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In vitro study of protective effect of *Pterocarpus erinaceus* Poir. stem bark and *Amaranthus spinosus* L. root extracts on cataractogenesis and glomerulopathy

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Abstract

Background: Cataracts and glomerulopathy are disabling complications of diabetes mellitus. The use of medicinal plants in the treatment of diabetes helps prevent or delay these complications. *Pterocarpus erinaceus* and *Amaranthus spinosus* are part of these medicinal plants used in traditional medicine to treat diabetes and its complications. The aim of this study was to evaluate in vitro the protective effect of the dried hydroethanolic extracts of the two plants against cataractogenesis and glomerulopathy induced by high glucose. Lenses and kidney fragments from Sprague–Dawley rats were cultured in artificial aqueous humor and in glomerular solution under high glucose, respectively. The extracts of the two plants at doses of 250 and 500 mg/kg bw were added to the culture medium and incubated for 72 h for the lenses and 96 h for the kidney fragments. Morphological and biochemical parameters were evaluated during the cultures.

Results: The lenses treated with extracts of both plants remained shiny and transparent in contrast to the opacity observed in toxic controls. The treatment by extract caused a significant ($p < 0.001$) decrease in malondialdehyde and a significant increase ($p < 0.001$) in glutathione and catalase levels in lenses and kidney fragments. The extracts also caused a significant increase ($p < 0.0001$) in glucose absorption in glomeruli and a decrease in tubular glucose reabsorption in kidney fragments. This led to a decrease in glucose in the incubation medium.

Conclusion: These findings showed that the dried hydroethanolic extract of *P. erinaceus* and *A. spinosus* can be used to reduce hyperglycemia effects by inhibiting oxidative stress pathways and then preventing or delaying the onset of cataracts and kidney failure in diabetes.

Keywords: Medicinal plants, Hydroethanolic extract, Diabetes complications, High glucose, Lens opacity, Kidney fragments

Background

Cataracts and glomerulopathy are diseases that lead to blindness and kidney failure. Cataract is characterized by opacity of the lens and results in blindness. Risk factors for developing cataracts can include age, diabetes mellitus, medications, ultraviolet radiation, smoking, alcoholism, nutrition, and sex (Zhang et al. 2020). Obesity and diabetes are an important risk factor for chronic kidney disease (Gnudi 2012; Xu et al. 2017). Glomeropathy is identified by persistent albuminuria, which is an important predictor for kidney disease progression and end stage (Karalliedde and Viberti 2010). Cataracts and glomerulopathy secondary to diabetes are complications caused by chronic hyperglycemia, which activates an abnormal metabolism and leads to

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endoplasmic reticulum (ER) stress (Naveen and Baskaran 2018). Activation of stress pathways induces an increase in membrane lipoperoxidation, a decrease in endogenous antioxidants and then tissue damage (Smith et al. 2015; Liang et al. 2020). Thus, drugs that cause decrease in hyperglycemia and stress pathways would prevent or delay the onset of cataractogenesis and glomerulopathy.

In traditional medicine, some of the plants used to treat diabetes have antihyperglycemic and antioxidant activities. *Pterocarpus erinaceus* Poir. and *Amaranthus spinosus* L., respectively, from the Fabaceae and Amaranthaceae families are medicinal plants traditionally used to treat several diseases including diabetes. The bark of the stem of *P. erinaceus* and the roots of *A. spinosus* are traditionally used as an infusion or decoction to treat this disease (Atchou et al. 2020; Lawson-Evi et al. 2021). Toxicological studies of *P. erinaceus* stem bark and *A. spinosus* roots reported that extracts of both were safe with a $LD_{50} > 5$ g/kg bw and no histopathological impact in subchronic oral administration, supporting their use in traditional medicine (Kumar et al. 2014; Atchou et al. 2021). Our previous study showed that extracts of *P. erinaceus* stem bark and *A. spinosus* roots decreased significantly in blood glucose levels in mice and exerted antioxidant activities (Atchou et al. 2020). Thus, we hypothesized that *P. erinaceus* and *A. spinosus* prevent against blindness and renal failure by decreasing in stress pathways and subsequently inhibit cells apoptosis. The aim of this study was to assess in vitro the effects of the dried hydroethanolic extracts of the two plants on diabetes complications such as glomerulopathy and cataracts. This study will prove, in addition to the hypoglycemic activity of *P. erinaceus* and *A. spinosus* previously reported, the effects of the extracts in the prevention and delay of diabetes complications.

Methods

Preparation of the extract

The fresh bark of the stem of *Pterocarpus erinaceus* Poir. and the roots of *Amaranthus spinosus* L. were collected, respectively, in the prefectures of Ave and Gulf (Togo). Both plants were identified in Laboratory of Botany and Plant Ecology of Faculty of Science, University of Lome. Voucher specimens were deposited in the herbarium as herbarium specimens No. 15515 TOGO (*P. erinaceus*) and No. 15516 TOGO (*A. spinosus*). Bark and roots were dried at laboratory temperature (20 ± 2 °C) protected from light for two weeks, then powdered. The powder was macerated in 50% hydroethanolic solution for 72 h and then filtered through Whatman filter paper (Ø 150 mm) as described in our previous study (Atchou et al. 2020). Finally, the filtrate was evaporated to dryness at a temperature of 45 °C, using a rotary evaporator. The choice of the bark of the stem of *P. erinaceus* and the roots of *A. spinosus* as plant material was based on their traditional use to treat diabetes (Atchou et al. 2020).

