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Original Research

Inhibitory effects of various solvent extracts from *Rhamnus frangula* leaves on some inflammatory and metabolic enzymes

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Abstract: Many enzymes are involved in numerous pathologies which are related to metabolic reactions and inflammatory diseases such as pancreatic lipase, α-amylase, α-glucosidase and xanthine oxidase and secreted phospholipases A_2 (Group IIA, V and X), respectively. Therefore, inhibiting these enzymes offer the potential to block production of more inflammatory substances and decrease the risk factors for cardiovascular diseases. The purpose of this study was to investigate some potent, bioavailable and selective inhibitors of some catalytic proteins implicated to metabolic syndrome and their antioxidant effects from various solvent extracts of *R. frangula* leaves. The anti-inflammatory, obesity, diabete and XO potentials were evaluated through analyses of inhibition activities of corresponding metabolites.The water extract exhibited an important inhibitory effect on human, dromedary and stingray $sPLA_2$-G IIA achieved an IC_{50} of 0.16±0.06, 0.19±0.05 and 0.07±0.01 mg/mL, respectively. Likewise, the same fraction demonstrated the highest pancreatic lipase inhibitory activity using two different substrates. Indeed, 50% of dromedary pancreatic lipase inhibition was demonstrated for 5 min and 15 min using olive oil and TC4 substrates, respectively. Besides, it was established that methanolic extract had more effective inhibitory lipase activity than ORLISTAT used as a specific inhibitor of gastric, pancreatic and carboxyl ester lipase for treating obesity, with an IC_{50} of 5.51±0.27 and 91.46±2.3 µg/mL, respectively. In the case of α-amylase, α-glucosidase and xanthine oxidase, the crude methanolic extract showed a potential inhibitory effect with an IC_{50} of 45±3.45, 3±0.15 and 27±1.71 µg/mL, respectively. Conclusively, *R. frangula* leaves extracts showed a potential value of some sPLA2, some metabolic enzymes and XO inhibitors as anti-inflammatory and metabolic syndrome drugs.

Key words: *R. frangula* leaves; Metabolic syndrome; $sPLA_2$; Pancreatic lipase; α-amylase, α-glucosidase; Xanthine oxidase; Inhibition.

Introduction

It is well known for many years that secreted phospholipases A_2 ($sPLA_2$) are detected in inflammatory cells and biological fluids (GIIA, GIID, GIIE, GIIF, GV, GX, GXIIA and GXIIB) (1-2), for example, in the synovial fluid of patients struggling from arthritis, especially serum and rheumatoid of septic patients (3-4). Indeed, it is generally accepted that some $sPLA_2$, by mobilizing free fatty acids, participate in inflammatory reactions, thus, initiating the metabolic cascade leading to the biosynthesis of lipid mediators like prostaglandins and leukotriens (5-6). Present anti-inflammatory therapies include the non-steroidal anti-inflammatory drugs that inhibit the cyclooxygenase ½ (COX-1/2) enzymes, thus preventing conversion of arachidonic acid to prostaglandins. However, these drugs have some limitations (7-9).

On the other hand, the predominance of metabolic syndrome (MS) is a serious health threat inflicting enormous economic burdens on society, due to increasing absorption of fat and carbohydrate. Unfortunately, many treatment strategies for many diseases related to metabolic reactions lead to adverse effects. For this reason, many studies were interested to search another therapies with an effective drugs without undesirable side effects. It has been demonstrated that many enzymes are implicated in MS leading to some cardiovascular diseases such as obesity, hypertension, hyperglycemia, hypertriglyceridemia and hypercholesterolemia (10-11). These metabolic abnormalities are related to some lipolytic and metabolic enzymes such as pancreatic lipase, α-glucosidase, α-amylase and xanthine oxidase (XO) (12). Indeed, pancreatic lipase is involved in important health problem of obesity via fat absorption. Some studies have discovered therapies inhibiting lipase activity like ORLISTAT (hydrogenated derivative of lipstatin obtained from *streptomyces toxitricini*) which plays an important role for obesity by blocking gastric and pancreatic lipases activities (13-17). Moreover, α-gucosidase and α-amylase act on hydrolysis of oligosaccharides and disaccharides producing easy absorption monosaccharides such as glucose. For the above mentioned, delaying absorption of glucose by inhibition of enzymatic hydrolysis of carbohydrates, could be another form of combating diabetes (18-19). Unfortunately, the most chemical drugs have undesirable effects like increasing risk of cardiovascular disease by using proliferator-activated receptor (PPAR)-gamma activators like therapy (10-11,20). Whereas, XO plays an important role in leading to uric acid and superoxidase radicals' generation inducing to the oxidative stress *in vivo*. Thus, it was well investigated in several studies that oxidative stress represents a crucial role in serious diseases such as cancer and others. Therefore, in previous studies, it was mentioned that XO inhibitors could

be benefic for treating hepatic diseases, gout, type 2 diabetes and others pathological processes related to these metabolites (21-23).

