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Biochemical and Histological Improvements in Alloxan-Intoxicated Diabetic Rats via Administration of *Vitex agnus*-cactus Seed Extract

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ABSTRACT: Natural resources constitute an exhaustible source of bioactive compounds well-known for their antidiabetic properties. This study investigated the antioxidant and antidiabetic activities of *Vitex agnus* citratus extract against alloxan-induced metabolic disorders in an animal model. The antioxidant ability of the extract was evaluated using three complementary assays (TAC, DPPH, and FRAP), and its total phenolic and flavonoid contents were determined calorimetrically. The in vivo experiment included five groups: group 1 received distilled water (10ml), group 2 received alloxan (150 mg/kg) and distilled water (10ml), group 3 received alloxan (150 mg/kg) and *V. agnus* cactus aqueous extract (200mg/kg bw), group 4 received alloxan (150 mg/kg) and VAC aqueous extract (300mg/kg bw), and group 5 received alloxan (150 mg/kg) and glibenclamide. The doses were administered once daily via gavage for four weeks. Blood glucose levels, hepatic enzymes, uric acid, urea, creatinine, and electrolytes were analyzed after four weeks of treatment. The antioxidant profile of VAC extract revealed considerable phenolic and flavonoid contents and an impressive antioxidant ability (37.6 ± 0 .73 mg EAA/g, 0.54±0.46 mg/mL, and 0.7±0.03 mg/mL for TAC, DPPH-IC50, and FRAP, respectively). Treatment of rats with *V. agnus* cactus extract significantly improved all changes induced by alloxan injection by controlling hyperglycemia, liver and kidney disorders, and serum electrolyte levels. Therefore, using *V. agnus* cactus would have pleiotropic effects in preventing metabolic disorders induced by alloxan.

1. INTRODUCTION

Diabetes mellitus is a common metabolic disorder involving carbohydrates, proteins, and fat metabolism, with micro-and macrovascular complications cr. It is considered one of the five major causes of death worldwide (Kumar et al., 2006; Vats et al., 2004). Diabetes presents a challenge to healthcare systems worldwide.

Diabetes mellitus can be classified into two major types: type 1 and type 2 diabetes. Type 1 or insulin-dependent diabetes mellitus (IDDM) results from autoimmune destruction of pancreatic islet β cells, and Type 2 or noninsulin-dependent diabetes mellitus (NIDDM) is characterized by the development of insulin resistance (Sánchez-Zamora & Rodriguez-Sosa, 2014).

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Several reports have indicated that chronic hyperglycemia associated with diabetes increases oxidative stress by over-producing reactive oxygen species (ROS), thereby decreasing antioxidant defense system activity and promoting free radical generation (Tiwari et al., 2014). The rise of free radicals leads to oxidative damage of the cell's components (Lipids, DNA, and proteins), which causes complications affecting multiple organs, such as the eyes, kidneys, nerves, and blood vessels (Robertson, 2004).

Diabetic nephropathy is the leading cause of late-stage kidney disease and is characterized by structural and functional renal abnormalities in patients with diabetes. It affects 20%–30% of patients with diabetes (Al-Waili et al., 2017; Thorp, 2005). Oxidative stress is an essential contributor to kidney damage (Al-Waili et al., 2017). Therefore, secondary metabolites with antioxidant properties can play a major role in preventing and reducing the progression of diabetic nephropathy.

Currently, there is an urgent demand among patients with diabetes to use natural products with antidiabetic effects as an alternative to synthetic hypoglycemic agents due to their lower side effects (Ahmed et al., 2010). Medicinal plants possess curative properties enhanced by secondary metabolites, such as flavonoids, essential oils, tannins, saponins, and alkaloids (K. Rashed & Butnariu, 2021; K.N. Rashed & Butnariu, 2014). Several traditional medicinal plants have hypoglycemic effects, such as Chamaerops humilis, Centaurium erythrea, Morinda lucida, Allium ursinumwatery and Zanthoxylum chalybeum (Agwaya & Nandutu, 2016; Gaamoussi et al., 2010; Kazeem et al., 2013; Putnoky et al., 2013; Sefi et al., 2011).