Preparation of solutions

Artificial aqueous humor (AAH) was prepared as described by Kurmi et al. (2014) followed by some modifications.

AAH; pH 7.8 = NaCl 140 mM; KCl 5 mM; $MgCl_2$ 2 mM; $NaHCO_3$ 0.5 mM; $NaH(PO_4)_2$ 0.5 mM; $CaCl_2$ 0.4 mM + 32 mg% penicillin + 25 mg% gentamicin.

Normal AAH (AAH_{Normal}); pH 7.8 = AAH + glucose 5.5 mM.

Toxic AAH (AAH_{Toxic}); pH 7.8 = AAH + glucose 55 mM.

Glomerular solution (GS) was prepared by combining the modified medium described by Economou et al. (2004) and the Kreb's-Ringer bicarbonate buffer.

GS = NaCl 118 mM + KCl 5 mM + $CaCl_2$ 1.28 mM + KH_2PO_4 1.2 mM + $MgSO_4$ 1.2 mM + $NaHCO_3$ 25 mM + 0.03% EDTA + insulin 5 µg/ml + 0.1% bovine albumin serum (BSA) + 5% animal serum (AS) + dexamethasone 50 nmol/ml + 32 mg% penicillin + 25 mg% gentamicin + 6 mg% fluconazole

Normal GS (GS_{Normal}) = GS + glucose 5 mM

Toxic GS (GS_{Toxic}) = GS + glucose 30 mM.

Lenses and kidneys extraction

Male and female Sprague–Dawley rats (150 ± 15 g) provided by the Laboratory of Animal Physiology of University of Lome were fasted for 18 h. The rats were anesthetized with ether before being killed by cervical dislocation. Then, lenses were immediately isolated by extracapsular extraction of the eyeball (Kurmi et al. 2014), and bilateral nephrectomy was performed only in male rats to remove the kidneys (Sharma et al. 2009). All experiments were performed in compliance with the institutional guidelines of the Laboratory of Physiology-Pharmacology, University of Lome (Togo) (001/2012/CB-FDS-UL).

In vitro study of cataractogenesis

Cataract was induced in vitro with a high glucose (HG) for 72 h (Kurmi et al. 2014). The isolated lenses were quickly rinsed and then pretreated in AAH_{Normal} for 24 h at the temperature of 37 °C in the presence of 95% O_2 and 5% CO_2. After incubation, lenses were observed under a magnifying glass and transparent lenses were selected. They were divided into 7 groups of 5 and then cultured in 1.0 ml of normal or toxic AAH as follows:

Group 1: AAH_{Normal} for normal control (NC);
Group 2: AAH_{Toxic} for toxic control (TC);

Groups 3 and 4: AAH_{Toxic} + *P. erinaceus* 500 and 250 mg/kg;
Groups 5 and 6: AAH_{Toxic} + *A. spinosus* 500 and 250 mg/kg;
Group 7: AAH_{Toxic} + Rutin 500 µg/kg for reference drug.

The groups were reincubated for 72 h under the same conditions, and the incubation solutions were renewed every 24 h.

Morphological evaluation of lens opacity

Lenses were wiped on absorbent paper and then weighed at the end of experiment. They were placed on the fine grids and observed with a magnifying glass for their transparency or opacity; then, images were taken (Kurmi et al. 2014).

Determination of biochemical parameters in lenses

Total proteins, malondialdehyde (MDA) and glutathione (GSH) and catalase (CAT) activity were determined in lens homogenates according to the following methods.

Total proteins were determined in lens homogenates by the method based on the binding of Coomassie brilliant blue to protein. The absorbance of the resulting complex was measured at 595 nm (Bradford 1976).

MDA, a marker of lipid peroxidation, was determined by reaction with thiobarbituric acid (TBA); then, the absorbance of the resulting color was measured at 586 nm (Patlolla et al. (2009).

GSH and catalase (CAT) are enzymes involved in the detoxification of cells. Ellman's method based on reduction of 5,5′-dithiobis- (2,-nitrobenzoic acid) by SH groups has been used to quantify GSH contained in homogenates. The yellow color resulting from the formation of nitromercaptobenzoic acid during the reaction was measured at 412 nm (Ellman 1959). Catalase activity was measured by a combination of optimized enzymatic conditions and spectrophotometric determination of hydrogen peroxide, which forms a stable complex with ammonium molybdate. The yellow color of the complex formed was measured at 405 nm (Goth 1991).

Ex vivo study of glomerulopathy

Glomerular damage was induced with high glucose (Katsoulieris et al. 2016), in kidney fragments of male rats for 96 h.

The extracted kidneys were immediately rinsed in the NaCl-EDTA solution; then, the outer capsule was removed. The decapsulated kidneys were cut into small pieces of 250 mg to expose the glomeruli and pretreated in normal glomerular solution for 2 h at the temperature of 37 °C in the presence of 95% O_2 and 5% CO_2. After incubation, kidney fragments were divided into 7 groups of 5 and then cultured in 1.0 ml of normal and toxic glomerular solution as the previously formed groups.

Groups were incubated for 96 h under the same conditions, and the incubation solutions were renewed every 24 h.