For this reason, searching a natural drug without undesirable side effects should be our urgent purpose. Thus, the aim of the present study was to evaluate the inhibitory effect on some pro-inflamatory $sPLA_2$, pancreatic lipase, α-amylase, α-glucosidase and XO activities of different solvents extracts from *R. frangula* leaves. This medicinal plant is considered as an important health component producing a variety of useful bioactive products. In fact, the leaves, bark and also fruit of several species of *R. frangula* were used as laxatives and also allowing an antifungal activity (24).

Materials and Methods

Enzymes

Group IIA, V, X, and XIIA $sPLA_2$ (EC 3.1.1.4) were prepared as described previously by Singer et al. (25). DrPL, dromedary and stingray group IB, IIA, and V $sPLA_2$ were purified as previously reported (26-30). α-glucosidase (EC 3.2.1.20), salivary amylase (EC 3.2.1.1) and XO (EC 1.17.3.2) were bought from Sigma Chemical Company (St. Louis, USA).

Extraction method

The plant tissue were collected in 2016 from the Riyadh region in Saudi Arabia, identified and vouchered by Dr. Mona suliman alwahibi at the Botany and Microbiology Department, College of Science, King saud university- Saudi Arabia. Then, it was washed in distilled water and air-dried at room temperature. Thus, from this vegetable powder, we dissolved 200 g with 100 mL of ethanol by homogenization at room temperature for 72 h and the homogenate was filtered through a Buchner funnel. After centrifugation (15 min at 10,000 rpm and at 4 ºC), the supernatant was lyophilized yielding the ethanolic extract which was then resuspended in water and partitioned successively with diverse solvents with different polarities (butanol, chloroform, ethyl acetate, and methanol). All resulting fractions including the remaining solution which is designated "water extract" were stored at 4 ºC before analysis.

Inhibition of $sPLA_2$ activity

According to the method reported by De Aranjo and Radvany (31), the inhibitory effect of various extracts was assayed using several $sPLA_2$s: hG- IIA, hG-V, hG-X, hG-XIIA, DrG-IIA, SG-IIA (0.02 µg/µL), DrG-IB and SG-IB (0.002 µg/µL) $sPLA_2$. Ten microliters of each extract solutions was mixed with 10 µL of these $sPLA_2$ and the obtained mixture was incubated for 20 min at room temperature. Finally, 1 mL of the PLA_2 substrate was added (3.5 mM lecithin solubilized in 100 mM NaCl, 3 mM sodium taurodeoxycholate (NaTDC), 10 mM $CaCl_2$, and 0.055 mM red phenol, pH 7.6). The hydrolysis reactions were followed spectrophotometrically at 558 nm for 5 min using a BIBBY, Anadéo RS232.UV–vis spectrophotometer. The inhibition percentage was calculated by comparison with a control experiment (absence of extract). The IC_{50} values were determined from the curve.

Inhibition of DrPL activity

The inhibitory activities of a positive control (ORLISTAT) and the methanolic extract of *R. frangula* against pancreatic lipase were investigated at different concentrations ranging from 5 to 200 µg/ml. DrPL was preincubated with the extract in order to measure the inhibitory effect of *R. frangula* extract. The assay contained 50 µL of the enzyme (12 U) and 10 µL of the extract mixed in the presence or in the absence of 4 mM NaTDC. Aliquots of 10 µL from the sample were used to assess the residual lipase activity, as indicated above. Inhibition of lipase activity was also expressed as the percentage decrease in the activity when pancreatic lipase was incubated with the test extracts. ORLISTAT was obtained commercially with the trade name Xenical (Hoffmann-La Roche). Besides, the lipase activity was measured, as previously described (32), on tributyrin (TC4) using a pH-stat at 37°C. One lipase unit (IU) was defined as 1 µmol of fatty acid titrated per minute. Lipase activity was measured on olive oil at 37°C (33).

α-amylase inhibitory activity in vitro

According to Subranian *et al*. (34), 10 µL of α-amylase (3,3 U), was preincubated with 10 µL of *R frangula* methanolic extract (5 to 160 µg/mL), methanol, or positive control (Acarbose) at 37°C for 5 min. The first reaction was measured at 620 nm after adding 180 µL of the amylase substrate (Labtest) and incubated for 8 min. Then, the second reaction was measured again after more incubation (5 min at 37°C). The reagent of the α-amylase (Labtest) was diluted in distilled water (1:1) before being added to the microplate (Bio-Tek ELX-800, USA). Acarbose was used as a positive control at various concentrations ranging from 1 to 10 mg/mL. The α-amylase inhibition was calculated using the equation: % inhibition = 100 – (A2 sample – A1 sample/ A2 control – A1 control)* 100 where A1 is the absorbance of the initial reading and A2 is the absorbance of the final reading.