Vitex agnus castus is a medicinal plant widely distributed in the Mediterranean, Europe, and Central Asia, commonly known as the chaste tree (Costa et al., 2015; Ono et al., 2008), and belongs to the Verbenaceae family. *Vitex agnus* castus is an intensely aromatic shrub along the rivers in Morocco. It is locally named "Angarf-lkrwaa" and "shajarat Mariam" (M.D.G. Miguel et al., 2014).

Traditionally, the chaste tree has been used to treat various women's conditions, such as uterine cramps, menstrual disorders, insufficient lactation, and acne (Chhabra & Kulkarni, 2011; Wuttke et al., 2003). Previous phytochemical analyses of *V. agnus*-castus L. revealed the presence of different bioactive compounds, including flavonoids, iridoids, and diterpenoids (Allahtavakoli et al., 2015; Neves et al., 2016). Many reports have demonstrated that different parts of *Vitex agnus* castus exhibit antifungal, antibacterial, antiepileptic, cytotoxic, and antioxidant activities (Asdadi et al., 2015; Aslantürk et al., 2013a; Kamari et al., 2018; Saberi et al., 2008). The diuretic properties of VAC seeds have recently been reported (F et al., 2021). Incorporating natural resources into the daily diet could be paramount in preventing the development and pathogenesis of various chronic diseases.

Until now, no study has been conducted on the aqueous extract of *Vitex agnus* castus in alloxan-induced diabetic rats. This study aimed to determine the total phenolic and flavonoid content antioxidant activities and to examine the antihyperglycemic and nephron-hepatoprotective effects of the aqueous extract of Moroccan *Vitex agnus* castus in non-diabetic and alloxan-induced diabetic rats.

2. MATERIAL AND METHOD

2.1. Collection and extract preparation

Seeds of *Vitex agnus* castus were collected in April 2019 from the Khenifra area (Latitude: 32°56′05″ Nord; Longitude: 5°39′42″ Ouest; altitude: 827 m). Pr. Bari Amina identified the botanical name. A voucher specimen with reference number 2298/4- 16-2/Kh was deposited in the Department of Biology of the same university.

Vitex agnus castus seed extract was prepared as it is traditionally used in Moroccan folk medicine. Briefly, seeds were dried in the dark at room temperature and ground to a coarse powder in an electric grinder. Then, 50 g of the dried powder was mixed with 500 mL of distilled water and boiled at 100 °C under reflux for 30 min. The decoction obtained was centrifuged, the final extract was filtered (Whatman, nº1), and the supernatant was collected for in vitro tests. Subsequently, the filtrate was concentrated in a rotary evaporator. Distilled water was added to prepare the chosen concentrations (200 and 300 mg/kg b.wt) for animals' treatments.

2.2. Bioactive Compounds and Antioxidant Activities

2.3. Total Phenolic Content (TPC)

The amount of polyphenol content was determined following the Folin–Ciocalteau method described by Laaroussi et al. (2020). 100 μL of aqueous seeds extract was mixed with 500 μL of Folin–Ciocalteau (0.2 N) reagent and 400 μL of sodium carbonate solution. Gallic acid was used as a standard to achieve the calibration curve, and the result was expressed in milligrams of Gallic acid equivalent per gram of the sample (mg GA/ g DW).

2.4. Total Flavonoid Content (TFC)

Total flavonoid content was quantified colourimetrically as described by Bakour et al. (2018). Briefly, 200 μL of aqueous seeds extract was mixed with sodium nitrite (5%) and 150 μL of Alcl3 solution (10%), 200 μL of NaOH (1%) 1 M was added after 5 min, and absorbance of the reaction mixture was measured at 510 nm. The results were expressed as milligrams of the quercetin equivalent per 100 grams of the sample (mg QE/g DW).

2.5. Total Antioxidant Capacity (TAC)

The total antioxidant capacity of seed extract was evaluated using the phosphomolybdenum method, following the procedure described by Bakour et al. (2018). 25 μL of aqueous seeds extract was mixed with 1 mL of reagent solution (6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After 90 min of incubation in a water bath at

95°C, the absorbance of the produced color was measured at 695 nm. Ascorbic acid was used as the standard calibration. The results were expressed as milligrams of ascorbic acid equivalent per gram of the sample (mg AAE/g DW).

