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In Vitro and *In Vivo* Antioxidant Properties of P-Coumaroyl Alphitolic Acid Extracted from Fruit of *Zizuphus mauritiana*

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ABSTRACT

Fruit of Zizuphus mauritiana has been commonly used in Traditional Medicine for its various pharmacological activities, such as its anticancer, antiepileptic, anti-inflammatory, anti-insomnia, neuroprotective effects and antioxidants. The aime of this study is to extract p-coumaroyl alphitolic acid compound present in fruit of Z. Mauritiana and to evaluate the antioxidant potential both in In-vitro and In-vivo effects. In-vitro antioxydant activities were assessed by DPPH, ABTS and FRAP methods. In-vivo antioxydant effects were evaluated in rats for 14 days, where different concentrations of p-coumaroyl alphitolic acid extracts (ACAE) (50, 100, 150, and 200 mg/kg/day) were orally administrated daily. Antioxidant profiles were performed by measuring malondialdehyde (MDA), blood proteins, reduced glutathione (GSH), glutathione peroxydase (GPX), catalase (CAT) and superoxide dismutase (SOD) activities in red blood cells. ACAE revealed excellent free radical scavenging activity (IC50) with DPPH (7.29 ± 0.94 µg/ml), ABTS (2.84 ± 0.07 µg/ml) and FRAP (28.32 ± 0.66 µg/ml) assay. Furthermore, CMAE decreased the level of MDA and SOD, and increased levels of GSH, GPX and CAT in blood. The results showed good antioxidant properties of ACAE of Zizuphus mauritiana fruit. Base on this results Jujube might be useful for the treatment of oxidative-stress related diseases such as chronic deseases.

Keywords

Zizuphus mauritiana, *p*-coumaroyl alphitolic acid, Antioxidant properties, Chronic deseases.

Introduction

Ziziphus mauritiana Lam. Wild occur on nearly every continent [1]. *Z. mauritiana* has very nutritious fruits and are usually eaten fresh. Plant materials are cheap and significantly contribute to the improvement of human health in terms of cure and prevention of diseases. This has been useful as food and medicine and a few have been studied especially African medicinal plants. They contain vitamins needed by human body for healthy living [2]. Fruit (Jujube) is widely recognized for its nutritional and nutraceutical values, making it an exceptional source of biologically active compounds. For centuries, dried jujube fruits have been utilized as food, food additives, and flavorings, owing to their remarkable nutritional value [1]. Jujube fruits are frequently processed into various forms such as paste, puree, syrup, and confections, which are consumed for their potential benefits in digestion improvement and overall health maintenance [3,4]. The fruits are applied on cuts and ulcers. They are also used to treat pulmonary ailments and fevers and to promote the healing of fresh wounds, for dysentery [5]. The fruit is abundant in numerous phytochemicals including phenolics, triterpenoids, alkaloids and sterols [6]. Among them, jujube phenolics are attracting more and more attentions for their, anticancer, antidiabetic, antimicrobial and anti-inflammatory activities [7,8]. As phenolics, triterpenoids are also a class of bioactive substances in medicinal jujube fruits, but their antitumor and antioxidant properties have been less studied than those of polysaccharides [9]. They have many functions, such as inhibiting histamine release, lowering blood pressure, and protecting the liver [10]. Based on its "targeted killing," triterpenoids have recently been studied as possible antitumor agents [11,12]; on the study of pharmacological bioactive compounds in the fruit of *zyzyphus*

jujuba p-coumaroyl alphitolic acid (ACA) was isolated [13]. Thus, the extraction process and the antioxidant capacity of this triterpenoid in jujube fruits could be very important. The present studies were performed to assess *In-vitro and In-vivo* antioxidant activities of *p*-coumaroyl alphitolic acid by using classic methods.

Material and Methods

Plant Material

This study was carried out on the fruit of *Ziziphus mauritiana*, because of its high nutritional and therapeutic value and the diversity of its uses. Dried jujube samples were purchased from farmers in Mokolo in the department of Mayo Tsanaga (Far North Region), an area where jujube is predominant.

Extraction and isolation of *p*-coumaroyl alphitolic acid

Triterpenoic acids were extracted using the method described by Yagi et al. [14]. After extraction with 95% ethanol (1.5 liters/500g sample), the alcohol is removed by vacuum evaporation. The suspension of the ethanolic extract in water was then extracted with butanol. This was separated by Chromatography on a silica gel 60 column using benzene-acetone (7/3) to obtain a resinous substance. The resinous material was partitioned with 2% Na_2CO_3 and ethyl acetate (EtOAc). The EtOAc phase was recovered and the acetate evaporated in vacuo. The residue was fractionated successively on a silica gel 60 column using the following solvents: - Chloroform - Ethyl acetate (85/5) - Benzene - acetone (8/1). The fractions collected were eluted on a TLC plate (solvent: Ethyl acetate - Methanol - Water; 10/1.35/1/) and the active fractions were identified by vaporisation of a methanolic solution of DPPH 0.2%. The active fractions were combined and quantified against a standard (Ursolic acid: 0-16.5 µg) by UV-Visible spectrophotometry at 220 nm. Figure 1 shows the structure of *p*-coumaroyl alphitolic acid [15].