α-glucosidase inhibitory activity

The test of α-glucosidase inhibitory activity was determined by the release of 4-nitrophenol α-D-glucopyranoside 4 NPGP. 180 µL of the α-glucosidase were preincubated with 20 µL of *R. frangula* methanolic extract added (5 to 160 µg/mL), methanol, or positive control (Acarbose), for 2 min at 37°C. Then, samples were incubated for more than 15 min at 37°C after the addition of 150 µL of the color reagent NPGP. The colorimetric test contained 10 mM of potassium phosphate buffer, pH 6.9, 5 mM of 4-NPGP, and 2U of α-glucosidase. Acarbose also was used as a positive control at various concentrations ranging from 0.5 to 5 mg/mL. The reading assay was performed by using a microplate reader (Bio-Tek ELX-800, USA) at 405 nm. The α-glucosidase inhibition was calculated using the equation: % inhibition = 100 – (A2 sample – A1 sample/ A2 control – A1 control)* 100 where A1 is the absorbance of the initial reading and A2 is the absorbance of the final reading, control is the absorbance of the assay with methanol (35).

XO inhibitory activity

According to Bondet *et al*. (36), XO activity was de-

termined by measuring the formation of uric acid from xanthine. The reagent 1 was composed by mixture of XO (667 mM), EDTA (0.1 mM), and hidroxilamine (0.2 mM) in 50 mM phosphate buffer solution (pH 7.5). In to each microplate well, 15 µL of extract (5 to 150 µg/mL), methanol, or control drug (allopurinol (0.25-3 µg/mL)) and 40 µL of XO were added and preincubated for 5 min at 37°C. After that, 95 µL from reagent 1 were added in to each microplate well and incubated at 37°C for 30 min. The absorbance was performed using a microplate reader (Bio-Tek ELX-800, USA) at 295 nm. Then, 150 µL of uric acid reagent was added in the mixture and the absorbance was measured again. Methanol was used as negative control and allopurinol as positive control. The XO inhibition was calculated using the following equation: % inhibition = 100 – (A2 sample – A1 sample/ A2 control – A1 control)* 100 where A1 is the absorbance of the initial reading and A2 is the absorbance of the final reading.

Statistical analysis

Data are presented as the mean value ± SD of at least three replicates for each sample and all

statistical comparisons were performed using independent Ttest by Microsoft Excel software. p values < 0.05 were considered to be significant.

Results

Evaluation of PLA_2 inhibitory effect

Preliminary experiments searching for the PLA2 inhibitory activity of various extracts were performed in order to evaluate the potential anti-inflammatory effect of *R. frangula* leaves, using human $sPLA_2$ IIA, V, X and XIIA groups, dromedary and stingray $sPLA_2$ IB, IIA and V groups.

The main objective of these experiments was to find an extract that was able to selectively inhibit the pro-inflammatory $sPLA_2$-IIA, V, X and XIIA with no or minimal inhibitory effects on the digestive $sPLA_2$-IB. Figure 1 showed that out of the 6 extracts screened, four extracts (ethanol, methanol, ethyl-acetate and water) showed significant inhibitory activities (Figure 1). The water extract showed the most promising potency in inhibiting the catalytic activity of human, dromedary and stingray pro-inflammatory $sPLA_2$-IIA, V, X and XIIA. The obtained data, as shown in Figure 1, clearly indicate that water extract effectively suppressed $sPLA_2$-IIA of these three species (human, dromedary and stingray) with an IC_{50} of 0.16±0.06, 0.19±0.05 and 0.07±0.01mg/mL, respectively. The ability of this biological extract to inhibit both mammalian and no mammalian $sPLA_2$ was demonstrated by the effectiveness of water extract at inhibiting PLA_2-GIIA and V of stingray. Similar results were obtained using human pro-inflammatory PLA_2 of group IIA, V, X and XIIA mediated conversion of arachidonic acid to prostaglandins, leukotrienes, and others with an IC_{50} of 0.16±0.06, 0.36±0.09, 0.25±0.25 and 0.1±0.1 mg/mL, respectively (Figure 1). These results suggested that water extract of *R. frangula* leaves may be used *in vivo* to suppress PLA_2 GIIA, V, X and XIIA inflammatory activities in the correspondent tissue that surexpressed these PLA_2. On the other hand, no inhibition of the dromedary and stingray $sPLA_2$-IB activity was recorded for the same extract, even at higher concentrations more than 5 mg/mL, which indicates a selective inhibition of the water extract against these two subgroups of $sPLA_2$ (digestive and inflammatory subgroups).

