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Research Article

# Acute toxicity, phenol content, antioxidant and postprandial anti-diabetic activity of *Echinops spinosus* extracts

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**Abstract:** *Echinops spinosus,* belonging to Asteraceae family, is used in folk medicine as an abortifacient and diuretic and for blood circulation, diabetes, stomach pain, indigestion and spasmolytic problems. The objective of this work is the study of acute toxicity, the content of phenolic compounds (polyphenols, flavonoids and tannins), antioxidant activity (DPPH, ABTS, FRAP, $H_2O_2$ and xanthine oxidase) and antidiabetic (α-amylase, α- glucosidase and lipase) *in vitro* and *ex-vivo* by studying the starch tolerance test. The phytochemical assay showed that the ethanolic extract is the richest in polyphenols, flavonoids and tannins with 77.01 mg GEA/g extract; 544.33 mg RE/g extract, and 32.20 mg EC/g extract, respectively. The ethanolic extract showed better antioxidant activity compared to the aqueous extract with ($IC_{50}$=13±0.25 µg/mL; $IC_{50}$=75.11±0.34 mg TE/g extract; $IC_{50}$=51.1±1.2 mg AAE/g extract; $IC_{50}$=28.2±2.87 µg/mL and 16.83 ± 0.72 µg/mL) in DPPH, ABTS, FRAP, $H_2O_2$ and xanthine oxidase. Extracts of *E. spinosus* have shown a remarkable inhibitory effect α-amylase and interesting inhibitory effect of α-glucosidase and lipase. The aqueous and ethanolic extract also lowered blood sugar levels to 0.96 and 0.93g/L, respectively, after 90 minutes in starch-loaded rats. Acute toxicity results indicate that *E. spinosus* extracts are non-toxic with an $LD_{50}$ greater than 2 g/kg in female Swiss mice. Therefore, the antioxidant and anti-diabetic activity may be at the origin of the bioactive compounds contained in the plant *E. spinosus.* However, *in vivo* studies on the mechanism of action are needed against oxidative stress associated with diabetes.

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## 1. INTRODUCTION

Diabetes mellitus is a complex metabolic disease characterized by impaired metabolism of carbohydrates, fats and proteins (Soliman and Abd El Raheim, 2015). They most commonly affect children and adolescents in developed and developing countries. Type 1 diabetes is the

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result of a deficiency in insulin production due to dysfunction of the pancreatic β cells and type 2 diabetes is a consequence of a low sensitivity to insulin in the target tissues and/or insufficient insulin secretion (Musabayane, 2012). Several scientific reports showed that hyperglycemia is a risk factor for micro-vascular complications (nephropathy, retinopathy and neuropathy) and macro-vascular (stroke) (Burton-Freeman 2010; Marc *et al*. 2008). Moreover, among these common symptoms are frequent urine, thirst, and overeating (Bahmani *et al*., 2014)**.**

Postprandial hyperglycemia is a factor in the development of type 2 diabetes and complications, it has been shown to cause glucose toxicity and damage the function of pancreatic beta cells (Shelly *et al*., 2010). The acute changes in blood sugar during the postprandial phase cause a state of oxidative stress, and diabetes management should adjust these postprandial changes as well (Shihabudeen *et al*., 2011). On the other hand, among the therapeutic strategies to control the development of diabetic complications is to delay the absorption of glucose from the intestine by suppressing the activity of digestive enzymes namely α-amylase and intestinal transporters of α-glucosidase and glucose such as SGLT 1 and GLUT 2 (Yusoff *et al*., 2015)**.**

Currently, the mode of action of some antidiabetic drug are available, such as inhibiting hepatic production of glucose (biguanides), triggering insulin secretion (sulfonylureas and glinides), slowing down digestion and absorption of intestinal carbohydrates to adjust the postprandial glucose level (α-glucosidase and α-amylase inhibitors), restore the sensitivity of the insulin receptor and peripheral uptake of glucose (thiazolidinediones and metformin) or insulin (Elya *et al*., 2015). Studies have reported that the use of plants (rich in active compounds such as polyphenols) in food to prevent chronic disease can help to regulate biological pathways and antioxidant balance (Etoundi *et al*., 2010)**.**

