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Protective effects of Spirulina powder and its ethyl acetate fractions against potassium dichromate-induced nephrotoxicity in Sprague-Dawley rats

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ABSTRACT

Background: Spirulina Dou Bogan (SPD) is a strain of the green algae Spirulina platensis produced at IREDESA (Pahou, Benin). We have previously shown that SPD (75 mg/kg, body weight, bw) protects against chronic high fructose administration-induced hyperglycemia and insulin resistance in Sprague-Dawley (SD) rats. **Objective:** This study aimed to evaluate the capacity of SPD to protect the nephrons of SD rats against acute renal injury induced by potassium dichromate ($K_2Cr_2O_7$). **Materials and Methods:** Five groups of eight SD rats each were formed and pretreated daily by gavage with SPD (75 mg/kg, bw), the ethyl acetate fraction of SPD (EAF 75 mg/kg, bw), and the aqueous fraction of SPD (AQF 75 mg/kg, bw) for 10 days consecutively. On day 8, animals were injected with a single dose of $K_2Cr_2O_7$ (15 mg/kg, s.c). After 48 hours, renal (creatinine and urea) and liver (AST and ALT) markers were monitored on blood samples, when malondialdehyde (MDA), glutathione (GSH) quantifications, and histopathological analyses were realized on kidney tissues. **Results:** SPD and EAF significantly reversed the deleterious effects of $K_2Cr_2O_7$ on urea and creatinine levels. EAF effects, in particular, indicate possible antioxidant mechanisms, as illustrated by the decrease in MDA production and enhancement of glutathione levels in the kidneys of animals treated with SPD and EAF compared to $K_2Cr_2O_7$-treated animals. As expected, SPD and EAF treatments restored the histopathological changes induced by $K_2Cr_2O_7$. **Conclusion:** In addition to its antidiabetic properties, SPD's nephroprotective properties make it an excellent nutraceutical for managing chronic diabetic conditions.

Keywords: Spirulina “Dou Bogan”, Potassium dichromate, Nephroprotection, Rats.

INTRODUCTION

Potassium chromates and dichromates are compounds of hexavalent chromium [chromium(VI), Cr(VI)]. There are various valence states for chromium, the most prevalent being the trivalent Cr (III) and the hexavalent Cr (VI). The chromic form Cr (III) is the predominant form in nature, while the chromate form Cr (VI), non-ubiquitous in nature, is produced essentially from industrial processes [1]. Chromates, particularly dichromates, are powerful oxidizing agents that can react strongly with reducing substances and organic materials. Many cellular metabolites reduce potassium dichromate (PD) to produce chromium, which triggers reactive oxygen species production, leading to oxidative damage in the liver, brain, and renal tissues [2-4]. Additionally, chromium causes DNA damage, lipid peroxidation, and the production of multiple pro-inflammatory cytokines, all of which contribute to the inflammatory process and ultimately result in severe nephrotoxicity [5,6].

The main cyanobacteria species responsible for creating natural green-blue dyes are found in the genus Arthrospira, also referred to as Spirulina, a blue-green alga [7]. Many reports have highlighted the presence of potential functional ingredients in *Spirulina platensis* such as β-complex, vitamins, minerals, proteins, γ-linoleic acid, and nutraceutical pigments like phycocyanin [8], polyphenols, and β-carotene, demonstrating the relevant role of this alga in protection against various diseases [9]. *S. platensis* has been shown to protect against pathological conditions, including anti-inflammatory, hypolipemiant, neuroprotective, hepatoprotective, immunomodulatory, and anticancer activities [10,11]. The alga has shown renoprotective effects in acute kidney injury conditions induced by cisplatin [12], and gentamicin [13], and in streptozotocin and alloxan-induced diabetes in rats [14,15]. *S. platensis* consists of closely connected, genetically and physiologically different groups, each with unique characteristics that allow them to thrive in particular environmental conditions. These groups also exhibit a range of medicinal properties. The strain of *S. platensis* developed at IREDESA (Pahou, Benin), marketed under the brand "Spiruline Dou Bogan" (SPD), is recognized in Benin. A previous study in our laboratory has shown that

SPD has protective and therapeutic effects against chronic high fructose administration (8 g/kg of fructose orally for 56 days)-induced hyperglycemia and insulin resistance in Sprague-Dawley (SD) rats [16]. As chronic diabetic conditions often lead to deterioration of kidney function, the present work aimed to explore the potential nephroprotective activity of the local spirulina strain SPD and its ethyl acetate fraction against potassium dichromate-induced acute nephrotoxicity in SD rats.