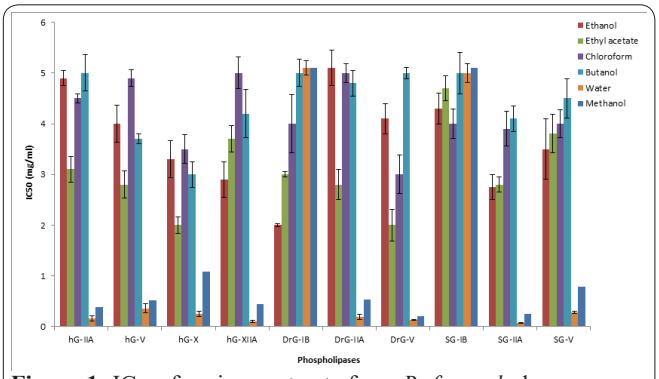

Figure 1. IC_{50} of various extracts from *R. frangula* leaves measured during the inhibition of hGIIA, hGV, hGX, and hGXIIA, DrIIA, SGIIA, DrGIB and SGIB $sPLA_2$. Experiments were performed in triplicate and are reported as the mean ± standard deviation (p< 0.05).

Effect of R. frangula extract on lipase inhibition

Obesity is one of the most provoking health burdens in the developed countries. One of the strategies to prevent obesity is the inhibition of pancreatic lipase enzyme. Indeed, it was necessary to search the suitable extract that should have the most potent activity to inhibit some enzymes which were involved in MS such as pancreatic lipase. As shown in Table 1, out of the 6 extracts screened, four extracts (ethanol, methanol, ethyl-acetate and water) showed significant results (Table 1). The water and methanolic extract exhibited the most promising results in inhibiting the catalytic activity of DrPL with 65±4.8, 90±1.7 % of inhibitory activity, re-

Table 1. Lipase, α-amylase, α-glucosidase, and XO inhibitory effects of different extracts from *R. Frangula* leaves (100 µg/mL) . Experiments were performed in triplicate and were reported as the mean ± standard deviation (p < 0.05).

	Inhibitory Activity (%)			
Enzyme / Extracts	Lipase	α-Amylase	α-Glucosidase	xanthine oxidase
Butanol	12±1.5	5±0.2	16±1	11±3.5
Chloroform	7±0.8	12±1.3	25±2.1	9±0.8
Ethanol	45±2.7	65±3.1	51±0.7	39±4.2
Ethyl acetate	38.5±1.2	65±4.6	60±1.7	58±3.8
Methanol	90±1.7	90±3.8	89±2.1	90.5±4.3
Water	65±4.8	75±7.6	79±4.1	58±3.7

Table 2: IC_{50} of the methanolic extract from *R. frangula* leaves measured during the inhibition of lipase, α-amylase, α-glucosidase, and XO. IC_{50} values were calculated from the curves (Figures 2, 4, 5 and 6). Allopurinol, ORLISTAT , and Acarbose were used as the standards. Experiments were performed in triplicate and are reported as the mean ± standard deviation ($p< 0.05$).

	IC_{50} (µg/mL)			
	Lipase	**α-Amylase**	**α-Glucosidase**	**xanthine oxidase**
Methanol	5.51±0.27	45.86±3.45	3.09±0.15	27.49±1.71
ORLISTAT	91.46±2.3	-	-	-
Acarbose	-	4989.5±213	2578±243.4	-
Allopurinol	-	-	-	0.53 ± 0.04

spectively (Table1). Hence, methanol extract was used for evaluating its effect on DrPL and ORLISTAT, as a positive control against pancreatic lipase (13,15) (Figure 2). As shown in Figure 2 and Table 2, methanolic extract had an inhibitory effect on DrPL with an IC_{50} 5.51±0.27 µg/mL while ORLISTAT, which was used as a specific inhibitor of gastric, pancreatic and carboxyl ester lipase for treating obesity (13,15), showed a lower level of inhibitory effect on DrPL in comparison with methanolic extract of *R. frangula* against this pancreatic lipase with an IC_{50} =91.46±2.3 µg/mL. Likewise, methanolic extract was also followed for inhibiting DrPL using TC4 or olive oil as substrate (Figure 3A). Depending on the substrate, the inhibition of DrPL activity started before 5 min of incubation with TC4 or olive oil. Fifty percent of DrPL inhibition was demonstrated at 5 min using olive oil and after 15 min using TC4 as substrates. Besides, total inhibition was observed after 40 min and 45 min in the case of olive oil and TC4, respectively (Figure 3A).