*E. spinosus* of the Asteraceae family contains about 120 species distributed throughout the Mediterranean regions, Central Asia and tropical Africa. The species *spinosus* thrives in desert conditions with rainfall between 20 and 100 mm, and a wide range of soil, widespread on coastal dunes, sandy and gravelly to rocky surfaces (Bouzabata *et al*., 2018)**.** It is a perennial herbaceous plant, reaching nearly 1 m, characterized by upright brownish to reddish stems with few leaves, 10 to 15 cm long, is hairy, arachnoid, and bears very long spines (Helal *et al.*, 2020). In folk medicine, it is used as an abortifacient, diuretic and for blood circulation, diabetes, gastric disorders, indigestion and sposmolytic problems (Khedher *et al*., 2014). In Morocco, it is used to facilitate childbirth. A decoction of the roots in water or olive oil is applied to help pregnant women to expel the placenta. Also administer before birth to stimulate contractions. In Marrakech and Salé, a root decoction is used for stomach pains, indigestion and lack of appetite, as well as diabetes. In Casablanca, the whole plant, in powder or decoction, is used as a diuretic or depurative and to treat liver diseases (Agyare *et al*., 2013). Here, the aim of this study was to examine acute toxicity of *E. spinosus* extracts and determine phenolic content as well as to evaluate antioxidant and postprandial antihyperglycemic activities of these extracts.

## 2. MATERIAL and METHODS

### 2.1. Standards and Reagents

$\alpha$-glucosidase from *Saccharomyces cerevisiae*, $\alpha$-amylase from *Bacillus licheniformis,* p-Nitrophenyl-$\alpha$-D-glucopyranoside (pNPG) were purchased from Sigma-Aldrich (France), phlorizin hydrate (Sigma Aldrich, USA), Acarbose.

### 2.2. Plant Material and Extraction

The roots of *E. spinosus* were collected from Oujda Region, Morocco. The collected plant was deposited under the voucher number HUMPOM 10051 in the herbarium at University Mohammed I., Oujda (Morocco). The roots of *E. spinosus* were dried at room temperature, ground into a powder and kept in the shade until use. In this study, two types of extraction

(infusion and maceration) were used. Indeed, the aqueous extract was prepared by the infusion method, by which 30g of *E. spinosus* powder was infused with 300 mL of distilled water for 1 hour and left to cool. The extract was filtered and evaporated at 50 °C using a rotary evaporator. Subsequently the extract was lyophilized and stored for later use.

For the ethanolic extract, 30 g of the root powder was macerated for 48 hours with stirring and at room temperature. The extract was filtered and evaporated at 40 ° C using a rotary evaporator.

## 2.3. Determination of Phenolic Contents

### 2.3.1. *Total phenolic content*

The determination of the total polyphenols is carried out by the method of Folin-Ciocalteau reagents described by Spanos and Wrolstad (1990). The gallic acid standard range has been evaluated at different concentrations ranging from 1.95 to 31.25 µg / mL, and the results are expressed in milligrams of gallic acid equivalent per one gram of extract (mg EAG / g extract). Briefly, 2.5 mL of 10% (v / v) of Folin Cioalteu reagent was mixed with 0.5 mL of sample solution. Subsequently 4 ml of sodium carbonate $Na_2CO_3$ at 7.5% (W / V) are added. The reaction mixture was incubated at 45 ° C for 30 minutes and the absorbance against the blank was determined by at 765 nm.

### 2.3.2. *Total flavonoid content*

The flavonoid content was evaluated by the aluminum trichloride ($AlCl_3$) colorimetric method described by Dewanto *et al*. (2002). Briefly, 0.5 mL of the sample at a concentration of 1 mg / ml was mixed with 3.2 mL of distilled water and 0.15 mL of 5% (w / v) sodium nitrite solution $NaNO_3$. The mixture is left to stand for 5 minutes. Then 0.15 mL of $AlCl_3$ is added. After standing for 6 minutes, 1 mL of 1M NaOH was added, then the mixture was incubated at room temperature for 30 minutes. Absorbance was determined at 510 nm. Rutin was used as a standard at final concentrations ranging from 50 to 400 g/mL and results are expressed in milligrams of gallic acid equivalent per gram of extract (mg RE/g of extract).