Determination of biochemical parameters in kidney fragments

Glucose from plasma is first filtered by glomeruli before being completely reabsorbed in the proximal tubule by sodium-glucose linked transporters (SGLT1, SGLT2) and glucose transporter (GLUT2) (Ghezzi et al. 2018). Glomerular absorption of glucose results in a decrease in plasma glucose. The tubular reabsorption of glucose on the contrary leads to an increase in plasma glucose.

The measurement of glucose in the incubation medium of kidney fragments makes it possible to know whether the glucose is initially absorbed by the glomeruli and then reabsorbed in a second step in the tubules. Thus, glucose was determined in the incubation solution at times t = 1 h; 2 h; 4 h; 12 h; and 24 h by the reaction with glucose oxidase (Trinder 1969).

Glucose absorbed = Initial glucose – Present glucose.

Since glucose does not appear in the urine under normal conditions,

Glucose reabsorbed = Glucose absorbed.

Initial glucose = initial concentration of glucose in incubation medium.

Present glucose = glucose concentration in the incubation medium during the assay.

Total proteins, MDA and GSH levels were determined in kidney homogenates at the end of 96-h incubation as previously.

Statistical analysis

Data were performed and analyzed by GraphPad Prism 6 Software Inc., USA. One-way and two-way ANOVA analysis was used to compare differences between groups and then considered significant at $p < 0.05$. Values were presented as mean ± SEM (standard errors of mean).

Results

Cataractogenesis

Effect of extracts on lens opacity

Lenses cultured in AAH_{Normal} under 5.5 mM glucose remained bright and transparent until the end of 72 h of incubation (Fig. 1a, b). In contrast, lenses cultured in AAH_{Toxic} under 55 mM glucose lost their brightness and developed dense opacities (Fig. 1c). At the same time, lenses cultured in AAH_{Toxic} with extracts (*P. erinaceus* and *A. spinosus*) and rutin (reference drug) remained bright and transparent (Fig. 1d–h). The extract of *P. erinaceus* and *A.*

spinosus at a dose of 500 mg/kg caused more decrease in lens opacity compared to a dose of 250 mg/kg.

Malondialdehyde levels in lenses

After 72 h of culture, MDA levels in lenses were significantly increased ($p < 0.01$) in toxic control (TC) compared to normal control (NC) (Fig. 2). Lenses cultivated in AAH_{Toxic} with extracts of *P. erinaceus* and *A. spinosus* had a significant ($p < 0.01$ to 0.0001) decrease in MDA levels compared to the toxic control (TC) (Fig. 2). Both doses of extracts (250 and 500 mg/kg) caused a significant decrease in MDA levels, but that of *A. spinosus* ($p < 0.001$) was more significant than that of *P. erinaceus* ($p < 0.01$) (Table 1). Rutin used as reference drug showed a significant ($p < 0.0001$) decrease in MDA levels in lenses (Fig. 2, Table 1).

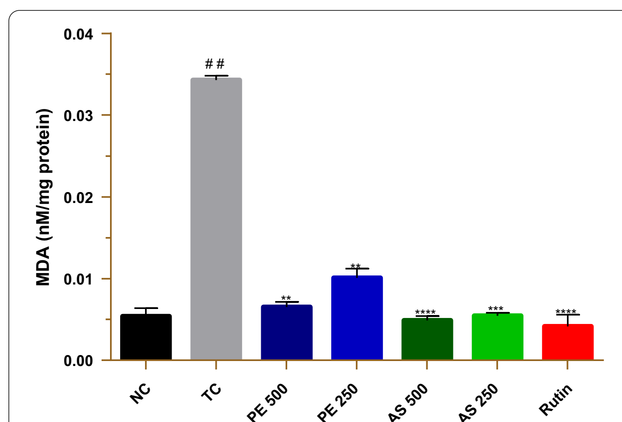

Fig. 2 Effect of *P. erinaceus* and *A. spinosus* in MDA levels in lenses. NC = normal control under 5.5 mM glucose; TC = toxic control under 55 mM glucose; PE and AS 500; 250 = *P. erinaceus* and *A. spinosus* at doses of 500 and 250 mg/kg bw + 55 mM glucose; Rutin = rutin at a dose of 500 µg/kg bw + 55 mM glucose. The values were analyzed with a one-way ANOVA and then expressed as mean ± SEM. $^{\#\#}p < 0.01$ compared to NC; $^{**}p < 0.01$; $^{****}p < 0.0001$, compared to TC. n = 5

Glutathione levels in lenses

Lenses cultured in AAH_{Toxic} under 55 mM glocose exhibited significant ($p < 0.001$) depletion in GSH compared to normal control (Fig. 3). Other hand, lenses cultivated in AAH_{Toxic} with extracts (*P. erinaceus* and *A. spinosus*) and rutin had significantly ($p < 0.01$ to 0.001) increase GSH levels compared to toxic control (Fig. 3). Extracts of *P. erinaceus* and *A. spinosus* at a dose of 500 mg/kg ($p < 0.001$) induced a greater increase in GSH level compared to the dose of 250 mg/kg ($p < 0.01$) (Fig. 3, Table 1).

Catalase activity in lenses

Catalase (CAT) activity was also significantly ($p < 0.0001$) decreased in toxic control lenses compared to normal control. In lenses treated with extracts of *P. erinaceus* at a dose of 500 mg/kg and *A. spinosus* at doses of 250 and 500 mg/kg, the CAT activity was significantly increased ($p < 0.0001$) compared to toxic control. *P. erinaceus* at a dose of 250 mg/kg induced a less increase in CAT activity. Rutin induced also significant ($p < 0.0001$) increase in catalase activity (Fig. 4, Table 1).

