixed dose procedure

The acute toxicity study of AFP was assessed in rats (n=10) using Organization for Economic Co-operation and Development (OECD) guideline 423 (OECD 2002). AFP suspended in distilled water and administered orally to 5 rats at a limit dose of 2000 mg/kg body weight. All the clinical signs and mortality were observed for 1 h after dosing, and then periodically during the first 24 h and daily for 14 days. The weight of vital organs and the liver function marker were recorded. AFP treated rats were compared with control rats (n=5) which administered distilled water for once.

Statistical analysis

Statistical analyses were carried out using statistical package for social sciences (SPSS) version 20 software. All data were expressed as means ± standard error (SEM). Acute toxicity data were analyzed using Student's *t* test. Values of $P < 0.05$ were considered as statistically of mean

Results

Bioactive constituents of AFP and AFG

Qualitative chemical screening of both AFP and AFG revealed the presence of alkaloids, flavonoids, phenols, glycosides, terpenoids, saponins, sterol, and protein. Meanwhile, tannins, resins, and anthraquinone were not detected (Table 1).

Table 1: Qualitative chemical screening of AFP and AFG.

Test	AFP	AFG
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Glycosides	+	+
Carbohydrates	+	+
Terpenoids	+	+
Saponins	+	+
Steroids and sterols	+	+
Resins	-	-
Protein and amino acids	+	+
Tannins	-	-
Anthraquinone	-	-

+ indicates the presence of the constituent; -indicates the complete absence of the constituent.

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Amino acid contents of AFP and AFG

The amino acid profile of the AFP and AFG indicates the presence of both essential and non-essential amino acids (Table 2). AFP and AFG have higher concentrations of some essential amino acids such as lysine, leucine, valine, and phenylalanine.While, glycine, alanine, aspartic acid, and glutamic acid are the highest non-essential amino acids of AFP and AFG.

Proteinase inhibitory activity of AFP

AFP exhibited proteinase inhibitory activity at various concentrations. The maximum inhibition of AFP was 400 µg/ml, as 55.17% inhibition was recorded and the minimum was 50 µg/ml as 27.85% inhibition was observed at this concentration. Standard Aspirin has shown maximum inhibitory activity at 400 µg/ml with 51.72% inhibition and minimum at 50 µg/ml with 31.03% proteinase inhibition (Fig.1).

Table 2: Amino acid contents of AFP and AFG.

Essential amino acid	Amino acid concentration in AFP (mg/g)	Amino acid concentration in AFG (mg/g)	Non -essential amino acid	Amino acid concentration in AFP (mg/g)	Amino acid concentration in AFG (mg/g)
Threonine	2.79	3.34	Aspartic acid	11.76	13.2
Leucine	8.34	6.43	Serine	3.42	3.05
Lysine	270.79	280.88	Glutamic acid	10.58	10.95
Valine	6.94	5.90	Alanine	12.49	10.03
Methionine	0.66	--	Glycine	14.88	12.09
Isoleucine	4.41	2.21	Proline	0.07	0.07
Histidine	2.75	2.65	Tyrosine	4.05	2.75
Phenylalanine	6.37	5.84	--	--	--
Total essential amino acids content (mg/g)	303.05	319.34	**Total non-essential amino acids content (mg/g)**	57.25	52.14
Essential amino acids (%)	84.11	85.96	**Non-essential amino acids (%)**	15.89	14.04

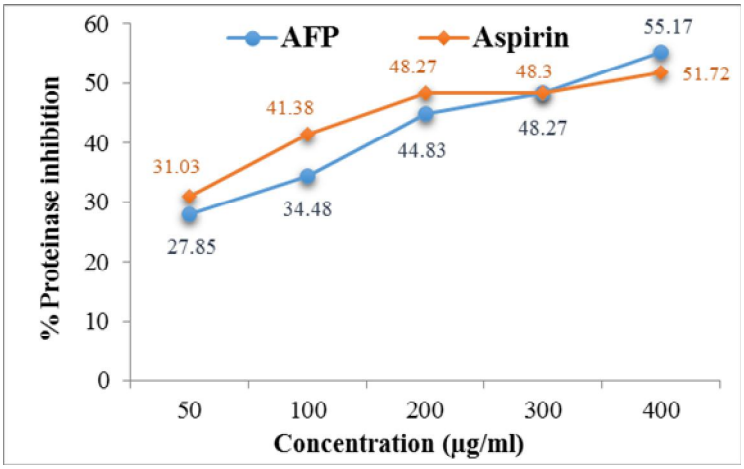

Figure 1: Effect of AFP on proteinase

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Anti-inflammatory activity of AFP

Figures 2&3 demonstrate that AFP stabilizes the HRBC membranes against lysis induced by heat effectively at different concentrations (50-400 µg/ml). Since, it able to resist hemolysis by 77.36% at highest concentration (400 µg/ml), as compared with Aspirin which prevent lysis by 88.68% at the same concentration.