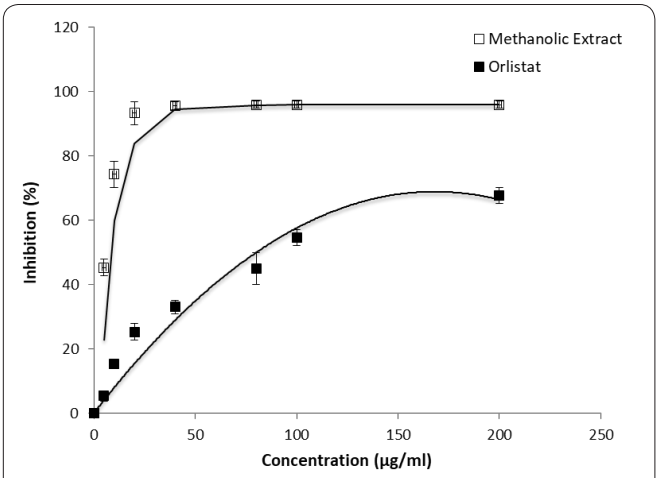

Figure 2. Dose-dependent inhibition of pancreatic lipase activity of ORLISTAT and methanolic extract of *R. Frangula* leaves. Values represent mean ± SD of triplicate measurements ($p< 0.05$).

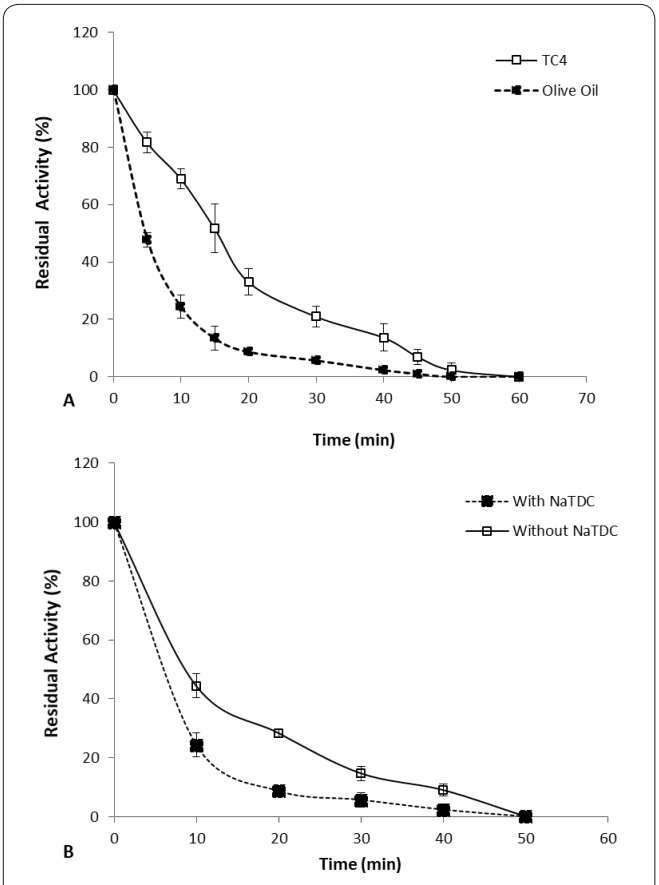

Figure 3. The inhibitory effect of *R. frangula* methanolic extract on DrPL on TC4 or olive oil **(A)** and in the presence or absence of NaTDC **(B)** ($p< 0.05$).

Effect of NaTDC on lipase inhibition

Under the same physiological conditions, we have explored the importance of bile salts NaTDC on inhibitory effect of methanol extract of *R. frangula*. Indeed, olive oil emulsion was incubated with DrPL and, with a supramicellar concentration (4 mM) of NaTDC in the presence of methanol extract. Figure 3 B has shown that residual activity of DrPL in the presence of NaTDC was lower compared to the data without NaTDC. Moreover, the total inhibition was reached after 40 min (with or without NaTDC). A similar effect of NaTDC was shown using TC4 emulsion as substrate (Figure 3 B). Therefore, the inhibitory effect of extract on lipase activity is accelerated by bile salts presence.