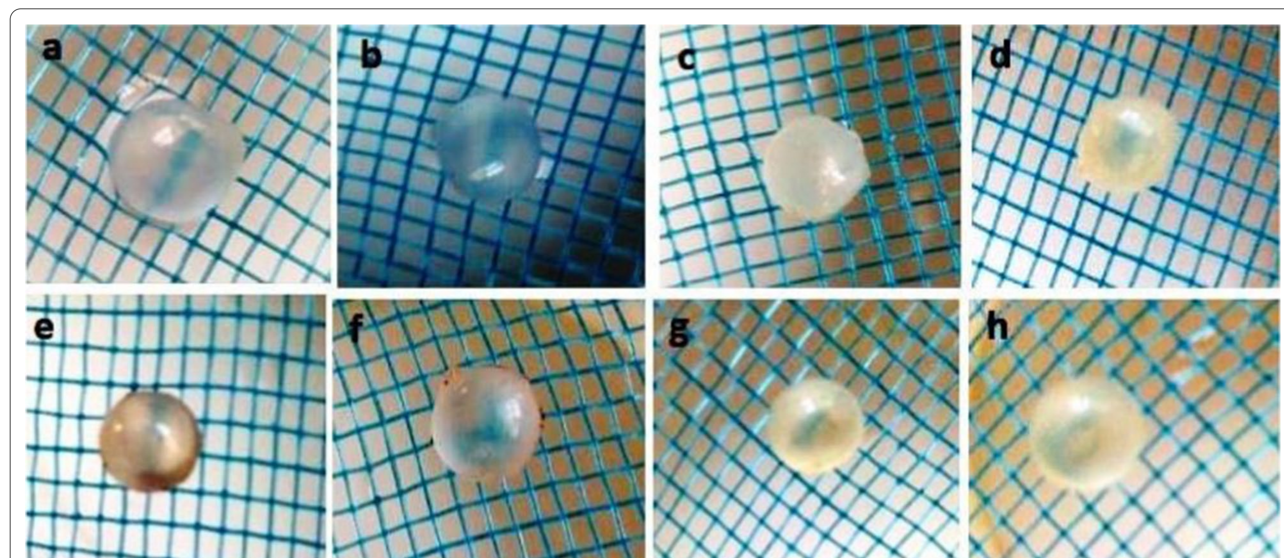

Fig. 1 Effect of *P. erinaceus* and *A. spinosus* on lens opacity. **a** = appearance before the induction of cataract. **b**; **c**; **d**; **e**; **f**; **g** and **h** = aspects after induction of cataract (72 h after). **b** = normal control uder 5.5 mM glucose; **c** = toxic control under 55 mM glucose; **d** = rutin at a dose of 500 µg/kg bw + 55 mM glucose; **e** and **f** = *P. erinaceus* at doses of 500 and 250 mg/kg bw + 55 mM glucose; **g** and **h** = *A. spinosus* at doses of 500 and 250 mg/kg bw + 55 mM glucose

Table 1 Biochemical parameters in lenses

Biochemical parameters	MDA (nM/mg protein)	GSH (µg/mg protein)	CAT (kU/l)
NC	0.0054 ± 0.0006	7.7233 ± 0.1308	6.5030 ± 0.4013
TC	0.0343 ± 0.0003##	2.8506 ± 0.1290###	1.3610 ± 0.2720####
P. erinaceus 500 mg/kg	0.0065 ± 0.0004**	6.8848 ± 0.1064***	8.8995 ± 0.6008****
P. erinaceus 250 mg/kg	0.0101 ± 0.0006**	5.0365 ± 0.1767**	4.5107 ± 0.4040**
A. spinosus 500 mg/kg	0.0049 ± 0.0003****	7.6180 ± 0.1014***	9.9106 ± 0.5719****
A. spinosus 250 mg/kg	0.0055 ± 0.0002***	3.7778 ± 0.1424**	8.6349 ± 0.4401****
Rutin 500 µg/kg	0.0041 ± 0.0008****	6.3668 ± 0.1160***	8.5134 ± 0.8131****

NC = normal control; TC = toxic control; MDA = malondialdehyde; GSH = glutathione; CAT = catalase. Values were analyzed with a one-way ANOVA and then expressed as mean ± SEM. ##$p < 0.01$; ###$p < 0.001$; ####$p < 0.0001$; compared to NC; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; compared to TC. n = 5