2.6. Free radical scavenging activity (DPPH assay)

Free radical scavenging activity (DPPH assay) was measured according to the method described by M. Miguel et al. (2014). Briefly, 875 μL of 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) solution (150 μM, prepared in methanol with an absorbance of 0.700 $\pm$ 0.01 at 515 nm) was added to 25 μL of different extract concentrations, and the mixture reaction was incubated in the dark for one hour. The absorbance was read at 517 nm, and the antiradical activity (% inhibition) was estimated using Equation 1. DPPH inhibition concentration at 50% (IC_{50}) was determined using six different aqueous seed extract concentrations, considering that the percent inhibition had to be between 20% and 80%.

% inhibition= (Abs control-Abs sample)/ (Abs control) $\times$100 (1).

2.7. Ferric Reducing Power Assay

The reducing capacity of the tested extract was determined by Oyaizu (1986). The absorbance was measured at 700 nm. Quercetin was used as a standard. The results were expressed as IC_{50} (mg/ml). IC_{50} (concentration corresponding to 0.5 absorbance) was calculated by plotting the absorbance against the corresponding concentration.

2.8. Induction of Diabetes Mellitus and Experimental Design

Hyperglycemia was induced in overnight fasted male Wistar rats via intraperitoneal administration of alloxan (150 mg/kg bwt) dissolved in normal saline. After 72h of alloxan injection, only rats with glycemia $\geq$ 250 mg/dl were considered people with diabetes and used in this study.

Thirty male Wistar rats weighing 160.07$\pm$6.15 g, obtained from the Animal Housing Breeding Center, Department of Biology, YY, were used for these experiments. Rats were kept in a ventilated room and lived in standard environmental conditions (22$\pm$3°C, 55 $\pm$ 5% humidity, 12h light/dark cycles).

Rats were randomly allocated into five groups, six rats in each group. The treatments and procedures applied are as follows:

Group1: The control group (NC) received distal water (10 ml/kg b.wt),

Group 2: The untreated diabetic group received distilled water.

Group 3: The diabetic untreated group received daily by gavage the aqueous extract of *Vitex agnus* castus seeds (200 mg/Kg /b.wt) for four weeks.

Group 4: The diabetic untreated group received daily by gavage the aqueous extract of *Vitex agnus* castus seeds (300 mg/Kg /b.wt) for four weeks.

Group 5: Diabetic untreated group, received daily by gavage glibenclamide (2.5 mg/Kg /b.wt).

At the end of the experiment, the rats fasted for 12 hours after their last feeding, the urine samples were collected, the blood samples were taken from each rat by retro-orbital bleeding under ether anaesthesia, and then plasma was separated by centrifugation (2000 x g) for 10 min.

2.9. Biochemical analysis

Blood samples were analyzed for glucose, Aspartate aminotransferases (AST), Alanine aminotransferases (ALT) using kit number 7D56-20, Lactate dehydrogenase (LDH) using kit number 7D69-20, Alkaline phosphatase (ALP) using kit number 7D55-20, Urea using kit number 7D75-30, urease/NADH method, Uric acid using kit number 7D76-20 uricase/POD method, Creatinine using kit number 7D64-20 picric acid/NaOH method, Albumin using kit number 7D53-20 bromocresol green method, Total protein using kit number 7D73-20 biuret method, Sodium (Na+), potassium (K+) and chloride (Cl^-). Urine samples were analyzed for sodium, potassium, and chloride.

2.10. Histopathological analysis

Rat livers were fixed in 10% formalin solution for 24h, after which the organs were dehydrated with ethanol at progressively higher concentrations, clarified in toluene, and finally embedded in paraffin. The acquired paraffin blocks were sectioned into fine slices (7mm) using a microtome. The slides were stained with hematoxylin and eosin to allow examination using an optical microscope.