α-amylase, α-glucosidase and XO inhibitory activity

One of the effective managements of MS is to retard the absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes in the digestives organs and also blocking XO activity. Thus, our purpose was to discover a new plant-based medicine that could modulate physiological effects in the inhibition of α-glucosidase, α-amylase and XO. Table 1 showed, such as the case of lipase, four of screened extracts (ethanol, methanol, ethyl-acetate and water) displayed a significant inhibitory effect (Table 1). Interestingly, methanolic extract was the most promising results in inhibiting the α-amylase activity with 90±3.8 % inhibitory activity. While, the water and methanolic extracts have shown comparable significant results in the case of α-glucosidase with 89±2.1 and 79±4.1 % inhibitory activity, respectively. Moreover, methanolic extract reduce XO activity by 90.5±4.3 of inhibition (Table 1). Hence, methanolic extract was selected for further analysis.

As shown in Figure 4 and Table 2, methanolic ex-

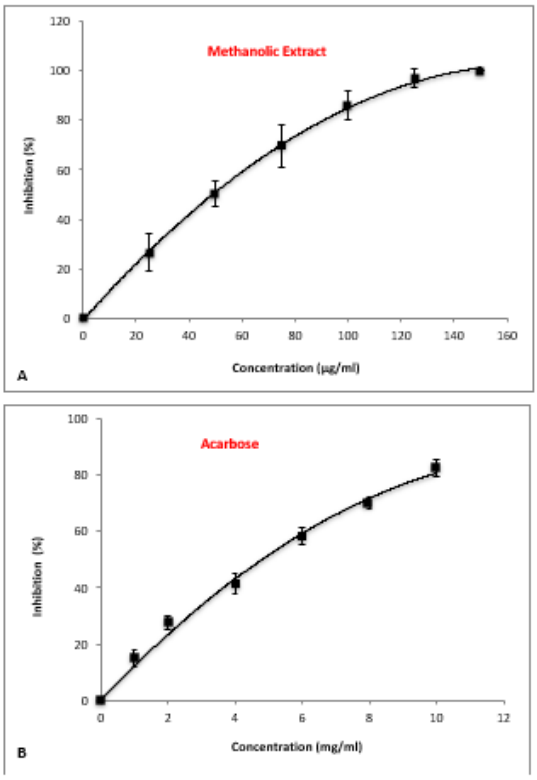

Figure 4. Inhibition of α-amylase by methanolic extract of *R. frangula* leaves **(A)** and Acarbose **(B)**. Data are expressed as inhibition percentage (%) values calculated by comparison with a control experiment (absence of extract). Values represent mean ± SD of triplicate measurements ($p< 0.05$).

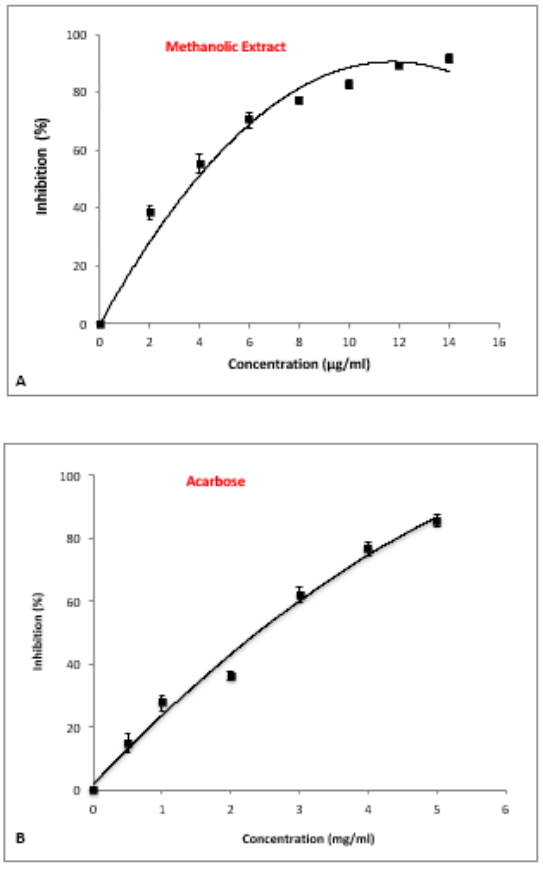

Figure 5. Inhibition of α-glucosidase by methanolic extract of *R. frangula* leaves **(A)** and Acarbose **(B)**. Data are expressed as inhibition percentage (%) values calculated by comparison with a control experiment (absence of extract). Values represent mean ± SD of triplicate measurements ($p< 0.05$).

tract of *R. frangula* leaves exerted an obvious inhibitory effect on α-amylase, with an IC_{50} value of 45.86 ± 3.45 µg/mL (Figure 4 A, Table 2). This value was better than quercetin assay, which was used as a positive control drug with an IC_{50} of 120 ± 9.91 µg/mL (Figure 4 B, Table 2). It was established that *R. frangula* leaves extract was able to inhibit α-glucosidase using pNPG as the reaction substrate with markedly higher values recorded for Acarbose (3.09 ± 0.15 and 4989.5±213 µg/mL, respectively) (Figure 5 A and B, Table 2).