### 2.3.3. *Total tannin content*

The tannin content was quantified by the vanillin method described by Julkunen-Tiitto (1985). Indeed, 50 µL of the sample or standard were mixed with 1.5 mL of 4% vanillin (prepared with methanol) then 750 µL of concentrated HCL were added. The mixture was stirred and incubated at temperature in the dark for 20 minutes. The absorbance was measured at 500 nm. The standard curve of the catechin was carried out under the same conditions and the results were determined in mg equivalent of catechin per gram of dry weight of extract (mg EC/g of extract).

## 2.4. Antioxidant Activity

### 2.4.1. *DPPH radical scavenging assay*

Antioxidant activity by the method of DPPH (2,2-Diphenyl-1-pierylhydrazyl) was achieved by the protocol of Huang *et al*., (2011). This method is based on the reduction of DPPH to DPPHH in the presence of radical scavengers. The extracts or the standard (BHT) were dissolved in a methanol solution of DPPH (0.02 M) and then incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517nm against a blank. The percentage of DPPH radical scavenging was calculated according to the following formula:

I% = [(Absorbance Negative Control- Absorbance Sample) / Absorbance Negative Control)] x 100

### 2.4.2. *Ferric reducing power assay* (FRAP)

The measurement of the ability to reduce ferric iron to ferrous iron was estimated according to the protocol described by Amarowicz *et al*. ( 2004). Briefly, 0.5 mL of the extracts were mixed with 1.25 mL of phosphate buffer solution (0.2M, pH 6.6) and 1.25 mL of 1% potassium

ferricyanide. The mixture was incubated at 50 °C for 20 minutes then 1.25 mL of 10% trichloroacetic acid was added in order to stop the reaction. The tubes are then centrifuged at 3000 rpm for 10 minutes. Then 1.25 mL of the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL of $FeCl_3$ (0.1%, w/v). The absorbance was determined at 700 nm. Ascorbic acid is used as a standard and the final results are expressed in milligrams of ascorbic acid equivalent per gram of extracts (mg AAE/g of extract).

#### 2.4.3. *Trolox equivalent antioxidant capacity* (TEAC) *assay*

The evaluation of the antioxidant capacity by the ABTS cation decolorization assay was estimated according to the method described by Tuberoso *et al*. (2013). The cationic radical ABTS was generated by oxidation of ABTS (2mM) with potassium persulfate (70mM) then the mixture was kept for 16 hours. The resulting solution was diluted with methanol to an absorbance of 0.70 at 734 nm. Subsequently, 2 mL of the diluted ABTS solution was mixed with 200 µL of each sample and allowed to react for 1 minute and the absorbance was measured at 734 nm. Trolox is used as a reference and sample results were expressed as milligram equivalent of Trolox per gram of extract (mg TE/g extract).

#### 2.4.4. $H_2O_2$ *trapping test*

The antioxidant activity of $H_2O_2$ was estimated by the method described by Muruhan *et al*. (2013). Briefly, 1 mL of the sample or ascorbic acid was mixed with 0.6 mL of $H_2O_2$ (40 mmol / L), then the mixed were incubated for 10 min and the absorbance was measured at 230nm. The percent inhibition of $H_2O_2$ was calculated using the following equation:

$$\% = [(A_0 - A_1) / A_0] \times 100$$

Where A0 was the absorbance of the control, and A1 was the absorbance in the presence of the sample or standard.