MATERIALS AND METHODS

Plant Material

The S. platensis strain SPD used in this investigation was obtained from IREDESA Institute, Pahou District (Cotonou, Benin), where it is usually cultured for commercialization ("Spiruline Dou bogan," SPD) in modified Zarrouk's medium at pH 9.5 under a light intensity of 2500 lux at 30 °C for 16 h light and 8 h dark periods. On the 20th day, the exponential growth phase of the algae is observed, and then the cultures are harvested, filtered, collected, overnight dried below 40 °C, and conserved in small aluminum-polyethylene containers for further use.

Chemicals

All the chemicals used in this experiment, including potassium dichromate, were of analytical grade and purchased from Sigma-Aldrich (St. Louis, USA).

Animals

Both male and female Sprague-Dawley (SD) rats aged 8–10 weeks (weighing about 150-200g) were used. They were obtained from the Nigerian Institute for Medical Research (Lagos, Nigeria) and housed in standard environmental conditions (temperature 24-25 °C, relative humidity, and a 12 t/12 h light-dark cycle). They were acclimatized to the laboratory conditions for 2 weeks with access to a standard diet and tap water ad libitum. All animals were treated according to the ethical guidelines approved by the Institutional Animal Ethics Committee, University of Lomé, Togo (approval no. 012/2021/CB-FDS-UL).

Induction of nephrotoxicity

The model used is inspired by the protocol of Khan et al [17]. Nephrotoxicity was induced by subcutaneous (s.c.) administration of a single dose of potassium dichromate ($K_2Cr_2O_7$, 15 mg/kg, bw). Spirulina powder and ethyl acetate fractions dosage (75 mg/kg) were chosen based on our previous findings in a model of chronic high fructose diet-induced diabetes in Sprague Dawley rats (16). Among three dosages tested over 56 days (18.75, 37.5, 75 mg/kg), 75 mg/kg was the most active. Five (05) groups of 8 rats were formed. Animals of Groupe I or Normal Controls (NC) received orally distilled water (5 mL/kg) for 7 days, followed by s.c. administration of a single dose of NaCl 9 g/L (10 mL/kg) on the 8th day and 5 mL/kg of distilled water again on the 9th and 10th days. Groupe II or Positive Controls (PC) rats received orally distilled water (5 mL/kg) for 7 days, followed on the 8th day by s.c. administration of a single dose of $K_2Cr_2O_7$ (15 mL/kg, bw, dissolved in NaCl 9 g/L at a rate of 10 mL/kg). On the 9th and 10th days, they received orally distilled water (5 mL/kg) again. Animals of Groups III, IV, and V were pretreated by gavage, respectively, with SPD (75 mg/kg, bw), the ethyl acetate fraction of SPD (EAF 75 mg/kg, bw), and the aqueous fraction of SPD (AQF 75 mg/kg, bw) for 7 days at a rate of 5 mL/kg. On the 8th day, they received a single dose of $K_2Cr_2O_7$ (15 mg/kg, bw dissolved in NaCl at a rate of 10 mL/kg), followed by oral administration of SPD and its fractions on days 9 and 10. On the 10th day, 12 hours after the last treatment, animals were anesthetized by exposure to diethyl ether, and blood was collected for the measurement of renal (creatinine and urea) and liver (AST and ALT) markers. SD rats were sacrificed, and rat kidneys were collected for oxidative stress marker (MDA, glutathione) quantification and histopathological analyses.

Blood renal and hepatic functions biomarkers assay

Serum renal (urea and creatinine) and hepatic markers (AST and ALT) were assayed using an automatic biochemistry analyzer, the Prietest Touch Plus Biochemistry Analyzer (Robonik India PVT LTD, India), and specific spectrophotometric diagnostic kits (PharmaLab, India).

Estimation of oxidative stress markers in kidney

The collected kidneys were rinsed in NaCl 9 g/L and homogenized in ice-cold 10 mM Tris-HCl buffer (pH 7.5) to prepare a 10% (w/v) tissue homogenate. The mixture was centrifuged at 4000 rpm for 30 minutes at 4 °C. Malondialdehyde (MDA) and reduced glutathione (GSH) were measured in the resulting supernatant.