2.11. Statistical analysis

Statistical comparisons between the groups were performed with a one-way analysis of variance (ANOVA) followed by a Turkey test using GraphPad Prism® software (version 5.0; GraphPad Software, Inc., San Diego, USA). Data were represented as mean $\pm$ SD (* p <0.05, ** p <0.01, *** p <0.001).

3. RESULTS

3.1. Total Phenol and flavonoid contents and antioxidant activity of *V. agnus* cactus aqueous extract

Table 1 summarizes the results of the antioxidant profile of VAC extract using five assays. The chemical analysis showed that the VAC extract contains 78.14$\pm$1.71 mg GAE/g DW of total phenolic content and total flavonoid content of 56 $\pm$ 0.91 mg QE/g DW. The total antioxidant capacity is 37.6 $\pm$ 0 .73 mg EAA/g DW. The antioxidant capacity of the aqueous VAC extract revealed significant antioxidant potential with an IC50 of 0.54$\pm$0.46 mg/mL and 0.7$\pm$0.03 mg/mL for the DPPH and FRAP assays, respectively.

3.2. The hypoglycemic effect of the *Vitex agnus* castus extract and glibenclamide

Table 2 represents the results of the glycemia levels obtained by the different groups. Results revealed that the administration of alloxan caused a significant increase in blood glucose level

Table 1
Total phenolic and flavonoid contents and antioxidant activities of the VAC extract.

Sample	TPC (mg GAE/g DW)	TFC (mg QE /g DW)	TAC (mg EAA/g DW)	IC_{50} DPPH (mg/ml)	IC_{50} FRAP (mg/ml)
Aqueous extract	78.14±1.71	56 ± 0.91	37.6 ± 0 .73	0.54 ± 0.46	0.7 ±0.03

($p<0.05$) (382 ± 19 mg/dl) compared with the control group. The administration of two doses of seed extract (200 and 300 mg/kg) for four weeks significantly decreased glycemia (186 ± 21 and 162± 14 mg/dl respectively) ($p<0.05$). There are no statistically significant differences between the groups that received two doses of seed extract and the group that received glibenclamide.

3.3. Effects of interventions on liver function and LDH

Table 3 shows plasmatic hepatic enzyme levels, including ALT, AST, LDH, and PAL. It clearly showed that alloxan induced a significant increase in hepatic enzyme levels (ALT, AST, LDH, and PAL) compared with the control group ($p<0.05$). Co-treatment with two doses of VAC extract reversed the changes in hepatic enzyme levels promoted by alloxan administration. Interestingly, the higher dose (300mg/kg) of VAC extract significantly reduced the hepatic enzyme levels. Both doses reduced the hepatic enzyme levels in a dose dependent manner.

3.4. Effects of interventions on kidney function

Table 4 summarizes the concentrations of urea, uric acid, creatinine, Albumin, and total protein in the serum of the different experimental groups. Regarding kidney function, alloxan significantly increased urea, uric acid, and creatinine levels and significantly decreased Albumin and total protein levels compared with group 1. The administration of both doses caused a significant decrease in urea, uric acid, and creatinine levels compared with group 2. The comparison of the effects of glibenclamide and the higher dose was significant only for urea and uric acid ($p<0.05$).

3.5. Effect of alloxan, glibenclamide and plant extract on plasma and urine electrolytes

Table 5 summarizes the results obtained for plasma and urine electrolytes (sodium, potassium, and chloride) of different groups under study. It is observed that there are no significant changes in serum electrolyte levels (sodium, potassium, and chloride) in the alloxan-intoxicated rats compared with the control group. However, the administration of alloxan significantly increased the urine sodium, potassium, and chloride levels. The administration of both seed extract doses significantly increased urine sodium, potassium, and chloride elimination without inducing hypokalemia. Electrolyte elimination was in a dose-dependent manner. The administration of glibenclamide restored electrolyte excretion to normal levels compared to the control group.

3.6. Effect of diabetes, glibenclamide and Plant Extract on the body weight

Table 6 representsthe changes in body weight of animals in the different studied groups. The group treated with alloxan showed a significant decrease in the body weight of rats ($p<0.05$). Body weight gain was mitigated dose-dependently by administering both dosages of seed extract.