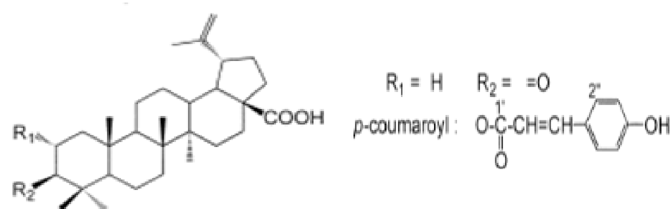

Figure1: *p*-coumaroyl alphitolic acid [15].

In vitro Measurement of Antioxidant Properties

2, 2-Diphenyl -1- Picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The free radical scavenging activity of the samples was determined according to the method of Kumari et al. [16].

A freshly prepared solution of DPPH in methanol (6×10^{-5}M) was used for the UV measurements. The samples of different concentrations (4–64 µg/mL) were added to DPPH solution in 1:1 ratio followed by vortexing. Then, it was allowed to take place in the dark at room temperature. Ursolic acid is taken as a standard. The inhibition percentage of DPPH radical scavenging activity was calculated using the following equation.

$$\text{Inhibition (\%)} = [(A0 - A)/A0] \times 100$$

Where, A0 is the absorbance of DPPH in the absence of the sample and A is the absorbance of DPPH in the presence of the sample.

The IC50 values (the concentration required to scavenge 50% of the free radical) were estimated from a plot of % inhibition against the concentration of the sample solutions.

Ferric Reducing Antioxidant Power assay (FRAP)

The total reduction capability of samples was determined according to the method of Kumari et al. [16]. 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferric cyanide were added to 1 mL of samples in different concentrations (4–64 µg/mL), followed by gentle mixing. The mixture was incubated at 50°C in a water bath for 20 min. The reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid and the mixture was centrifuged at 4000 rpm for 10 min. From the top layer, 2.5 mL was transferred into the tube containing 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3.6H_2O$), mixed thoroughly. After 5 min, the absorbance was measured at 700 nm against blank. Ursolic acid is taken as a standard.

ABTS+ Radical Cation Decolorization Assay

The antioxidant activities of the extracts were determined by the improved ABTS+ radical cation scavenging ability with the slight modification [17]. ABTS+radical cation was produced by mixing 7 mM 2, 2′- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate ($K_2S_2O_8$), incubated at room temperature in the dark. To determine the ABTS radical scavenging activity, 3 mL of ABTS+ solution was mixed thoroughly with 0.2 mL of different concentration (4–64 µg/mL) of extracts. Ursolic acid is taken as a standard. The reaction mixture was allowed to stand at room temperature for 6 min. The percentage inhibition was calculated by the following formula:

%Inhibition = (OD of control − OD of sample) × 100/OD of control

In vivo Measurement of Antioxidant Properties

Animals and ethics

Male albinos Sprague Dawley rats (160-180 g) were housed in polycarbonate cages in a controlled environment with a temperature of 25 ± 2°C, relative humidity (40–60%), with a 12-h light–dark cycle (12h/12h: 7 – 19 h light and 19 – 7h dark) [18]. The investigation conforms to the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). During an acclimatization period of 1 week, the rats received tap water and a commercial rat diet *ad libitu* [19]. At the end of this period, the rats were weighed and randomly assigned to one of the different groups (n = 6 / group) according to the experimental studies. Experimental studies received approval of the local ethical committee for animal handling and experimental procedure. Each animal was used only once.

Experimental Procedure
The triterpenoids fraction from *Ziziphus mauritiana*, ACA (50, 100, 150 and 200 mg/kg; b.w), were used in this study. Thirty six male rats were randomly divided into five groups, each consisting of 6 animals, which included:
- Group 1: Untreated control rats which received distilled water for 14 days.
- Group 2: Positive control received 50mg/kg bw of vitamin C for 14 days.
- Group 3: Received 50 mg/kg bw of ACA extract for 14 days.
- Group 4: Received 100 mg/kg bw of ACA extract for 14 days.
- Group 5: Received 150 mg/kg bw of ACA extract for 14 days.
- Group 6: Received 200 mg/kg bw of ACA extract for 14 days.

Administrations were done orally, the extract dissolved in 1 mL of distilled water per 100 g of body weight. 24 h after the last dose, all animals were anesthetized with chloroform and blood samples were immediately collected from the heart by cardiac puncture in two tubes to obtain serum and plasma (heparin tubes). Serum was separated by centrifugation at 3000 rpm for 5 min (4°C) and plasma was separated by centrifugation at 1500 rpm for 10 min (4°C). Serum was used for protein assessment and plasma was used for MDA determinations and Oxidative stress which was performed by measuring GSH; GPX; CAT and SOD activities in red blood cells.