#### 2.4.5. *Xanthine oxidase inhibition test*

The method of Umamaheswari *et al*. (2007), was used to determine the percent inhibition of xanthine oxidase (xo). Allopurinol was used as a positive control. Indeed, 1mL of the sample was mixed with 1.9 mL of phosphate buffer (pH 7.5), 0.1 mL of enzymatic solution (0.2 unit / mL) and 1.0 mL of 0.5 mM xanthine solution. Subsequently, 1mL of 1M HCl was added after incubation for 15 minutes at 25 ° C. The absorbance was read at 295 nm and the results were calculated using the following formula:

$$I (\%) = [((Ac-Acb) - (As-Asb)/ (Ac-Acb)) \times 100]$$

Where Ac was the absorbance of control; Acb was the absorbance of control blank; as was the absorbance of sample; and Asb was the absorbance of sample blank.

### 2.5. Antihyperglycemic Activity

#### 2.5.1. *α-Amylase inhibitory assay*

The inhibition of α-amylase was evaluated by the starch-iodine method as described by Chakrabarti *et al*. (2014) with certain modifications. Briefly, 250 µL of the sample or standard (Acarbose) and mixed with 100 µL of the phosphate buffer solution (20mM, PH 6.9) containing the α-amylase enzyme. The mixture was incubated at 37 °C for 10 min, then 600 µL of starch substrate (1%) was added and the mixture was re-incubated at 37 °C for 10 min. At the end of the reaction, 250 µL of the HCl solution and 100 µL of iodine were added. Absorbance was determined by spectrophotometer at 630 nm. The results were calculated as a percentage according to the following formula:

$$\text{Inhibition } (\%) = (1-(OD_{\text{Test sample}}/OD_{\text{Control}})) \times 100$$

### 2.5.2. *α-Glucosidase inhibition assay*

The inhibitory activity of α-glucosidase was determined by the protocol described by Kee *et al*. (2013). In fact, 150 µL of sample solution or acarbose were mixed with 100 µL of the α-glucosidase enzyme. (0.1U), the reaction is incubated for 10 minutes, 200 µL of ρ-nitrophenyl-α-D-glocopyranoside (pNPG) substrate were added, Then, a second incubation was carried out for 30 minutes, and at the end of the reaction 1 ml of 0.1 M $Na_2CO_3$ was added. The absorbance was determined at 405 nm.

The percentage of inhibition was calculated according to the following formula:

$$\text{Inhibitory activity (\%)} = [OD_{Control}\text{-}OD_{Test\ sample})/OD_{Controle}] \times 100$$

### 2.5.3. *Lipase inhibition assay*

Lipase inhibitory activity was determined by Hu *et al*. (2015) with some modifications. Briefly, 150 µL of the extract or orlistat were mixed with 500 µL of Tris-HCl buffer (1mM, pH8) containing the lipase enzyme (2U), the reaction was incubated for 30 minutes at 37 °C, then 450 µL of 1 mM of -4-Nitrophenyl butyrate substrate were added followed by a second incubation at 30 minutes for 37 ° C. Absorbance was determined at 405 nm. The percentage inhibition of lipase was calculated using the following formula:

$$\text{Inhibition (\%)} = [((Ac - Acb) - (As - Asb)) / (Ac - Acb)] \times 100$$

Where Ac refers to the absorbance of the control, Acb refers to the absorbance of the control blank, As the absorbance of the sample, and Asb is the absorbance of the blank sample.

### 2.6. Experimental Animals

Male and female Wistar rats weighing 150-250 g were used in the experiments. The animals were kept in cages at the Faculty of Medicine and Pharmacy in Rabat. They were maintained under standard conditions. The experiment was carried out according to the principles described in the "Guide to the care and use of laboratory animals", 8th edition prepared by the National Academy of Sciences (National Research Council of the National Academies). Every effort has been made to minimize animal suffering and the number of animals used. Ethics approval was obtained from Mohammed V University in Rabat

### 2.7. Oral Starch Tolerance in Normal Rats

The *ex-vivo* antihyperglycemic activity of the *E. spinosus* samples was determined according to the method as described by Beejmohun *et al*. (2014). Briefly, six groups of rats each composed of five rats ($n$ = 5) were put on an empty stomach for 18 hours with free access to water. The control group received the vehicle (distilled water); the negative control group treated with the vehicle (starch); the positive control group treated with the acarbose vehicle at 50 mg / kg and the other two groups were treated with the aqueous and ethanolic extract of *E. spinosus* at 150 mg/kg orally (p.o). Thirty minutes later, all animals were loaded with starch orally at a dose of 2.5 mg/kg. Blood was drawn from the tail vein before (t = 0), and at 30, 60, 90 and 120 min after starch administration.