3.7. Histopathological study

Figure 1 displays the histological analysis of the livers of animals from different groups. Figure 1A presents the liver tissue of normal rats characterized by hexagonal lobules and acini centered on the vein with normal structure. The administration of alloxan at a dose of 150 mg/kg bw induced mild congestion with disorganization of liver architecture and central vein dilatation (Figure 1B). Organ sections from diabetic rats treated with the extract (150 mg/kg dw and 300 mg/kg dw) restored the normal liver architecture dose-dependent (Figure 1C and D). At the same time, the histopathological analysis of the liver of animals treated with glibenclamide revealed disorganization of liver tissue (Figure 11E).

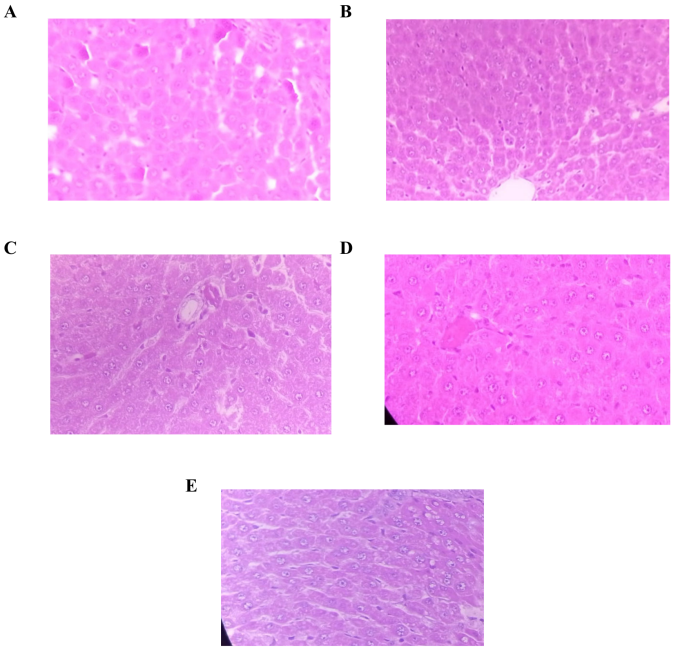

Figure 1. Histopathological liver tissue (A): group control, (B): Untreated diabeticrats, (C): Diabetic rats treated with 150 mg/kg, (D): Diabetic rats treatedwith 300 mg/kg, and (E): Diabetic rats treated with glibenclamide.

Table 2
The effect of daily oral administration of the interventions on blood glucose levels.

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Blood glucose levels (mg/dl)	105 ± 5 b***	382 ± 19 a***	186 ± 21 a** b***	162± 14 a* b***	154 ± 10 b***

a: comparison between group 1 and all groups, b: comparison between group 2 and all groups, c: comparison between group 3 and group 5, d: comparison between group 4 and group 5 (The significance started at p< 0.05).

Table 3
Effect of the interventions on liver function and lactate dehydrogenase in control and experimental animals.

Experiment groups	ALT(U/L)	AST(U/L)	LDH(U/L)	PAL(U/L)
Group1	71.18 ± 3.85 b**	131.14 ± 5.45 b**	45.00 ± 3.17 b**	40.52 ± 4.65 b*
Group 2	93.58 ± 4.01 a**	175.40 ± 7.17 a**	72.19 ± 4.15 a**	47.25 ± 4.16 a*
Group 3	82.36 ± 3.46	150.13 ± 5.38 b*	67.46 ± 2.75 a**	44.69 ± 3.57
Group 4	77.10 ± 4.15 b*	139.37 ± 6.22 b**	45.58 ± 2.87 b**	40.63 ± 2.82 b*
Group 5	72.11 ± 4.70 b**	135.51 ± 5.91 b**	45.95 ± 3.49 b** d**	40.97 ± 4.11 b*

ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: Lactate dehydrogenase; PAL: Alkaline phosphatase, a: comparison between the group 1 and all groups; b: comparison between group 2 and all groups, c: comparison between group 3 and group 5, d: comparison between group 4 and group 5 (the significance started at p < 0.05).