Estimation of lipid peroxidation (MDA)
Lipid peroxidation was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) using the modification method of Niehius and Samuelsson [20]. In brief, 0.1 ml of sample (10% w/v) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25 N HCl). After vortex mixing, samples were maintained at 95°C for 20 min. Afterwards samples were centrifuged at 3000 rpm for 10 min and supernatants were read at 532 nm against reference blank. A calibration curve was constructed using MDA as standard and the results were expressed as nmol/mg protein. Percentage inhibition was calculated using the equation:
% lipids Inhibition = {Ao- A1}/Ao × 100
Where; Ao is the absorbance of the control and A1 is the absorbance of the sample extract.

Estimation of protein concentration
The protein content was estimated by Biuret method [21] using bovine serum albumin as a standard.

Determination of Superoxide dismutase (SOD)
The activity of superoxide dismutase was assayed by monitoring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT). Superoxide dismutase was assayed as described by Misra and Fridovich [22]. The assay mixture contained 0.5 ml of hepatic PMS, 1 ml of 50 mM sodium carbonate, 0.4 ml of 25 μm nitroblue tetrazolium and 0.2 ml of freshly prepared 0.1 mM hydroxylamine-hydrochloride. The reaction mixture was mixed quickly by inversion followed by the addition of clear supernatant of 0.1 ml of sample (10% w/v). The change in absorbance was recorded at 560 nm. Percentage inhibition was calculated using this equation:
% superoxide dismutase inhibition = [(normal activity – inhibited activity)/(normal activity)] × 100%.

Determination of reduced glutathione (GSH)
Reduced glutathione was quantified by the GSH assay, as reported in Ellman [23]. The assay is based on the oxidation of GSH by 5,5-dithiobis (2-nitrobenzoic acid) (DTNB). DTNB and GSH react together, producing 2-nitro-5- thiobenzoic acid (TNB) having a yellow color. Briefly, the sample was prepared by mixing DTNB (2.4 mL), buffer (0.5 mL), and respective dilution of the supernatant (0.1 mL). The GSH concentration was determined by measuring absorbance at 412 nm using a UV-Vis spectrophotometer using phosphate buffer as a blank.

Determination of glutathione peroxidase (GPX)
Glutathione peroxidase (GPX) activity was analyzed by the method of Rotruck et al., [24]. To 0.2 ml of buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of tissue homogenate were added. To that mixture, 0.2 ml of glutathione solution and 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37°C for 10 min along with the control tubes containing all the reagents but no enzyme. After 10 minutes, the reaction was arrested by the addition of 0.4 ml of 10% TCA. 0.2 ml of tissue homogenate was added to the control tubes. The tubes were centrifuged and supernatant was assayed for glutathione content by adding Ellman's reagent.

Determination of catalase (CAT)
Catalase activity was assessed by the method of previously reported by Naz et al., [25]. Phosphate buffer (0.1 M, pH 7.4) was taken as a blank. The samples were analyzed against a control. A measure of 40 μL of the plant supernatant was added to the H_2O_2 solution (40 mM; 0.6 mL), and the total volume was made up to 3 mL and mixed thoroughly. The final reading was noted at 240 nm in triplicate. The results were expressed as micromoles of hydrogen peroxide decomposed/min/mg of protein.

Statistical Analysis
The experimental results were expressed as means ± standard deviation. For each group, the result obtained was the mean for 6 rats. All results were analyzed using a one-way analysis of variance. Duncan's Multiple Range test was performed to evaluate differences between groups. Differences between means were considered to be significant at $p < 0.05$.

Results and Discussion
Aerobic organisms produce reactive oxygen species (ROS) as a consequence of aerobic respiration and substrate oxidation. Low levels of ROS are helpful in many processes including cell differentiation, cell growth, apoptosis, immunity and defence against micro-organism. In contrast, high levels of ROS result in oxidative stress, which may cause metabolic malfunction and macromolecular, cellular and tissue damages. The reports from several studies have produced a clear evidence that there exist

a good correlation between type and severity of disease and antioxidant level in the blood. In order to deal with damaging activities of ROS, aerobic organisms process antioxidant defence systems. The enzymatic antioxidant defences include SOD, GPX and CAT [26]. These activities play an important role in the progress of the disease, therefore in the treatment of chronic diseases, food antioxidant treatment may useful and should be added to combined therapy for these patients. Antioxydant activities of p-coumaroyl alphitolic acid was done by assessing *in-vitro* and *in-vivo* antioxyant activities.