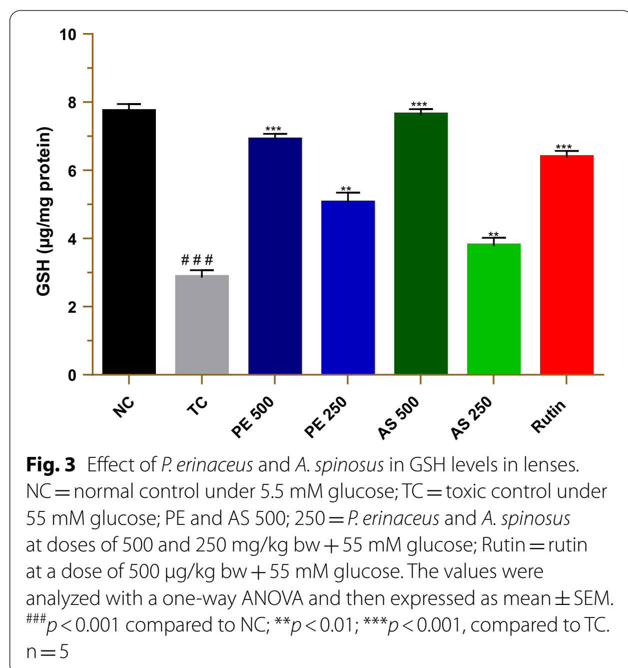

Fig. 3 Effect of *P. erinaceus* and *A. spinosus* in GSH levels in lenses. NC = normal control under 5.5 mM glucose; TC = toxic control under 55 mM glucose; PE and AS 500; 250 = *P. erinaceus* and *A. spinosus* at doses of 500 and 250 mg/kg bw + 55 mM glucose; Rutin = rutin at a dose of 500 µg/kg bw + 55 mM glucose. The values were analyzed with a one-way ANOVA and then expressed as mean ± SEM. ###$p < 0.001$ compared to NC; **$p < 0.01$; ***$p < 0.001$, compared to TC. n = 5

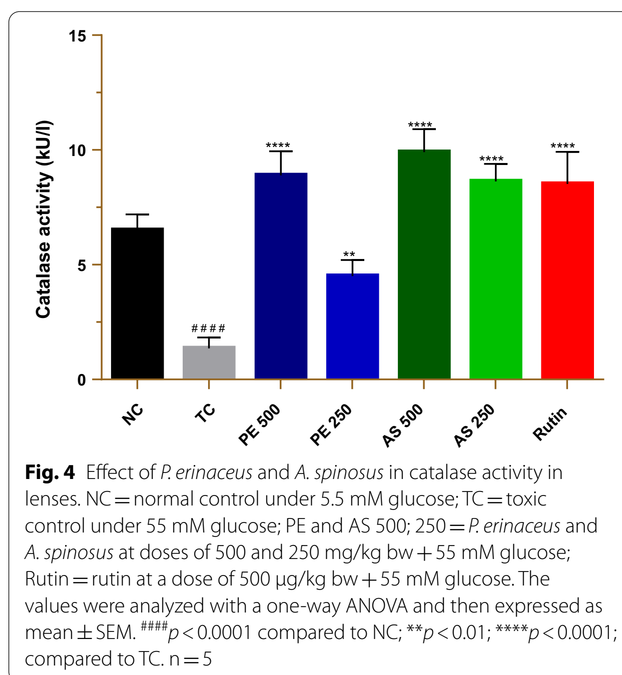

Fig. 4 Effect of *P. erinaceus* and *A. spinosus* in catalase activity in lenses. NC = normal control under 5.5 mM glucose; TC = toxic control under 55 mM glucose; PE and AS 500; 250 = *P. erinaceus* and *A. spinosus* at doses of 500 and 250 mg/kg bw + 55 mM glucose; Rutin = rutin at a dose of 500 µg/kg bw + 55 mM glucose. The values were analyzed with a one-way ANOVA and then expressed as mean ± SEM. ####$p < 0.0001$ compared to NC; **$p < 0.01$; ****$p < 0.0001$; compared to TC. n = 5

Glomerulopathy ex vivo

Effect of extracts on glucose absorption and reabsorption in kidney fragments

The kidney fragments were cultured in glomerular solution for 96 h, and glucose absorption was evaluated during the first 24 h. In normal control cultured under normal glucose (NG) of 5 mM, the absorption of glucose was maximal at the 12th h and tended to a plateau around the 24th h (Fig. 5). In toxic control treated with a HG of 30 mM, the absorption of glucose was significantly ($p < 0.0001$) increased over 24 h compared to normal control. At time t = 4 h, this absorption of glucose was maximal in toxic control before gradually decreasing (Fig. 5). Decreased glucose absorption involves increased glucose reabsorption, which subsequently increases glucose levels in the kidney fragments incubation medium (Fig. 5). Fragments cultivated under HG with *P. erinaceus* extract at doses of 500 and 250 mg/kg bw showed a significant ($p < 0.0001$) increase in glucose absorption at time t = 2 h and 4 h, respectively, before moving toward a plateau. *P. erinaceus* extract at a dose of 500 mg/kg caused a more significant increase in glucose absorption than a dose of 250 mg/kg (Fig. 5). *A. spinosus* (250 and 500 mg/kg bw) and rutin induced maximal glucose absorption at time t = 4 h and moving toward a plateau from 12th h (Fig. 5). The dose 250 mg/kg ($p < 0.0001$) of the extract of *A. spinosus* was slightly more effective at time t = 24 h than the dose 500 mg/kg ($p < 0.001$) compared to toxic control. The decrease in glomerular glucose absorption in the toxic controls at the 12th and 24th h reflects a glucose reabsorption