GSH levels were estimated colorimetrically using Ellman's reagent as described previously by Sedlak and Lindsay [18]. Malondialdehyde (MDA) concentration was measured according to the method described by Kantati et al [19]. Protein concentrations were determined using the Bradford method with bovine serum albumin as the standard [20].

Histopathological analysis

Parts of kidneys from all groups were fixed in 10% buffered formalin for 24 h at room temperature, then washed under running water, dehydrated in ascending concentrations of ethanol (60°, 80°, 95°, and 100°), cleared in toluene, and finally embedded in paraffin. Then, sections of 5 µm thickness were stained with hematoxylin and eosin and evaluated by light microscopy.

Statistical analysis

Collected data were statistically analyzed by one-way ANOVA, followed by a Tukey test, to determine statistical significance between different groups using GraphPad Prism 6.05 software.

RESULTS

Blood renal and hepatic function biomarkers

Renal marker creatinine

Creatinine is an excellent marker of the efficiency of glomerular filtration. Injection of $K_2Cr_2O_7$ (15 mg/kg, *bw)* significantly raised the levels of creatinine ($P<0.001$, compared to NC animals). SPD 75 mg/kg and its organic fraction (EAF 75 mg/kg) significantly reverse this effect ($P<0.05$ and $P<0.001$, respectively, compared to PC animals). Creatinine values decreased, in the best way, from 3.54 ± 0.44 mg/dL in PC rats treated with $K_2Cr_2O_7$ alone to 0.85 ± 0.07 mg/dL ($P<0.001$) in the serum of $K_2Cr_2O_7$ + EAF 75 mg/kg treated animals (Figure 1).

Renal marker urea

When compared to PC animals (Figure 2), the ethyl acetate fraction (EAF 75 mg/kg) was the most effective in reducing the urea level augmentations caused by potassium dichromate (-72.51%, $P<0.001$).

Hepatic biomarker AST

Despite the presence of potassium dichromate, SPD and its fractions decreased aspartate aminotransferase (AST) levels in serum (Figure 3). The EAF produced the best results (-41.31%, $P<0.001$, compared to PC rats).

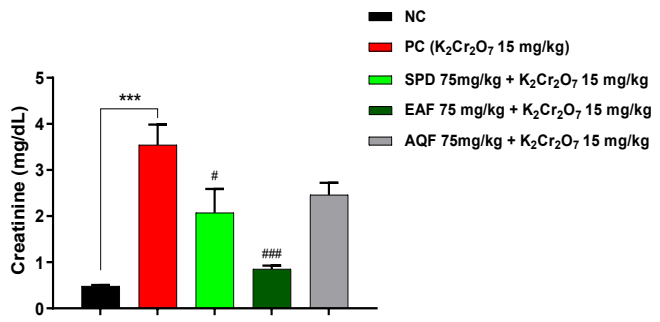

Figure 1: Effect of SPD and its fractions on serum creatinine levels

SD rats of both sexes were divided into five groups, pretreated with SPD and its fractions for 7 days, and received on the 8th day s.c: NaCl 9 g/L (Normal Control, NC); $K_2Cr_2O_7$ (15 mg/kg) alone (Positive Control, PC); SPD 75 mg/kg + $K_2Cr_2O_7$ (15 mg/kg); ethyl acetate fraction (EAF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg; and aqueous fraction (AQF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg. 48 h post-injection, creatinine levels were monitored in blood samples. The values are expressed as Means ± SEM, n = 8. *** P<0.001: PC vs NC. # P<0.05: SPD 75 mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC. ### P<0.001: EAF 75mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC.

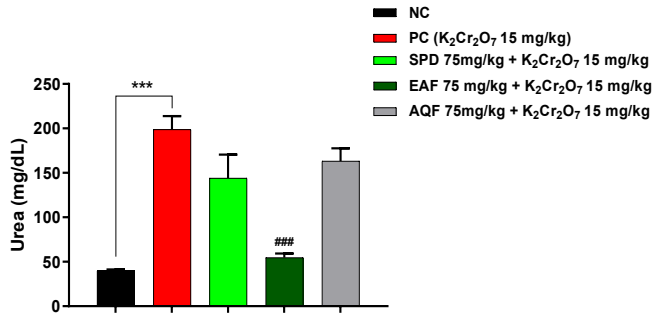

Figure 2: Effect of SPD and its fractions on serum urea levels

SD rats of both sexes were divided into five groups, pretreated with SPD and its fractions for 7 days, and received on the 8th day s.c: NaCl 9 g/L (Normal Control, NC); $K_2Cr_2O_7$ (15 mg/kg) alone (Positive Control, PC); SPD 75mg/kg + $K_2Cr_2O_7$ (15 mg/kg); ethyl acetate fraction (EAF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg; and aqueous fraction (AQF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg. 48 h post-injection, urea levels were monitored in blood samples. The values are expressed as Means ± SEM, n = 8. *** P<0.001: PC vs NC. ### P<0.001: EAF 75 mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC.