Oxidant inhibition potency of AFP

Table 3 and figure 4 illustrate that AFP acts as oxidant inhibitor. As it possesses (1) the ability to scavenge DPPH with high percentage (98.02%) approximately as ascorbic acid which scavenge DPPH by 99.69% at 80 mg/ml. (2) TAC was 478.60 at 10 mg/ml AFP. TAC of the phosphomolybdenum method verified that AFP has both water-soluble and fat-soluble antioxidant capacity. These findings revealed that the AFP contains antioxidants compounds that able to reduce the oxidant agent effectively.

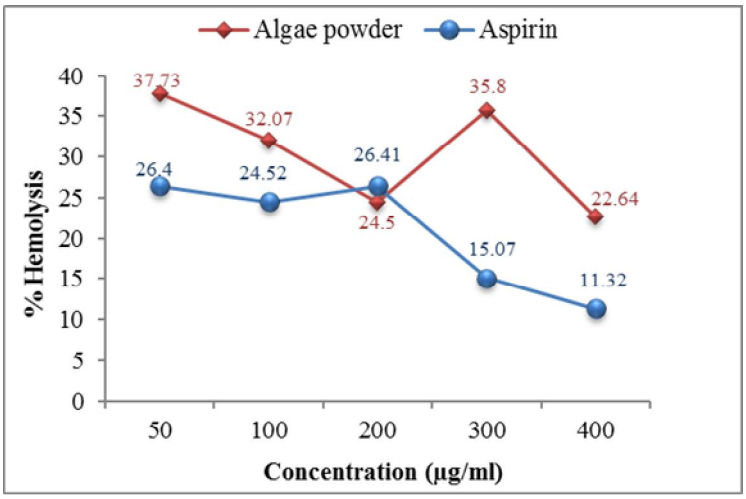

Figure 2: Effect of AFP on HRBC membrane hemolysis.

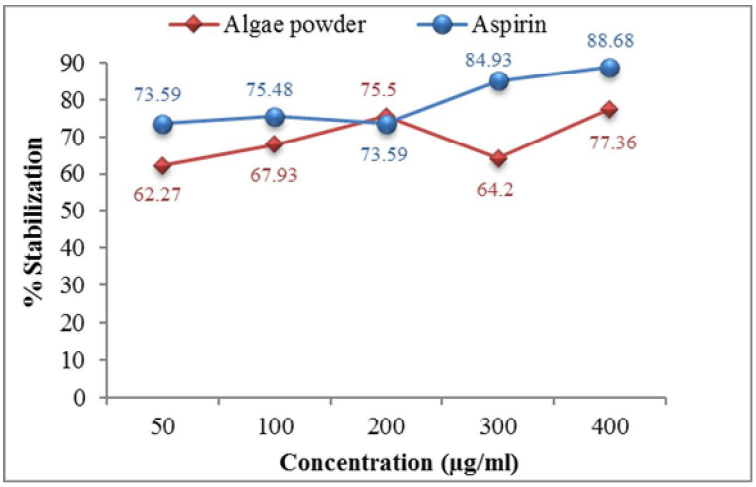

Figure 3: Effect of AFP on HRBC membrane stabilization.

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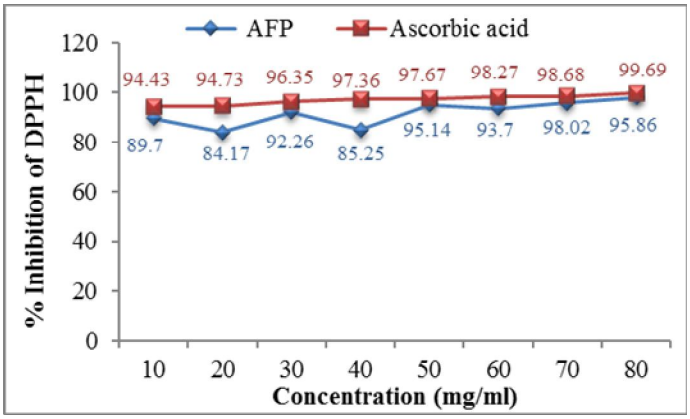

Figure 4: DPPH free radical scavenging assay of AFP.