### 2.8. Acute Oral Toxicity

Acute oral toxicity was achieved using the method described in European guideline OECD-425 (Guideline, 2012). Swiss albino mice weighing 20 to 30 g were used in this experiment. Each group received the extracts orally at a dose of 2 g/kg. After treatment, the animals were observed for 14 days in order to assess the behavioral toxic effects.

### 2.9. Statistical Analysis

Data were expressed as mean± SEM. Statistical analysis and comparison of means were evaluated by one-way analysis of variance (ANOVA). The differences were considered statistically significant at $p< 0.05$. Analysis was performed with GraphPad Prism 6.

## 3. RESULTS

The results of the assays of polyphenols, tannins and flavonoids are summarized in the Table 1. In the aqueous extract, TPC is (34±0.58 mg GEA/g extract), TFC (10.33 ± 4.2 mg RE/g extract) and TCC (42 ± 13.3 mg EC / g extract). Thus, the ethanolic extract was found to be richer with TPC (77.01±2.25 mg GEA/g extract), TFC (544.33 ± 26.33 mg RE / g extract) and TCC (32.20±2.49 mg EC/g extract). These results reveal the richness of *E. spinosus* in polyphenols, tannins and in particular in flavonoids with a difference in variability between the two extracts. The comparison with other study reveals that the content of polyphenols and tannins in the ethanolic extract of *E. spinosus* in this study is higher than those obtained in the same species with TPC (19.3 mg GAE / 100g), TCC (10.5 mg EC/100g) (Khedher, Moussaoui and Salem, 2014).

**Table 1.** Total phenolic, flavonoid and condensed tannin content of *E. spinosus* extracts.

| Aqueous extract | | | Ethanol extract | | |
|---|---|---|---|---|---|
| TPC (mg GEA/g extract) | TFC (mg RE/g extract) | TCC (mg CE/g extract) | TPC (mg GEA/g extract) | TFC (mg RE/g extract) | TCC (mg CE/g extract) |
| 34±0.58 | 10.33± 4.2 | 42± 13.3 | 77.01±2.25 | 544.33±26.33 | 32.20± 2.49 |

TPC : total phenolic content
TFC: total flavonoid content
TCC: total condensed tannins
mg GEA/g extract: mg Galic Acid equivalent per gram of extract
mg RE/g extract: mg of Rutin equivalent per gram of extract
mg CE/g extract: mg Catechin equivalent per gram of extract

The antioxidant activity of the different extracts was evaluated using five methods: anti-radical power with (DPPH), reducing power (FRAP), antioxidant power by ABTS, trapping of peroxide dismutase ($H_2O_2$) and inhibition of xanthine oxidase from the aqueous and ethanolic extracts of *E. spinosus* is shown in the Table 2. The study of the antioxidant activity by the DPPH test shows that the aqueous and ethanolic extract have significant anti-free radicals with $IC_{50}$ respectively of (25 ± 10.69 and 13 ± 0.25 µg / mL). The study reported by Khedher *et al.* (2014) of the same species showed lower activity than the extracts of *E. spinosus* obtained by our study with an $IC_{50}$ of 147 µg/mL using the DPPH test. Likewise, the extracts exhibited an interesting effect towards the ABTS cation, with better activity of the ethanolic extract (75.11±0.34 mg TE/g extract). The mode of action of antioxidants towards the DPPH radical is particularly linked to the hydroxyl group responsible for this action. We can see that this anti-free radical activity of the ethanolic extract is due to their richness in substance with a hydroxyl group (Boylan *et al.*, 2015). Likewise, the study of the reducing power of $Fe^{3+}$ into $Fe^{2+}$ has shown that the aqueous and ethanolic extracts have a reducing effect respectively of (20.61±2.72 and 51.1±1.2 mg AAE/g extract). It has been reported that flavonoids not only react as an antioxidant, some of them have the power to break down deoxyribose as a result of the reduction of $Fe^{3+}$ to $Fe^{2+}$ (Schinella *et al.*, 2002).