Moreover, this inhibitory activity was higher than that of α-amylase. Notably, obtained results clearly showed that methanolic extract of *R. frangula* leaves significantly inhibited α-glucosidase and α-amylase activities in a concentration-dependent manner (IC_{50} values of 3.74± 0.08 µg/mL and 2578± 243.4 µg/mL), respectively, (Figures 4 A and 5 A, Table 2). However, as shown in Figure 6, methanolic extract of *R. frangula* leaves established a lower activity of XO enzyme compared to allopurinol with an IC_{50} of 27.49 ±1.71 µg/mL and 0.53 ± 0.04 µg/mL, respectively (Figures 6A and B, Table 2).

Discussion

Natural metabolic and inflammatory enzymes inhibitors from plant sources offer an attractive therapeutic approach to the treatment of numerous diseases by decreasing or blocking inflammatory diseases risk involving some $sPLA_2$ which were highly expressed. Powerful synthetic $sPLA_2$ inhibitors are not sufficiently

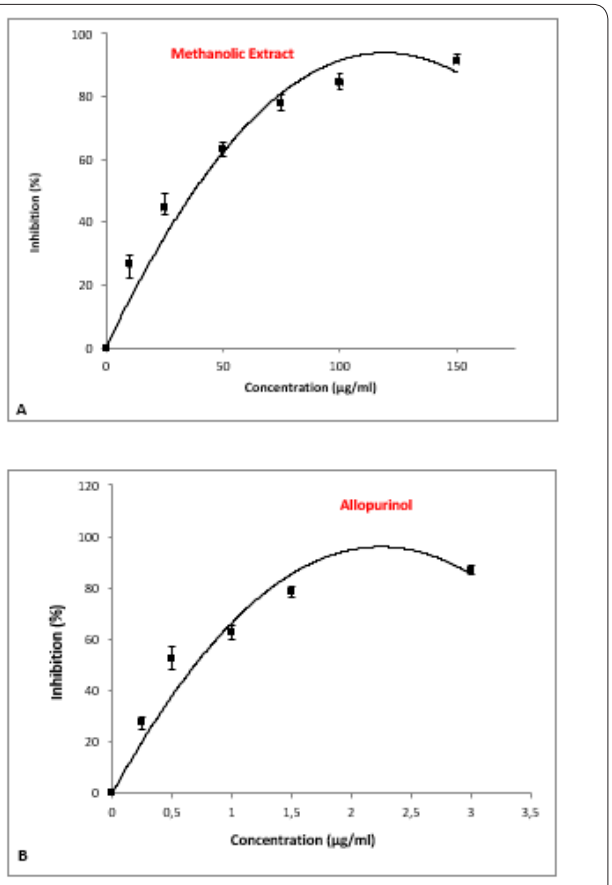

Figure 6. Inhibition of XO by methanolic extract of *R. frangula* leaves **(A)** and allopurinol **(B)**. Data are expressed as inhibition percentage (%) values calculated by comparison with a control experiment (absence of extract). Values represent mean ± SD of triplicate measurements ($p< 0.05$).

effective used as therapy in studies with mammalian cells or animals because addition of micromolar and important concentration of agents to cells often leads to off-target effects. Furthermore, synthetic metabolic enzymes inhibitors are also available, but could cause various disorders and numerous negative gastrointestinal symptoms at high doses. Some studies have shown that $sPLA_2$-IB is present at high levels in pancreatic juice that enters the gastrointestinal tract (2,37). Also, mice lacking in the $sPLA_2$-IB induced by feeding a diabetogenic high-fat/-carbohydrate diet, were resistant to obesity and diabetes (2,38-39). The obtained data, as shown in Figure 1, suggested that water extract of *R. frangula* leaves may be used *in vivo* to suppress PLA_2 GIIA, V, X and XIIA inflammatory activities in the correspondent tissue that surexpressed these PLA_2. On the other hand, no inhibition of the dromedary and stingray $sPLA_2$-IB activity was recorded for the same extract which indicates a selective inhibition of the water extract against these two subgroups of $sPLA_2$ (digestive and inflammatory subgroups).

The physiological role of these $sPLA_2$s was always thought to be related to numerous diseases. Importantly, there is a large amount of studies showing that human group IIA $sPLA_2$ was highly expressed during inflammation, for example, in the synovial fluid of patients suffering from arthritis (2,40-41). Indeed, group IIA, V and X $sPLA_2$s, exert several proatherogenic properties in the vessel wall and may act by generating proinflammatory lipid mediators, such as prostaglandins, thromboxanes leukotrienes, and lysophospholipids hydrolyzing low-density lipoprotein (LDL) particles and converting them into more proatherogenic particles (42-45). Specifically, $sPLA_2$-IIA and X have a potential role in atherosclerosis (46-47). These studies provided the impetus for the present study that explores the possibility of these $sPLA_2$s inhibition as a potential therapy to surpress or decrease the risk of inflammatory diseases.