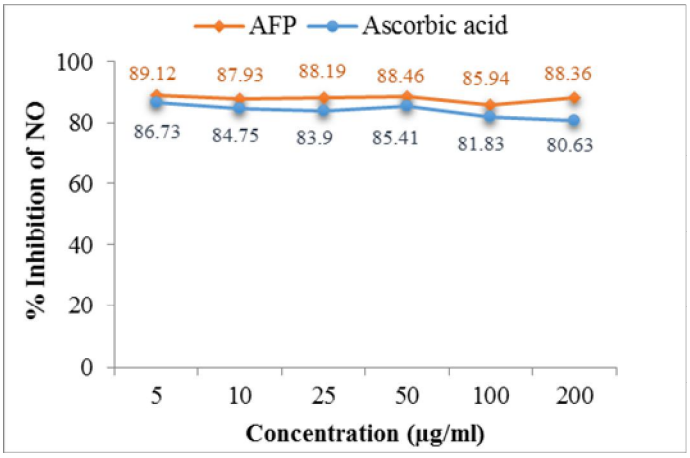

Figure 5: Nitric oxide radical scavenging ability of AFP.

Table 3: Total antioxidant capacity (TAC) in AFP. AAE: Ascorbic acid equivalent

	Sample	Total antioxidant capacity (mg AAE/g powder)
	AFP (10 mg/ml)	478.60

Nitric oxide radical scavenging ability of AFP

Figure 5 displays that AFP has a potent nitric oxide scavenging activity. Maximum inhibition of nitric oxide (NO) was recorded at 5 µg/ml of AFP. At this concentration, NO inhibition was found to be 89.12% regarding AFP in comparison with 86.73% of ascorbic acid. Again, the minimum NO % inhibition of AFP and ascorbic acid were 85.94% and 81.83%, respectively.

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***In vivo* study**
Acute toxicity study

No mortality or toxic symptoms were observed in either control or AFP-treated rats. Meanwhile, there were no significant changes in relative organs weight between control and AFP-treated rats (Table 4). It seems that liver, kidney, heart, lung and spleen were not adversely affected by AFP acute administration. Furthermore, no drastic differences in the liver function markers (GGT, ALAT, and ASAT) were recorded between AFP and control rats (Table 5). The present results indicate the safety of AFP, since no observable adverse effect level (NOAEL) at dosage of 2000 mg/kg body weight was noticed. Therefore, it seems that the LD_{50} dose of the AFP is higher than this dose.

Table 4: Effect of AFP on relative organ-to-body weight index (%) of control and experimental rats.

Organ	Organ body weight ratio (g/100 g)	
	Control	AFP
Liver	3.45 ± 0.090	3.68 ± 0.130
Kidney	0.68 ± 0.015	0.70 ± 0.018
Heart	0.43 ± 0.042	0.45 ± 0.016
Lung	0.75 ± 0.075	0.67 ± 0.026
Spleen	0.44 ± 0.037	0.52 ± 0.046

Values are mean ± SEM (n = 5).

Table 5: Effect of AFP on GGT, ALAT, and ASAT in rats.

Parameters / Groups	GGT (U/L)	ALAT (U/mL)	ASAT (U/mL)
Control	3.28 ± 0.18	31.91 ± 0.69	104.05 ± 3.25
AFP	3.47 ± 0.24	37.08 ± 1.11*	116.00 ± 4.44

Values are mean ± SEM (n = 5). * refers to significant change ($P<0.05$).

Discussion

Osteoarthritis (OA) is a degenerative joint disease; it causes discomfort due to inflamed joints (Patel et al., 2015). OA resulted in destruction of articular cartilage which ends with impaired joint motion and disability (Arjmandi et al., 2014). Many causative aspects contributed in OA development; some of them are inflammation, proteinases and reactive oxygen or nitrogen species (ROS/RNS) generation which ultimately led to synovitis (Berenbaum, 2013; Narayani et al., 2014; Ferrándiz et al., 2015; Jeong et al., 2015). Most commonly prescribed pharmaceuticals modalities used for treating OA are symptomatic; however, it is not block or reverse the cartilage and joint destructions besides their side effects (Akhtar and Haqqi, 2012).