In vitro antioxydant activities of *p*-coumaroyl alphitolic acid

In-vitro antioxidant activity of *p*-coumaroyl alphitolic acid of *Ziziphus mauritiana* fruit was done by ABTS, DPPH and FRAP methods. Table 1 presents calculated IC50 values of *in vitro* antioxidant assay of p-coumaroyl alphitolic acid of *Ziziphus mauritiana* fruit.

Table 1: IC_{50} for *in vitro* antioxidant assay of p-coumaroyl alphitolic acid of *Z. mauritiana* fruit.

		IC_{50} Values	
Triterpenoic acids	ABTS (µg/ml)	DPPH (µg/ml)	FRAP (µg/ml)
p-coumaroyl alphitolic acid (ACA)	2.84 ± 0.07	7.29 ± 0.94	28.32 ± 0.66
Ursolic acid (standard)	47.92 ± 0.83	47.92 ± 0.83	47.92 ± 0.83

ABTS Radical Cation Decolourization Assay

Table 1 showed result of assessment of ABTS method of ACA. From this result it is noted that the calculated IC50 of ABTS method was found to be lower (2.84 ± 0.07 µg/ml) than Ursolic acid (standard) (47.92 ± 0.83 µg/ml). This value is lesser than those reported by Abalaka et al. [27] (18-124 µg/ml); Ramar Mohan et al. [28] (38.07µg/ml) on extract of *Z. mauritiana* leaves. Kumari et al. [16] demonstrate that extract of *Z. mauritiana* leaves plays an important role in NO suppression. The presence of hydroxyl radical in the body may lead to the oxidative DNA damage. Therefore, it is very important to find the solution using natural products with good scavenging activity against this ROS. Overproduction of NO causes cancer, inflammation, neurodegenerative, chronic inflammatory diseases, ischemiareperfusion and other pathological conditions [29]. In the present study, the extracts scavenging the NO which results in the ABTS activity, confirm that the extract of ACA showed a good radical scavenging capacity and can be used against oxydative stress and chronic deseases. According to Kumari et al. [16] there is a significant correlation between ABTS and FRAP methods.

Total Reduction Capability

Total Reduction Capability is presented in table 1. As oberved with ABTS method, FRAP method showed good reducing capabilities of A**CA (**28.32 ± 0.66 **µg/ml)**. This value is lower comparable with previous studies of Ramar Mohan et al. [28] (90.70 µg/ml) observed with methanol extract of *Z. mauritiana lam* leaves. These authors believe that the antioxidants in the extract are determined by assessing the ability of extract to reduce the ferric cyanide complex to the ferrous form. Higher reducing power means better abilities to donate the electron and the free radical form stable substances by accepting the donated electrons, resulting in the termination of radical chain reaction [30]. This may serve as a significant indicator of its potential antioxidant activity [31]. It is thus possible to say that ACA have good antioxydant activity and con be used in the treatment of chronic deseases. According to Kumari et al., [16] there is a strongly correlation between FRAP and DPPH.

DPPH Radical Scavenging Activity

DPPH Radical Scavenging Activity is presented in table 1. Results showed good IC50 value (7.29 ± 0.94 µg/ml). The calculated IC50 were found to be lower than those observed by Ramar et al. [28] (38.07µg/ml) in methanol extract of *Z. mauritiana lam* leaves. Diverse biological activities such as anti-inflammatory, anti-atherosclerotic and anti-arcinogenic activities are documented by these plant derived compounds [32]. ACA which is derived compound confirm that the extract has a good radical scavenging capacity and can be used against oxydative stress and chronic deseases.

In vivo antioxydant activities of p-coumaroyl alphitolic acid

The biochemical assessment is conducted to show the benefic effects of ACA on management of hypertension, inflammatory and cardiovascular diseases which can be linked to the oxidative stresses and blood lipdic parameters [33]. Antioxidant activities of different samples were evaluated *in vivo* by determination of protein, SOD, GSH, CAT and GPX in plasma after 14 days.

Evaluation of Proteins

Another marker of oxidative stress and protein oxidation is the Advanced Oxidation Protein Product (AOPP) (Witko-Sarsat & Gausson, 2003) [34]. Figure 2 shows the evolution of protein levels in plasma.

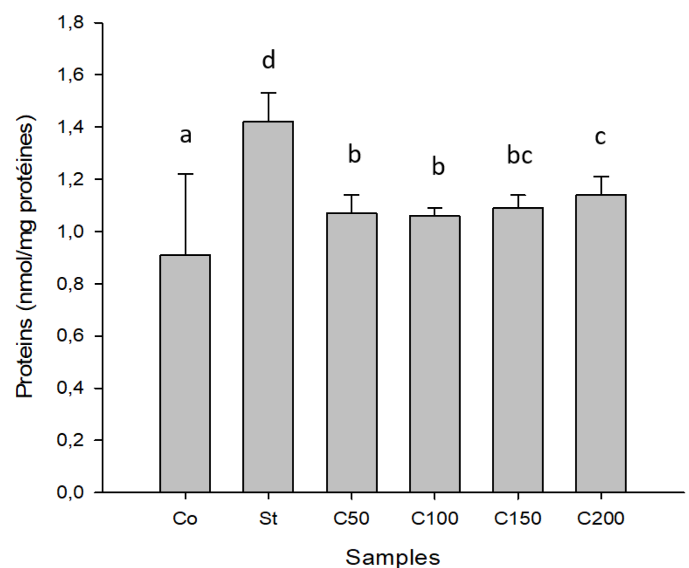

Figure 2: Effect of ACA consumption on proteins.