Besides, methanolic extract was selected for further analysis as it was found to show the most promising results in inhibiting the α-amylase, α-glucosidase, and XO activities (Table 1). Interestingly, one can see from results presented in Figure 2 and Table 2 that methanolic extract had a better inhibitory effect on DrPL than ORLISTAT, which was used as a specific inhibitor of gastric, pancreatic and carboxyl ester lipase for treating obesity (13,15). It seems that *R. frangula* leaves may contain also lipase inhibitors which can be used as a treatment for obesity like found in many natural products in other studies (48-50). In fact, it was established that green tea extract (48) and soybean seeds proteins (49-50) were able to inhibit lipases. Similar study has shown that ethanol extract of Marine algae interestingly reduced human pancreatic and dog gastric lipase activity with 86±4, 70±3 % of inhibition, respectively (51). Further, recent research discovered that the fruit of *Cudrania tricuspidata* inhibited porcine pancreatic lipase activity (~ 60% of inhibition). It was investigated that the potent inhibitory activity of this fruit was due to its various chemical constituents of this fruit including their maturation stages (52). The variability of the inhibition rates of DrPL on the two substrates TC4 and olive oil, as shown in Figure 3A, is related to the fact that this enzyme hydrolyses more efficiently the shorter than the long chain triacylglycerols contrary to the other mammal pancreatic lipases (25). In fact, the catalytic effectiveness of DrPL is 11.8 higher than that of turkey pancreatic lipase using the TC4 as substrate. A previous study has shown that DrPL should have a structure allowing it to hydrolyse efficiently triacylglycerols at high interfacial energy without any denaturation and also tolerate the presence of long chain free fatty acid at olive oil/water interface (53). Indeed, the obtained data in Figure 3B, clealy demonstrated that the inhibitory effect of extract on lipase activity is accelerated by bile salts presence. In 2008, Ben Rebah et al. had suggested that added NaTDC could creat a mixed bile salts inhibitory compound interface where lipase may be adsorbed and inactivated as previously reported (51).

On the other hand, it is well known that α-glucosidase plays a central role in modulating postprandial hyperglycemia (18-19). A Previous study has reported the established α-glucosidase inhibitors and their effects on delaying the expeditious generation of blood glucose and food uptake (54-55). Notably, the obtained resultants, in Figures 4, 5 and Table 2, collaborated with some previous studies (56). Infact, it showed that methanolic extract of *R. frangula* leaves significantly inhibited α-glucosidase and α-amylase activities. Various studies have reported that some food and herbs have potential beneficial effects on diabetic glycemic control by inhibiting these enzymes without effectiveness (19, 57-58).

However, as shown in Figure 6, methanolic extract of *R. frangula* leaves established a lower activity of XO enzyme compared to allopurinol. In fact, it was well investigated in recent studies that curcumin derivatives targeted XO activity and also urate transporter. Thus, it seems be a potent agent anti-hyperuricemic and uricosuric activity *in vivo* (59).

To summarize, it was investigated the effectiveness of methanolic extract of *R. frangula* leaves to inhibit metabolic enzymes particularly α-glucosidase, α-amylase and XO which could be approved drug for modulating the homeostasis of MS. Others studies have shown the importance of flavones and coumarins screened out from an herbal medecines to inhibit XO activity and thus its cooperation in drug discovery (60).

Here, we report that leaves extract of *R. frangula* significantly showed a potential value of some $sPLA_2$, pancreatic lipase, α-glucosidase, α-amylase and XO inhibitors as anti-inflammatory and metabolic syndrome drugs and therefore may be a preferred alternative for modulation of physiological homeostasis. Indeed, water extract of *R. frangula* leaves possess potential anti-inflammatory effect methanolic fractions possess potential anti-obesity activity better than that of synthetic pancreatic and gastric lipase inhibitor (ORLISTAT). We also report that anti-diabetic activity has the ability to inhibit α-glucosidase and α-amylase and these were associated with extract antioxidant activity which showed a strong dose-dependent at the concentrations tested.

Availability of data and material

The datasets supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing inter-

ests

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Authors' contributions
ABB designed the study and revised the manuscript, IK carried out the research and wrote the first draft of the manuscript, RB performed the statistical analysis and revised the manuscript, MO assisted in the research work. All authors read and approved the final manuscript.

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