Chemical compounds in plants are electron donors, they help speed up the conversion of $H_2O_2$ to $H_2O$. The results of *E. spinosus* extracts on $H_2O_2$ scavenging showed that the aqueous extract exhibited antioxidant activity with an $IC_{50}$ of (36.04±1.65 µg/mL) and the ethanolic extract with an $IC_{50}$ of (28.2 ± 2.87 µg / mL). Likewise, extracts of *E. spinosus* showed lower

activity than that of ascorbic acid ($IC_{50}$=5.98±0.47µg / mL). For xanthine oxidase inhibitory activity, the ethanolic extract ($IC_{50}$=16.83±0.72 µg / mL) showed a higher inhibitory activity than that of the aqueous extract ($IC_{50}$=20.14±1.28 µg/mL). This minor variation between the different antioxidant methods may be due to the intrinsic mode of action of the antioxidant reactions, or to certain factors namely the stereoselectivity of the radicals and the solubility of the antioxidant components (Yahyaoui *et al*., 2018).

**Table 2.** Antioxidant activity by DPPH, FRAP, ABTS, $H_2O_2$ and xanthine oxidase (XO) methods of *E. spinosus*; Average of three replicates.

| | DPPH $IC_{50}$ (µg/mL) | ABTS (mg TE/g extract) | FRAP (mg AAE/g extract) | $H_2O_2$ $IC_{50}$ (µg/mL) | Xanthine oxydase $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|
| Aqueous extract | 25±10.69 | 38.13±1.76 | 20.61± 2.72 | 36.04±1.65 | 20.14±1.28 |
| Ethanol extract | 13±0.25 | 75.11± 0.34 | 51.1± 1.2 | 28.2±2.87 | 16.83±0.72 |
| BHT | 3.28± 0.79 | - | - | - | - |
| Ascorbic acid | - | - | - | 5.98± 0.47 | - |
| Allopurinol | - | - | - | - | 0.78±0.01 |

*E. spinosus* extracts have also been evaluated for their inhibitory effect on α-amylase, α-glucosidase and lipase. Salivary and pancreatic $\alpha$-amylase hydrolyzes the $\alpha$-1,4-glucosidic bonds of polysaccharides, such as starch. Subsequently, $\alpha$-glucosidase located in the brush borders of intestinal cells hydrolyzes the resulting oligosaccharides into glucose, which is then transported in the blood. Moreover, the main function of pancreatic lipase is the breakdown of triacylglycerides into glycerol and free fatty acids (Loizzo *et al.*, 2008). The results obtained have been summarized in the Table 3. The macerated ethanolic extract showed a better inhibitory effect against the three anti-diabetic enzymes with $IC_{50}$ of 371±0.62, 18.6±1.2, and 10.44±1.08 µg/mL, respectively. The aqueous extract was less effective against the enzymes α-amylase, α-glucosidase and lipase compared to the ethanolic extract with $IC_{50}$ of 668.8 ± 1.45; 19.68 ± 0.46 and 24.96 ± 1.52 µg / mL, respectively. Likewise, the ethanolic extract exhibited an inhibitory power greater than that of orlistat (12.55 ± 4.17 µg / mL) for lipase and an almost similar activity of acarbose (18.01 ± 2.00 µg / mL) for α-glucosidase. The study of Dammak et al (2020) showed that *E. spinosus* has lipid-lowering activity in mice. The inhibition of digestive enzymes ($\alpha$-amylase, $\alpha$-glucosidase and lipase) responsible for the degradation of carbohydrates and lipids can therefore be one of the strategies in the management of the postprandial state in diabetics and their ability to prevent type 2 diabetes. Similarly, the different phenolic compounds have been identified for their ability to inhibit the enzyme $\alpha$-amylase due to their action to bind to proteins (Shobana, Sreerama and Malleshi, 2009).