The concentration values obtained for C50 (1.07 ± 0.07); C100 (1.06 ± 0.03); C150 (1.09 ± 0.05); C200 (1.14 ± 0.07) were significantly different ($p<0.05$) from the standard (St) (1.42 ± 0.11). This result suggests that the protective action of ACA on proteins is greater than the standard. However, it should be noted that all the experimental values obtained are higher than the control (0.91 ± 0.04). These values are similar to those obtained by Ben Saad [35] (0.65-1.8 nmol/mg protein). The increase observed of protein level could be explained by stimulation of the cells to secrete antioxidant enzymes following consumption of ACA extracts. This confirms the protective action of ACA against protein oxidation by free radicals. Indeed, the imbalance between defence systems and the production of free radicals leads to biochemical alterations in the body's cells, such as the appearance of DNA breaks or damage to the integrity of the cell membrane by the induction of lipid peroxidation and alterations to proteins [36]. These modifications to peptides by the addition of products of lipid peroxidation generally lead to a loss of the catalytic or structural function of the proteins affected [37]. These cells are generally more sensitive to the action of proteases, leading to their elimination. This could explain the low levels obtained in the control samples, in contrast to the treated samples.

Evaluation of Malondialdehyde (MDA)

Oxidative stress can be correlated to the lipid molecular peroxydation and can be assessed by determination of inherent products yielded. This can be done by evaluation of MDA which is one of the best indexes of cell destruction due to the fact that this product is rapidly formed in serum than in normal cell. MDA concentration can be then reflected the oxidative stress degree of rats [26] and constitutes a biological marker of lipid peroxidation. Figure 3 shows the effect of ACA consumeption on Malondialdehyde (MDA).

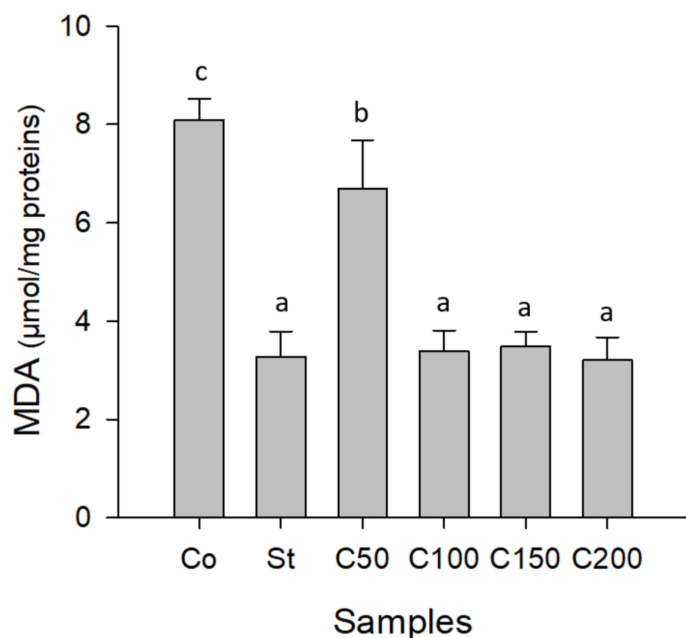

Figure 3: Effect of ACA consumption on Malondialdehyde (MDA).