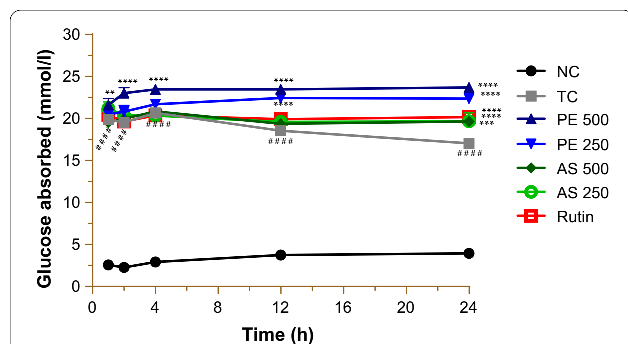

Fig. 5 Effect of *P. erinaceus* and *A. spinosus* on glucose absorption. NC = normal control under 5 mM glucose; TC = toxic control under 30 mM glucose; PE and AS 500; 250 = *P. erinaceus* and *A. spinosus* at doses of 500 and 250 mg/kg bw + 30 mM glucose; Rutin = rutin at a dose of 500 µg/kg bw + 30 mM glucose. The values were analyzed with a two-way ANOVA and then expressed as mean ± SEM. ####$p < 0.0001$ compared to NC; **$p < 0.05$; ***$p < 0.01$; ****$p < 0.0001$; compared to TC. n = 5

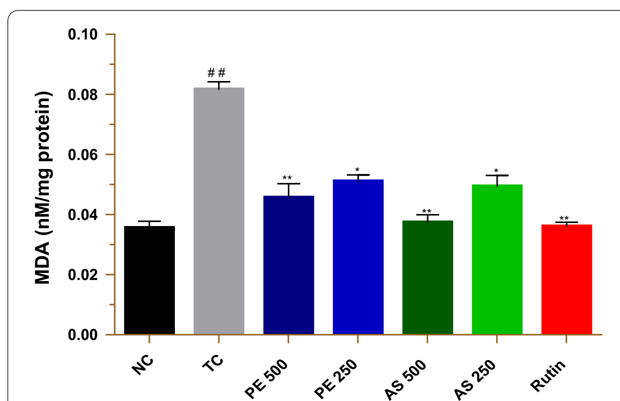

Fig. 6 Effect of *P. erinaceus* and *A. spinosus* in MDA levels in kidney fragments. NC = normal control under 5 mM glucose; TC = toxic control under 30 mM glucose; PE and AS 500; 250 = *P. erinaceus* and *A. spinosus* at doses of 500 and 250 mg/kg bw + 30 mM glucose; Rutin = rutin at a dose of 500 µg/kg bw + 30 mM glucose. The values were analyzed with a one-way ANOVA and then expressed as mean ± SEM. ##$p < 0.01$ compared to NC; *$p < 0.05$; **$p < 0.01$; compared to TC. n = 5

in the tubules, which tends to re-establish the equilibrium "glucose absorbed = glucose reabsorbed" (Fig. 5). When considering this formula, it is deduced therefrom at the 24th h that the extracts of *P. erinaceus* and *A. spinosus* at doses of 250 and 500 mg/kg induced a significant ($p < 0.001$) inhibition of glucose reabsorption. The effect of *P. erinaceus* was greater than that of *A. spinosus*. The rutin used as a reference drug also induced a significant inhibition of glucose reabsorption (Fig. 5).

Malondialdehyde levels in kidney fragments

MDA levels in toxic control cultured under HG were significantly increased ($p < 0.01$) compared to normal control cultured under NG (Fig. 6). In kidney fragments cultured under HG with *P. erinaceus* and *A. spinosus* extracts, MDA levels were significantly ($p < 0.05$ to 0.01) reduced compared to toxic control (Fig. 6). Extracts of both plants at dose of 500 mg/kg bw induced more significant ($p < 0.01$) decrease in MDA levels in fragments than a dose of 250 mg/kg bw ($p < 0.05$) (Fig. 6, Table 2). Rutin also induced a significant ($p < 0.01$) decrease in MDA levels (Fig. 6, Table 2).

Glutathione levels in kidney fragments

Kidney fragments cultured under HG (TC) showed a significant ($p < 0.01$) depletion in GSH store compared to normal control (NC). However, fragments cultured under HG with *P. erinaceus* and *A. spinosus* extracts showed a significant increase ($p < 0.05$ to 0.001) in GSH levels compared to toxic control (Fig. 7). GSH level increased significantly in *P. erinaceus* extract at doses of 250 mg/kg ($p < 0.05$) and 500 mg/kg ($p < 0.01$). This level of GHS was more significantly increased in *A. spinosus* extract at doses of 250 mg/kg ($p < 0.01$) and 500 mg/kg ($p < 0.001$) than the two doses of *P. erinaceus* extract (Table 2). Rutin at a dose of 500 µg/kg bw also induced a significant ($p < 0.01$) increase in GSH levels (Fig. 7, Table 2).

Discussion

A high glucose (HG) induces cataracts in lenses and glomerular damage in kidneys. Thus, the incubation of lenses and kidney fragments under HG in the presence of extracts provides access to the efficacy of *P. erinaceus* and *A. spinosus* against these two complications of diabetes.