**Table 3.** $IC_{50}$ values of ESA and ESE extracts on $\alpha$-amylase, $\alpha$-glucosidase and lipase inhibition.

| | $IC_{50}$ (µg/mL) | | |
|---|---|---|---|
| | α-amylase | α-glucosidase | Lipase |
| ESA | 668.8±1.45 | 19.68±0.46 | 24.96±1.52 |
| ESE | 371±0.62 | 18.6±1.2 | 10.44±1.08 |
| Acarbose | 44.75±0.54 | 18.01±2.00 | - |
| Orlistat | - | - | 12.55±4.17 |

ESA: aqueous extract of *E. spinosus*

ESE: ethanolic extract of *E. spinosus*

The *ex vivo* oral starch tolerance test study showed that groups increased blood glucose 30 minutes after starch loading, ESA significantly increased ($p$<0.05) hyperglycemia at 0.83g/L and ESE at 0.91g/L while acarbose increased insignificantly at 1.03g/L. Also, acarbose lowered blood sugar to 0.98 mg/dL after 60 minutes, and then gradually reduced to 0.88 g/L after 120 minutes. Rats treated with starch caused an increase in blood sugar of 1.26g / L after 30 minutes. Then, it is reduced to 0.97g/L after 120 minutes. On the other hand, ESA and ESE reduced blood sugar to 0.96 and 0.93g / L, respectively, only after 90 minutes. Thus, all groups decreased blood sugar compared to control. Furthermore, the area under the curve (AUCglucose) for the ESA and ESE treated groups was significantly lower than that of the control group. Likewise, the AUC values of the acarbose group were significantly lower than those of the control group (Figure 1). The hypoglycemic results obtained *ex vivo* confirm the results obtained in vitro. Therefore, this can be explained by the inhibition of α-amylase and α-glucosidase observed *in vitro*. Thus, more experimentation will be necessary in order to validate the same thing and to elucidate other diabetic pathways as well. Natural resources have been known by these therapeutic effects some of them have been confirmed by its action to slow down absorption of glucose by reversibly modulating the action of enzymes (α-amylase and α-glucosidase) responsible for the breakdown of complex carbohydrates into monosaccharides. On the other hand, some have the ability to slow gastric emptying to the stomach (Ali *et al.*, 2013). The decrease in blood sugar levels can be caused by the excessive secretion of insulin causing the deposition of intracellular glycogen (Uddin *et al*., 2014).

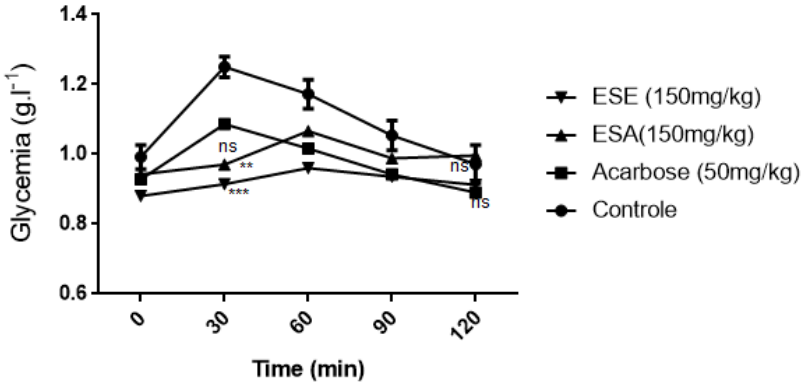
There is evidence that the generation of oxidative stress occurs as a result of depletion of antioxidants and may contribute to pancreatic cell apoptosis and hence increased diabetic complications (Khadayat *et al*., 2020).

The study of the phytochemical profile showed that *E. spinosus* is rich in terpenoids, thiophenes and sterols namely lupeyl acetate, taraxasterile acetate, lupeol, stigmasterol-bD-glucoside and b-sitosterol -bD-glucoside and two sesquiterpenoids and Echinopines A as well as fatty acids and alkanes. In addition, phytochemical analysis by HPLC-UV identified phenolic acids, the most abundant being p-coumaric (8.59 mg / kg DW), cinnamic (4.68 mg / kg DW). The most abundant flavonoids are kaempferol (30.37 mg/kg DM), quercetin and rutin in ethanol extract (Khedher *et al*., 2020).