MDA is one of the end products formed when polyunsaturated fatty acids are broken down by free radicals. Assessing the importance of oxidative stress in many diseases requires the use of different assays, the most widely used of which is MDA. Following oxidative stress, lipoperoxides are formed [38], including MDA, which results from the fragmentation of polyunsaturated fatty acids [39]. The results obtained in this study show that the different treatments given to the rats resulted in a reduction in MDA levels compared with the control. The values obtained were respectively 8.09 ± 1.30 (CO); 6.69 ± 0.51 (C50) 3.39 ± 0.42 (C100); 3.49 ± 0.29 (C150); 3.20 ± 0.47 (C200) and 3.28 ± 0.99 (St). These results are higher than those of Tajudeen et al. [40] (7.67 - 10.83 nmol.10-7/ml) obtained with jujube leaves extracts. The values obtained are similar to those found by Saeed et al. [41] who evaluated the effect of long-term morphine treatment on oxidative stress enzymes in male rats. The decrease in levels observed in this study could be explained by the inhibition of reactive oxygen species following ingestion of ACA extracts. Compared to the standard (St), there is no significant difference ($p<0.05$) between the C100, C150 and C200 doses. Therefore, it is possible to say that regarding MDA test, vitamin C and ACA could have the same results for doses of 100, 150 and 200. Given that lipid peroxidation is associated with several types of biological damage, jujube consumption could therefore help combat numerous human diseases, including cancer, ageing and atherosclerosis [42]. Lipid hydroperoxides formed by free radical attack on polyunsaturated fatty acid residues in phospholipids can still react with redox metals (such as Fe^{2+}, Fe^{3+}), ultimately producing mutagenic and carcinogenic malondialdehyde (MDA) [43]. It is also well known that the peroxidation of polyunsaturated fatty acids in membrane phospholipids leads to a deficit in membrane functions, notably through a reduction in fluidity and the inactivation of membrane receptors and enzymes [44]. This can lead to changes in membrane permeability. Free radicals, acting as pro-oxidants, are involved in the genesis of oxidative stress and the imbalance of the oxidant-antioxidant balance. Oxidative stress induces a high production of Reactive Oxygen Species (ROS), which are highly toxic to cells, particularly cell membranes. These ROS interact with the lipid bilayer to produce lipoperoxides [45]. However, endogenous enzymatic and non-enzymatic antioxidants are responsible for detoxifying the body by combating the deleterious effects of free radicals [46]. When ROS generation exceeds cellular antioxidant capacity, oxidative stress develops, leading to cellular dysfunction and tissue damage.

Evaluation of GSH

The cell's first antioxidant defence system is glutathione (GSH), which has a sulphydryl (-SH) function. Thanks to this free -SH function, it binds to toxic metabolites. Glutathione (GSH) performs its role in synergy with antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GSH-R), glutathione S-transferase (GSH-ST) and catalase (CAT), which are effective at eliminating toxic free radicals generated by oxidative stress [47-49]. In this work, SOD; GPX and CAT were assayed in blood plasma as a target

fluid specialised in the transport of toxification products. Figure 4 shows the effect of ACA consumed on glutathion réductase (GSH-R).

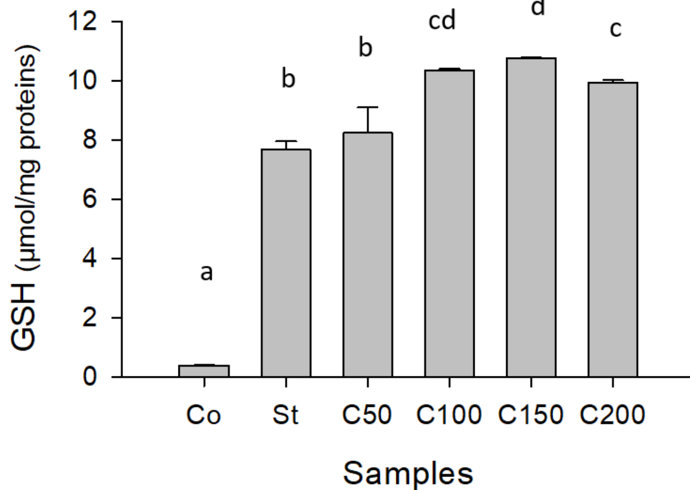

Figure 4: Effect of ACA consumption on glutathion réductase (GSH-R).

GSH levels increased in the ACA-treated groups compared with the controls. This increase varied from 0.39 ± 0.01(CO) to 10.75 ± 0.03 (C150). The values obtained with the different doses were higher than the standard (7.68 ± 0.27). However, analysis of variance showed that only doses C100 (10.35 ± 0.04); C150 (10.75 ± 0.03) and C200 (9.93 ± 0.09) were significantly different ($p<0.05$) compared to control (Co). These results are similar to those determined by Kalidoss and Krishnamoorthy [50], but lower than those evaluated by Merghem et al. [51] (0.44-1.02 µmol / g tissue) obtained with *Ruta montana* extracts. According to Taleb-Senouci et al. [52], the changes in GSH levels observed with the different doses can be considered a particularly sensitive indicator of oxidative stress. This suggests that the increase in GSH levels reflects the cell's defensive action against ROS. Such an increase should lead to inhibition of the toxic effect of free radicals. GSH acts as a direct scavenger of free radicals, is a cosubstrate required for GPX and glutathione-s-transferase activity [53] and is involved in the regeneration of oxidised vitamin E. In addition, GSH can react with H_2O_2 and lipid peroxides through the action of glutathione peroxidase (GPX) to eliminate reactive intermediates by reducing hydroperoxides [47-49]. It therefore acts as a protective physiological antioxidant in biological systems [54]. Consumption of jujube would therefore help to protect biological systems.