Table 4
Effect of interventions on Urea, Uric Acid, Creatinine, Albumin and Total protein levels in control and experimental animals.

Experiment groups	Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)	Albumin (mg/dl)	Total protein (mg/dl)
Group 1	37.18 ± 2.57 b***	23.72 ± 1.18 b**	0.90 ± 0.04 b***	3.9 ± 0.08 b***	6.4 ± 0.08 b*
Group 2	85.09 ± 6.82 a***	35.86 ± 1.42 a**	1.78 ± 0.18 a***	2.7 ± 0.05 a***	4.3 ± 0.04 a*
Group 3	58.26 ± 5.13 a* b**	30.10 ± 1.94 a*	1.21 ± 0.11 a* b**	3.4 ± 0.05 a** b***	5.7 ± 0.07
Group 4	49.36 ± 2.72 b**	29.25 ± 1.36 a* b*	1.16 ± 0.05 b**	3.5 ± 0.08 a* b***	6.0 ± 0.05 b*
Group 5	38.12 ± 3.37 b*** d*	25.34 ± 1.13 b** d*	0.97 ± 0.08 b***	3.6 ± 0.07 a* b***	6.6 ± 0.04 b*

a: comparison between group 1 and all groups, b: comparison between group 2 and all groups, c: comparison between group 3 and group 5, d: comparison between group 4 and group 5 (the significance started at p < 0.05).

Table 5
Effect of alloxan,Glibenclamide and plant extract on Plasma and urine Electrolytes (Sodium,Potassium, and Chloride).

Groups	Plasma concentration of ions (mEq/L)			Urinary concentration of ions (mEq/L)		
	Sodium	Potassium	Chloride	Sodium	Potassium	chloride
Group 1	140.53 ± 1.9	8.11 ± 0.5	119.88 ± 1.8	86.58 ± 1.0 b***	67.32 ± 1.1 b***	98.12 ± 0.9 b**
Group 2	141.28 ± 2.1	8.18 ± 0.4 a	117.56 ± 1.4	130.12 ± 2.1 a***	101.03 ± 1.4 a***	108.54 ± 1.3 a**
Group 3	140.64 ± 1.7	8.09 ± 0.9	120.01 ±1.5	128.22 ± 1.8 a***	80.67 ± 1.2 a*** b***	104.86 ± 1.5 a**
Group 4	141.01 ± 1.6	8.13 ± 0.4	119.92 ± 1.7	98.76 ± 1.3 a** b***	72.26 ± 0.9 a* b***	98.40 ± 1.1 b**
Group 5	140.59 ± 1.9	8.18 ± 0.4	112.10 ± 1.1	85.27 ± 1.2 b*** C***d**	68.02 ± 1.3 b*** C***	98.56 ± 1.4 b** C*

a: comparison between group 1 and all groups, b: comparison between group 2 and all groups, c: comparison between group 3 and group 5, d: comparison between group 4 and group 5 (the significance started at p < 0.05).

Table 6
Effect of Interventions on body weight

Experimental groups	Initial BW	Final BW	BW gain	% of change
Group 1	152.15 ± 4.87	175.84 ± 6.42b***	23.69 ± 4.34	-
Group 2	163.27 ± 5.26	122.18 ± 5.94a***	-41.09 ± 3.11	-30.52
Group 3	161.18 ± 8.13	169.34 ± 5.60a* b***	8.16 ± 2.04	38.59
Group 4	155.59 ± 6.45	168.50 ± 6.24a* b***	12.59 ± 1.63	37.91
Group 5	168.20 ± 5.95	185.76 ±7.34a** b*** c***d***	17.56 ± 3.35	52.03

a: comparison between group 1 and all groups, b: comparison between group 2 and all groups, c: comparison between group 3 and group 5, d: comparison between group 4 and group 5 (the significance started at p < 0.05).