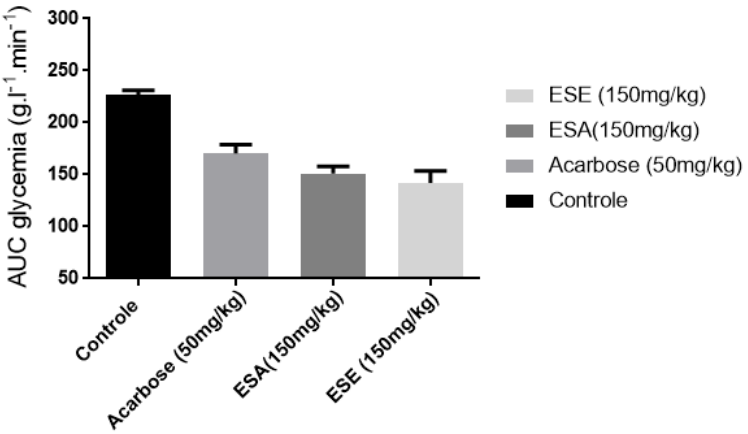
Cinnamic acid and its derivatives have been reported to be known antioxidants for their contribution to free radical scavenging, restoration of beta cell function, increased expression of glucose transporters (GLUT) and a the regulation or inhibition of enzymes involved in glucose metabolism (Ferreira *et al*., 2019). Terpenoid has been reported to have the same function of insulin, promotes intracellular glycogen deposition by stimulating glycogen synthesis and blocking glycogen phosphorylase, also improves glycogen metabolism when hepatic glycogen level is reduced (Uddin *et al*., 2014). Rutin induces reduced absorption of carbohydrates from the small intestine, suppression of tissue gluconeogenesis and formation of sorbitol, reactive oxygen species and advanced glycation end product precursors (Ghorbani, 2017).

The study of the acute toxicity of aqueous and ethanolic extracts of *E. spinosus* at a dose of 2 g/kg, no mortality was recorded and no behavioral or other changes were observed. Therefore, the oral $LD_{50}$ of *E. spinosus* is greater than 2000 mg/kg.

**Figure 1.** Effect of *Echinops spinosus* extracts and acarbose on glycemia after starch intake in normal rats (a) and with presentation in the area under curve (b). The values are means ± SEM (n = 5). *** $p$ <0.001; ** $p$ <0.01 compared with normal controls. Ns = not significant to the normal controls. ESA: aqueous extract of *E. spinosus*; ESE: ethanolic extract of *E. spinosus* and AUC: area under the curve.




(a)




(b)

## 4. CONCLUSION

This work aims to evaluate the acute toxicity, the content of phenolic compounds, the antioxidant activity with five methods (DPPH, FRAP, ABTS, $H_2O_2$ and xanthine oxidase), antihyperglycemic with three methods (α-amylase and α-glucosidase and lipase) and *ex-vivo* by the starch tolerance test of aqueous and ethanolic extracts of *E. Spinosus*. The study of *E. spinosus* extracts has demonstrated their richness in phenolic compounds, in particular flavonoids. The extracts have also demonstrated antioxidant power in vitro, postprandial antihyperglycemic. Further studies and *in vivo* antioxidant and antidiabetic pathways must be performed to confirm the effect. Moreover, a chronic toxicological and phytochemical study is necessary.

### Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

### Authorship contribution statement

**Kaoutar Benrahou**: Investigation, Resources, and Writing - original draft. **Otman El Guourrami**: Methodology, Supervision, and Validation. **Hanaa Naceiri Mrabti**: Visualization, Software, Formal Analysis. **Gokhan Zengin**: Validation. **Abdelhakim**

**Bouyahya**: Investigation, Resources, and Writing - original draft. **Yahia Cherrah**: Software, Formal Analysis. **My El Abbes Faouzi**: Software, Formal Analysis.

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