Evaluation of catalase (CAT)

Catalase (CAT) is an antioxidant enzyme involved in defending the cell against the toxic effects of hydrogen peroxide (H_2O_2) by catalysing its breakdown into water (H_2O) and molecular oxygen (O_2), and is a biomarker of oxidative stress [55]. Figure 5 shows the results of the effect of ACA consumption on CAT.

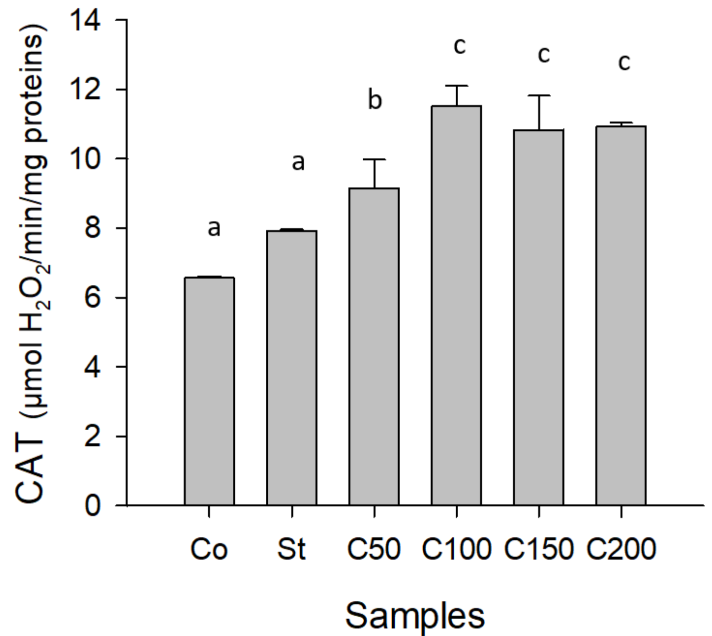

Figure 5: Effect of ACA consumption on catalase (CAT).

The results of this study showed an increase in CAT activity. The values obtained show a significant difference ($p<0.05$) between the value of the standard (St) (7.92 ± 0.04) and the concentrations C50 (9.16 ± 0.81); C100 (11.51 ± 0.59); C150 (10.83 ± 0.98) and C200 (10.93 ± 0.11). These values are close to those estimated by Ben Saad et al. [35] (8-16 µmol H_2O_2/min/mg protein) and lower than those of Si et al. [56] (12-20 µmol H_2O_2/min/mg protein) and Kalidoss and Krishnamoorthy [50] (16-21 µmol H_2O_2/min/mg protein). The variation observed with the different doses could be explained by a stimulation of the cells to secrete antioxidant enzymes following consumption of ACA extracts. In fact, the increase in CAT activity could be the result of activation of the enzyme by superoxide anion. This also explains SOD's ability to protect CAT. Some authors believe that the induction of specific CAT activity in correlation with SOD constitutes the first line of defence in the cell against oxidative stress under the pro-oxidant effect of free radicals [57,58].

However, increased production of hydrogen peroxide can exceed the antioxidant capacities of this enzyme, resulting in inhibition of their activity [59]. In this context, supplementing food with ACA could help solve this problem. The low activity of the standard in relation to the doses studied suggests that ACA extract has greater antioxidant activity than the vitamin C used as standard.

Evaluation of Superoxide dismutase (SOD)

SOD is one of the most important antioxidant enzymes in the body's defence system. The major function of SOD is to catalyse the dismutation of superoxide anion (O2--) into hydrogen peroxide (H_2O_2), thereby reducing the toxic effects of this free radical [60]. This test permits to evaluate the effect of ACA consumption on the dismutation of free radicals. Figure 6 shows the effect of ACA consomption on Superoxide dismutase (SOD).

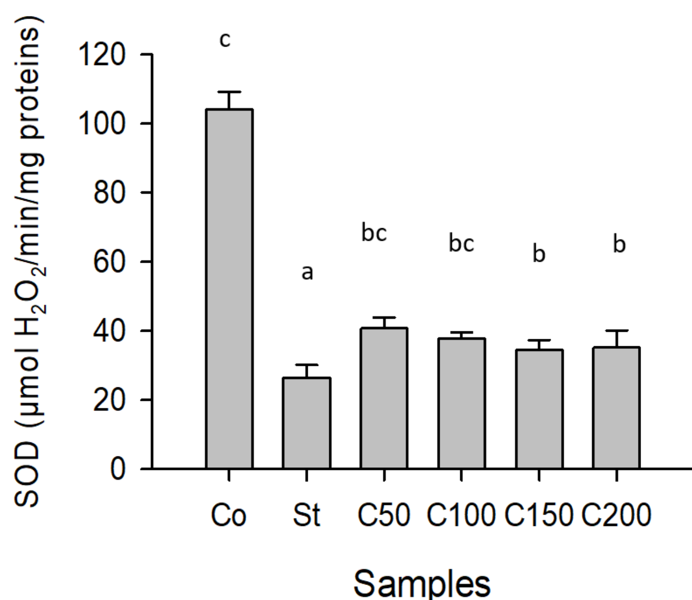

Figure 6: Effect of ACA consomption on Superoxide dismutase (SOD).