The present study showed that extracts of *P. erinaceus* and *A. spinosus* alleviated dose-depending lens opacity, which causes blindness. Lenses treated with the 500 mg/kg dose of extracts of both plants were brighter and more transparent compared to the 250 mg/kg dose. Biochemical parameters

Table 2 Biochemical parameters in kidney fragments

Biochemical parameters	MDA (nM/mg protein)	GSH (µg/mg protein)
NC	0.0355 ± 0.0014	6.7555 ± 0.2725
TC	0.0816 ± 0.0015##	0.3767 ± 0.0625##
P. erinaceus 500 mg/kg	0.0455 ± 0.0028**	3.7991 ± 0.0715**
P. erinaceus 250 mg/kg	0.0509 ± 0.0013*	3.5779 ± 0.2172*
A. spinosus 500 mg/kg	0.0373 ± 0.0015**	5.3670 ± 0.1345***
A. spinosus 250 mg/kg	0.0493 ± 0.0021*	2.8165 ± 0.1058**
Rutin 500 µg/kg	0.0360 ± 0.0009**	4.6966 ± 0.2016**

NC = normal control; TC = toxic control; MDA = malondialdehyde; GSH = glutathione. The values were analyzed with a 1-way ANOVA and then expressed as mean ± SEM. ##$p < 0.01$ compared to NC; *$p < 0.05$;**$p < 0.01$; ***$p < 0.001$; compared to TC. n = 5

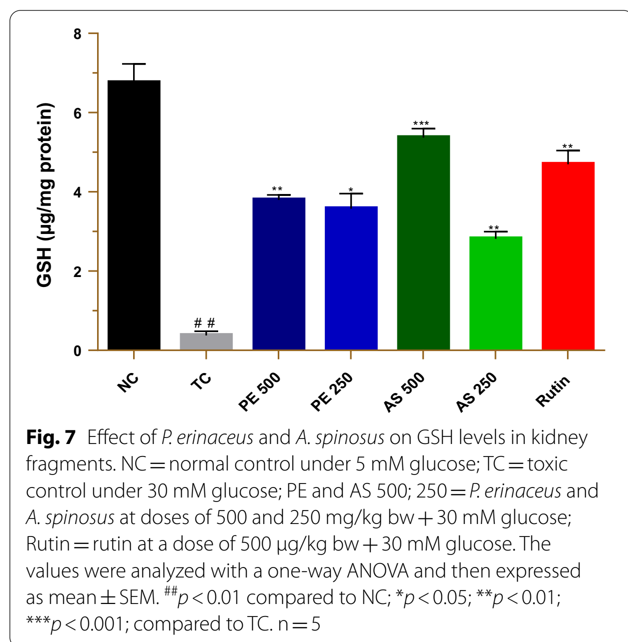

Fig. 7 Effect of *P. erinaceus* and *A. spinosus* on GSH levels in kidney fragments. NC = normal control under 5 mM glucose; TC = toxic control under 30 mM glucose; PE and AS 500; 250 = *P. erinaceus* and *A. spinosus* at doses of 500 and 250 mg/kg bw + 30 mM glucose; Rutin = rutin at a dose of 500 µg/kg bw + 30 mM glucose. The values were analyzed with a one-way ANOVA and then expressed as mean ± SEM. $^{\#\#}p < 0.01$ compared to NC; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; compared to TC. n = 5

showed that extracts of *P. erinaceus* and *A. spinosus* at doses of 250 mg/kg ($p < 0.01$) and 500 mg/kg ($p < 0.001$) decreased significantly in MDA levels and increase in GSH store and CAT activity. This means that *P. erinaceus* and *A. spinosus* alleviated lens opacity by inhibiting oxidative stress and subsequently enhancing the activities of detoxification enzymes in cells. GSH and CAT (which catalyzes H_2O_2 into $H_2O + O2$) are enzymes involved in lenses detoxification and maintain the integrity of phospholipid bilayer by reducing lipid peroxidation (oxidative stress) (Pirie 1965). A decrease in these enzymes leads to a decrease in the endogenous antioxidant system and consequently an increase in lipoperoxidation (MDA), which leads to ER stress. Oxidative stress from RE induces lens epithelial cell apoptosis and cataract formation.

The protective effect of *P. erinaceus* and *A. spinosus* extracts against glomerular function, and damage was observed in kidney fragments cultured under HG. The fragments treated with extracts of both plants at doses of 250 and 500 mg/kg bw for 96 h under 30 mM glucose resulted in an improvement in insulin action and an inhibition of oxidative stress in kidney fragments. The measurement of glucose levels in culture solution for 24 h showed that HG induces in glomeruli an increase in glucose absorption. Later, this glomerular glucose absorption decrease with increasing tubular glucose reabsorption (in toxic control). It was demonstrated that in glomeruli, insulin receptors (IR) phosphorylation was induced in response to a HG (Katsoulieris et al. 2016). Prolonged treatment of glomeruli with HG downregulates insulin signaling pathways that contribute to insulin resistance and glycosuria (Katsoulieris et al. 2016). This explains the significant ($p < 0.0001$) increase in glucose absorption in kidney fragments cultured under HG at the start of the experiment, before decreasing from the 12th h. The integrity of the glomerular filtration barrier is maintained by the podocytes. Thus, the loss of insulin signaling in this cells leads to glomerular damage and progressively to renal failure (Welsh et al. 2010). Both plants potentiated the action of insulin in glomeruli probably by increasing the sensitivity of podocytes to insulin, which are particularly capable of absorbing glucose via the translocation of the glucose transporters GLUT1 and GLUT4 (Coward et al. 2005) and lead to increased glucose absorption. Extracts at doses of 250 and 500 mg/kg of both plants and rutin significantly ($p < 0.001$) inhibited glucose reabsorption at 24th h. In fact, the glucose filtered by the glomeruli is completely reabsorbed in the renal tubules at the level of the S1 and S2 segments (SGLT2 and GLUT2) and later in the S3 segment (SGLT1 and GLUT2) (Ghezzi et al. 2018). The inhibition of these glucose transporters by extracts of the two plants could be at the origin of the decrease in the reabsorption of glucose in the kidney fragments. Increased glucose transport by glomeruli enhances glucose metabolism in cells and leads to ER stress, which are responsible for apoptosis of glomerular endothelial cells and podocytes in the filtration barrier. Extracts of *P. erinaceus* and *A. spinosus* inhibited dose-depending oxidative stress. Treatment of kidney fragments with extracts at doses of 250 and 500 mg/kg bw under HG for 96 h, showed that both plants significantly reduce MDA levels, then increase in GSH store. *A. spinosus* was more effective; in particular in the induction of an increase GSH levels ($p < 0.01$ to 0.001) compared to *P. erinaceus* ($p < 0.05$ to 0.01). This means that the extracts of both plants could prevent or delay apoptosis of glomeruli cells by reducing oxidative stress.