4. DISCUSSION

Quantifying the phytochemical content of natural products constitutes the first step in predicting their beneficial properties in vitro and in vivo. Preliminary quantification of the phytochemical content of *Vitex agnus* castus revealed considerable phenolic and flavonoid contents. These compounds are well known for their tremendous health benefits and preventive and curative effects (Asdadi et al., 2015; Aslantürk et al., 2013b; M. Miguel et al., 2014; Tiwari et al., 2014). This plant is expected to have numerous beneficial properties within this framework, such as antihyperglycemia and antioxidant ability. The extraction was performed using water, widely used in Moroccan traditional medicine (Bellakhdar, 1997).

Among the various extracts of different parts of Vitex, the seeds and stem extracts revealed the lowest phenolic content compared with other parts (Leaf, root, and flower) (Berrani et al., 2021). The results obtained from this study are higher than those reported by Berrani et al. (2021). A previous study found that *Vitex agnus* contains 34 chemical compounds, including luteolin, 3,4-dihydroxybenzoic acid, vanillic acid, chlorogenic acid, hesperidin, 4,5-Dichloro-1,3-dioxolan-2-one, 1H-Indene, 2,3-dihydro-1,1,2,3,3-pentamethyl, and Isobutyl 4-hydroxybenzoate, which were suggested to have high antioxidant potential (Ababutain & Alghamdi, 2021; Berrani et al., 2021; Fo et al., 2022). Plant extract acts as a multifaceted agent through its ability to delay or inhibit the actions of reactive oxygen species. The examination of the antioxidant ability of Vitex seed extracts revealed an important antioxidant ability as evaluated by two complementary tests. Our results agree with those reported by Berrani et al. (2021).

Several studies have explored the association between the bioactive compounds of different natural products and diabetes and its complications (Gowd et al., 2017; Oh & Jun, 2014; Santangelo et al., 2016; Sharifuddin et al., 2015). The antidiabetic effect of *Vitex agnus* has been evoked by several authors, and its ability to counteract the adverse effects of Streptozotocin and D-galactose (Ahangarpour et al., 2017; Berrani et al., 2018; Soleymanzadeh et al., 2020). Our study evaluated the hypoglycemic effect of *Vitex agnus* seed extract in diabetic rats induced by alloxan. The current study showed that the administration of both studied doses (200 and 300 mg/kg bw) sustained for four weeks decreased blood glucose, hepatic enzyme, urea, creatinine, and uric acid levels in a dose-dependent manner.

The administration of VAC hydroalcoholic extract twice a day during the last seven days of D-galactose treatment in mice sustained 45 days later significantly decreased serum glucose levels and increased insulin levels (Ahangarpour et al., 2017). In the same context, Stella et al. found that Vitex extract controls blood glucose levels in different ways, including modification of glucose cell use and stimulation of the regeneration process of pancreatic cells (Stella et al., 2011). Vitex extract has been demonstrated to improve D-galactose-induced metabolic disorder in mice aging-model by affecting glucose transport carriers and insulin resistance (Ahangarpour et al., 2017). Postprandial hyperglycemia control is a target of multiple diabetes therapeutic drugs that delay glucose intestinal absorption (Perry et al., 2018). A previous study reported that Vitex extracts affect α-glucosidase and β-galactosidase activities, decreasing serum glucose levels (Berrani et al., 2021).

VAC could significantly reduce hepatic enzyme levels in a dose-dependent manner. In contrast, alloxan increases hepatic enzyme levels as a sign of liver damage, elevating hepatic enzyme leakage (Bakour et al., 2018). Alloxan was found to induce ß-cell islet destruction and oxidative stress with severe pancreatic damage surpassing the ability of the self-defence antioxidant system (Jahan et al., 2021; Meeran, 2019). Oxidative stress is a common factor that induces organ problems through the overproduction of reactive oxygen species (ROS). Within this frame, we investigated in the first step the ability of VAC to attenuate free radicals in vitro using three complementary tests (DPPH, FRAP, and TAC). The findings revealed an exciting antioxidant ability of the studied extract (0.37± 0 .73 mg EAA/g, 0.54± 0.46 mg/ml, 0.7 ±0.03 mg/ml for TAC, DPPH-IC_{50}, and FRAP-IC_{50} respectively). Indeed, administration of VAC extract for four weeks significantly decreased the plasma levels of hepatic enzymes in a dose-dependent manner (Groups 3 and 4). An experimental study on ovariectomized rats treated with the crude and butanol fraction of *Vitex agnus* reduced the adiposity index. It ameliorated nonalcoholic fatty liver disease by restoring the activities of antioxidant enzymes and reducing hydrogen peroxide production in mitochondria (Moreno et al., 2015). Similarly, Hamza et al. tested the impact of VAC extract on serum oxidative stress markers. The authors found that the VAC extract significantly downregulated MDA and elevated catalase levels in an experimental rat model (Hamza et al., 2019).