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Regarding OA-associated inflammation, the present study showed the potency of the AFP and AFG to resist the HRBC lysis even in small concentrations as compared to the standard Aspirin drug. Since, the RBC membrane is analogous to the lysosomal membrane; AFP or AFG can be considered as membrane stabilizer and consequently has anti-inflammatory action. Therefore, the stabilization of lysosomal membrane (RBC analogous herein) limit the inflammatory response by preventing the secretion of lysosomal components which cause tissue damage (Rajendran and Lakshmi, 2008). Further, the present results exhibited that AFP or AFG markedly inhibited the trypsin enzyme like the standard Aspirin drug which affirms the ability of AFP to inhibit proteinases. Patel and Zaveri (2015) clarified that the inhibition of trypsin represents the ability of test drugs to confer significant defense against cartilage damage during arthritic reactions. Thus, even if the lysosomal leakage is occurred for any reason; the AFP or AFG can protect the area of leakage from further damage by its proteinase inhibitor activity. Chou (1997) clarified that the lysosomal constituents of neutrophils is the storage house of proteinases which cause further tissue inflammation and damage when released. Several types of proteinases contributed in arthritic reactions by degradation of proteoglycan of the cartilage (Narayani et al., 2014). As the AFP markedly inhibited the proteinase enzyme; the present result can consider AFP as anti-osteoarthritic agent. Again, the current study revealed that AFP or AFG may have the similar effect *in vivo* and can stabilize the lysosomal membranes and inhibiting the release of lysosomal content of neutrophils at the inflammatory area.

Regarding the relationship between OA and ROS, the present study revealed that AFP scavenge DPPH and has total antioxidant capacity. Since, AFP can reduce Mo (VI) to Mo (V) which means that the AFP contains antioxidant compounds that reduce the oxidants in the reaction mixture. Additionally, NO or RNS contributed to the OA development (Davies et al., 2008). Nitrite and other very reactive species formed during the reaction of NO with oxygen or with superoxides. These reactive substances alter the structure and function of many cellular constituents (Parul et al., 2012). Incubation of sodium nitroprusside in phosphate buffer saline resulted in nitrite production, which is reduced by the AFP. This may be due to the antioxidant activity of AFP which enables it to compete with oxygen in order to react with NO thereby inhibiting the generation of nitrite. Thus, AFP or AFG can be considered as oxidant inhibitor or potent scavenger as it can scavenge DPPH and NO free radicals in addition to its TAC content.

The phenolic compounds, flavonoids and other bioactive constituents of AFP or AFG able to neutralize free radicle, mitigate inflammation and inhibit proteinase as Zia et al. (2014) recorded generally. Also, the current study claimed that the antioxidant potency of AFP or AFG back to its sulphated polysaccharides (Usov, 2011). Zhang et al. (2004) disclosed that algal polysaccharides are free radical scavengers *in vitro* and act as antioxidants for the prevention of oxidative damage in living organisms. Furthermore, the antioxidant amino acids (cysteine, glutamic acid, glycine and methionine) of the AFP and AFG may add the potency of their antioxidant activities besides their phenolic compounds (Kerksick and Willoughby, 2005). Additionally, the current study attributes the anti-inflammatory activity of the AFP or AFG to its histidine content. As Farshid et al. (2010) revealed that histidine is a potent anti-inflammatory compound that can stabilize lysosomal membrane (RBC membrane analogous herein) effectively. Thus, the ability of AFP or AFG to inhibit inflammation, proteinases, and oxidants may enable them to preserve articular cartilage and alleviate the osteoarthritis. Cheeke et al. (2006) and Miller et al. (2011) added that saponin and leucine which considered bioactive constituents presented in AFP and AFG also have the potency to diminsh inflammation/proteinases/oxidants.

Jothy et al. (2011) reported that one of the false believes that natural products are safe just because they are naturally in origin. Therefore, evaluation of the toxic is the so called of AFP or AFG is crucial particularly after its candidacy as anti-osteoarthritic agent. Generally, change of relative organ weights is a fundamental index reflects the toxicity of tested substance and determines whether the organ was exposed to the injury or not (Carol, 1995). Dybing et al. (2002) disclosed that the heart, liver, kidney, spleen, and lungs are the primary organs influenced by toxicant. The present study recorded that AFP or AFG did not show any adverse effect on weight of these vital organs. Moreover, the present study assesses some liver function markers as liver is the chief target organ of acute toxicity (Rhiouania et al., 2008). The existing study revealed that AFP did not cause any functional change to the hepatocytes, as no obvious difference was observed between AFP and control rats. Hence, the present study suggested that AFP and AFG are virtually nontoxic and can be utilized for pharmaceutical products.

Conclusion

From the aforementioned findings, the present study suggests that the AFP or AFG may be a promising candidate as chondroprotective agent and may be used as anti-osteoarthritic agent in the future. The potency of AFP and AFG to be inflammatory-proteinase-oxidant inhibitor may be due to the cumulative synergistic relationship among its bioactive constituents. This study postulates that these bioactive compounds may prevent OA initiation, or arrest its progression, or enhance the repair of the joint cartilage. However, further studies on the *in vivo* anti-osteoarthritic effect of AFP and AFG are needed.

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