The mechanism by which HG induces damage in cells through several processes among which oxidative stress plays an important role (Mohammadi et al. 2020). The polyol pathway in which aldose reductase, a rate-limiting enzyme, catalyzes a NADPH-dependent reduction of glucose to sorbitol, remains the primary mechanism for the development of cataracts and glomerular damage (Sies et al. 2017; Kiziltoprak et al. 2019). Under diabetic conditions, about 30% of glucose enters this pathway instead of 5% under normal physiological conditions (Kanchan et al. 2016; Alsahli and Gerich 2017). Increase of intracellular activity of aldose reductase leads to an accumulation of sorbitol in cells and causes a significant decrease in the NADPH/NADP ratio, which subsequently decrease in GSH and nitric oxide synthase activities and then increase ER stress (Lee and Chung 1999). Osmotic swelling resulting from ER stress leads to hydropic degeneration of lens fibers and glomeruli (Kiziltoprak et al. 2019). Oxidation of proteins in cells under HG also causes oxidative stress and generates

reactive oxygen species (ROS) such as superoxide ($O2^-$), hydroxyl (OH^-) and nitric oxide ($NO^·$). Accumulation of ROS increases membrane lipid peroxidation and cell apoptosis, resulting in cataracts (Nishimoto et al. 2003; Kim et al. 2010) and glomerular dysfunction (Pérez-Rodríguez et al. 2015; Sies et al. 2017).

Some authors had previously reported the effect of *P. erinaceus* and *A. spinosus* against oxidative stress. Akinmoladun et al. (2015) reported that ethanolic extract of *P. erinaceus* stem bark restores MDA level and GSH depletion in rats acetaminophen-induced liver injury. The antioxidant activity of the methanolic extract of *Pterocarpus erinaceus* stem bark was proved on the ulcer induced by indomethacin in albino rats by Patrick (2018). The author demonstrated that the methanolic extract of the plant decreased in MDA and increased in GSH and CAT levels dose-depending. Previous studies have also reported the antioxidant activities of *A. spinosus*. Mishra et al. (2012) demonstrated that the hydroethanolic extract of *A. spinosus* leaves causes significant increase in GSH and CAT activities in vivo in kidneys of rats. The ethanolic extract of the whole plant induced a significant decrease in lipoperoxidation and a significant increase in GSH and CAT activities in wound tissues of rats (Paswan et al. 2020). These effects of extracts of the two plants on oxidative stress were also observed in our in vitro study when determining the levels of MDA, GSH and CAT. The antioxidant activity of dried hydroethanolic extracts of *P. erinaceus* stem bark and *A. spinosus* roots could be due to the presence of phenolic compounds such as flavonoids and tannins which were revealed during our previous phytochemical screening (Atchou et al. 2020).

The present study showed that extracts of both plants alleviate lens opacity and reduced oxidative stress dose-depending. Thus, the treatment with extracts of *P. erinaceus* stem bark and *A. spinosus* roots at doses of 250 and 500 mg/kg bw may prevent or delay the effects of hyperglycemia and improve in lens and glomeruli, cells survivors. The dose of 500 mg/kg bw was more effective and could be used against cataracts and renal failure in diabetes complications.

Conclusion

This study demonstrated that a prolonged treatment of lens and kidney fragments under high glucose leads to cataracts and glomerulopathy. Cultured lens and kidney fragments under high glucose with extracts of *P. erinaceus* and *A. spinosus* alleviated lens opacity and improved insulin sensitivity in glomeruli through increased glucose absorption. The extracts prevent cataracts and glomerular damage by significantly reducing oxidative stress, which leads to membrane lipoperoxidation and cell apoptosis. These findings suggest that the dried hydroethanolic extracts of *P. erinaceus* stem bark and *A. spinosus* roots at a dose of 500 mg/kg bw were effective and could be used to prevent or delay cataracts and glomerulopathy in diabetics. However, further studies can be performed in vivo to confirm the effect of the two plants on diabetes complications.

Abbreviations
AAH: Artificial aqueous humor; bw: Body weight; CAT: Catalase; ER: Endoplasmic reticulum; GLUT: Glucose transporter; GS: Glomerular solution; GSH: Glutathione; HG: High glucose; MDA: Malondialdehyde; NC: Normal control; SEM: Standard errors of mean; SGLT: Sodium-glucose linked transporters; TBA: Thiobarbituric acid; TC: Toxic control.

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Authors' contributions
KA designed the research and wrote the original draft of this article. PL approved the design and supervised the research. KE coordinated the research. All authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

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Competing interests
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