Regarding kidney function, the administration of *V. agnus* cactus extract significantly reversed the adverse effects of alloxan on kidney function, reducing urea, uric acid, and creatinine. Results agree with those reported by Elsemelawy et al. (2017). Oroojan et al. found that administering *V. agnus* cactus hydroalcoholic extract attenuated histopathological damage in D-galactose-induced aging model mice (Oroojan et al., 2016). The nephroprotective effect of *V. agnus* cactus against different toxic agents was established by reducing ROS production and suppressing inflammation in kidney tissue samples. These effects may be related to the antioxidant effects of VAC. Several studies have proved that *V. agnus* cactus contains numerous bioactive compounds, particularly Pendleton, casticin, orientin, vitexin, aucubin agnus, luteolin 6-C-(4"-methyl-6"-O-transcaffeoylglucoside), luteolin 6-C-(6"-Otranscaffeoylglucoside), luteolin 6-C-(2"-O-transcaffeoylglucoside), and luteolin 7-O-(6"-pbenzoylglucoside), together with four known compounds 5, 4'- dihydroxy-3, 6, 7, 3-tetramethoxyflavone, luteolin, artemetin, and isorhamnetin (Berrani et al., 2021; Certo et al., 2017; Mari et al., 2015, 2012). Reducing postprandial hyperglycemia is a treatment strategy that impedes glucose absorption (Perry et al., 2018). Numerous authors have

previously claimed that C.agnus cactus can significantly impact food digestion by controlling α-glucosidase and α-amylase activities (Berrani et al., 2021; Boujbiha et al., 2023). It has been found that one of the major components of VAC (chlorogenic acid) significantly increases insulin expression in islet cells, inhibits apoptosis and downregulates the expression of related mRNA in the advanced glycation end products (AGE-RAGE) signaling pathway in RIN-m5f cells (Neves et al., 2016). A recent study revealed that treatment of streptozotocin-intoxicated rats with luteolin restored the physiological structure of pancreatic tissues by suppressing streptozotocin-induced congestion and necrosis of tissues (Kahksha et al., 2023). All phytochemical compounds of VAC act synergistically to attenuate the harmful effects of oxidative stress through their ability to scavenge and attenuate free radicals.

5. CONCLUSIONS

Vitex agnus citratus contains considerable phytochemical contents that are highly associated with its health benefits. The outcomes of this study show that *V. agnus* cactus exhibits high antioxidant and antidiabetic potential. This plant could be used as a natural remedy to prevent and/or treat the negative consequences of oxidative stress and toxic agents due to its dense chemical composition, which exhibits high antioxidant potential.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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ETHICAL APPROVAL

Ethical approval (USMBA-SNAMOPEQ 2019–05) was obtained from Sidi Mohamed Ben Abdellah University, Fez, under the responsibility of the Animal Facility and the Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life, University of Sidi Mohamed Ben Abdellah, Fez, Morocco. The animals care and handling were in accordance with the internationally accepted standard guidelines for the use of animals.

AUTHOR CONTRIBUTIONS

Fatima El Kamari, Driss Ousaaid, and Hassan Laaroussi: Research concept and design, collection and/or assembly of data, Formal analysis, Data curation, Investigation, Writing-original draft, Writing-review & editing, and Visualization. Abdelghani Bouchyoua, Amina Chlouchi, Anjoud Harmouzi, Najia El Hamzaoui, and Jihane El Amrani: Writing—review and editing and visualization. Badiaa Lyoussi: supervision, editing, and review.

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