The plasma SOD of the control group (104.10 ± 5.06) was higher than that of the Standard (26.42 ± 3.66 µmole/l) and experimental groups. The values obtained for the different doses were 40.70 ± 3.13 (C50); 37.75 ± 1.72 (C100); 34.45 ± 2.92 (C150) and 35.15 ± 4.90 (C200) respectively. According to the Duncan test there is no significant difference ($p < 0.05$) between the value of plasma SOD for different experimental groups studied. These results are in agreement with the values obtained by Si et al. [56] (23-35 µmol H_2O_2/min/mg protein) and Kalidoss and Krishnamoorthy [50] (36-43 µmol H_2O_2/min/mg protein). SOD activity decreased, but catalase activity increased in plasma for all groups treated with ACA, compared with the control. This may be due to the inactivation of catalase (an enzyme involved in the detoxification of hydrogen peroxide by superoxide anion). Thus, the reduction in SOD activity could indirectly play an important role in the protection of tissues by catalase from highly reactive hydroxyl radicals [61]. In view of the results of this analysis, we can say that ACA extract is more antioxidant than vitamin C.

Evaluation of Glutathion peroxidase (GPX)

Glutathion peroxydase is an enzyme which destroys the free radicals, takes free radical from cell and protects the cell membrane against oxidation. GPX allows the conversion of oxygenate water into water. Figure 7 shows the effect of ACA consumption on Glutathion peroxidase (GPX).

The results of this study showed a clear increase in GPX activity in plasma following treatment of rats with ACA extracts. The values obtained showed no significant difference ($p<0.05$) for the concentrations C100 (7.20 ± 0.18); C150 (7.49 ± 0.56); C200 (7.28 ± 0.29) compared with the standard (7.03 ± 0.52). These results are similar to those obtained by Ben Saad [35] (4-8 µmol H_2O_2/min/mg protein), but lower than those of Shtukmaster et al. [62] (40-80 µmol H_2O_2/min/mg protein). It is thought that the increase in GPX levels could be explained by a stimulation of cell secretion of antioxidant enzymes following consumption of ACA extracts. These enzymatic antioxidants act as lines of defence in the cell against the pro-oxidant effect of free radicals [57,58]. In fact, the increase in lipid peroxidation, which is reflected here by the increase in GPX, weakens both the functioning of membranes by reducing their fluidity and the activity of membrane and cytoplasmic enzymes [63]. Consumption of jujube would therefore be indicated against oxidative stress.

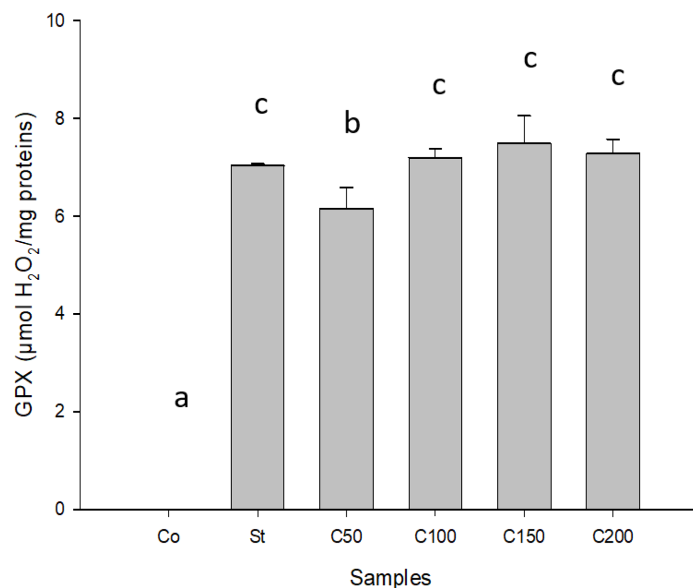

Figure 7: Effect of ACA consumption on Glutathion peroxidase (GPX).

Conclusion

The present study was carried out to evaluate the Antioxidant activity of *p*-coumaroylates of alphitolic acid of *Zizuphus mauritiana* fruit, this activity was tested *in vitro* and *in vivo* by using classic methods. The results showed that extracts leads to increased antioxidant capacity toward DPPH, ABTS and FRAP radicals, and caused amelioration in the bloob antioxidant status, by decreasing the MDA concentration, Superoxide dismutase (SOD) and increasing the rate of reduced glutathione (GSH), Glutathion peroxidase (GPX); catalase activity (CAT) and proteins in blood. In view of the above, we believe that eating jujube could help fight against many chronic diseases, including cancer, ageing and atherosclerosis.

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