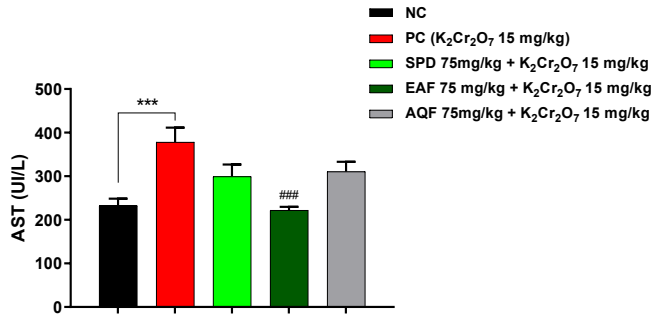

Figure 3: Effect of SPD and its fractions on serum AST levels

SD rats of both sexes were divided into five groups, pretreated with SPD and its fractions for 7 days, and received on the 8th day s.c: NaCl 9 g/L (Normal Control, NC); $K_2Cr_2O_7$ (15 mg/kg) alone (Positive Control, PC); SPD 75 mg/kg + $K_2Cr_2O_7$ (15 mg/kg); ethyl acetate fraction (EAF 75mg/kg) + $K_2Cr_2O_7$ 15 mg/kg; and aqueous fraction (AQF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg. 48 h post-injection, AST levels were monitored in blood samples. The values are expressed as Means ± SEM, n = 8. *** P<0.001: PC vs NC. ### P<0.001: EAF 75 mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC.

Hepatic biomarker ALT

Animals treated with $K_2Cr_2O_7$ alone had significantly higher alanine aminotransferase (ALT) levels, as seen in Figure 4 (+60.47%, P<0.001, compared to NC rats). The concomitant administration of EAF 75 mg/kg and SPD 75 mg/kg resulted in decreased ALT values to levels similar to those seen in NC animals (-41.47%, P<0.01, and -35.59%, P<0.05, respectively, compared to PC animals).

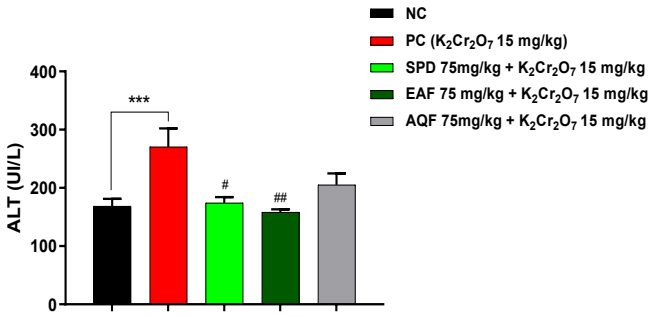

Figure 4: Effect of SPD and its fractions on serum ALT levels

SD rats of both sexes were divided into five groups, pretreated with SPD and its fractions for 7 days, and received on the 8th day s.c: NaCl 9 g/L (Normal Control, NC); $K_2Cr_2O_7$ (15 mg/kg) alone (Positive Control, PC); SPD 75 mg/kg + $K_2Cr_2O_7$ (15 mg/kg); ethyl acetate fraction (EAF 75mg/kg) + $K_2Cr_2O_7$ 15 mg/kg; and aqueous fraction (AQF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg. 48 h post-injection, ALT levels were monitored in blood samples. The values are expressed as Means ± SEM, n = 8. *** P<0.001: PC vs NC. # P<0.05: SPD 75 mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC; ## P<0.01: EAF 75mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC.

Oxidative stress markers in kidney homogenates

Malondialdehyde (MDA) levels

MDA is a marker of lipid peroxidation. Figure 5 shows a significant increase in the level of renal MDA (P<0.05) in rats treated with potassium dichromate alone, compared to NC rats. The concurrent administration of SPD 75 mg/kg and EAF 75 mg/kg reduced lipid oxidation in rats' kidneys and maintained the MDA level at values similar to those observed in the NC group.

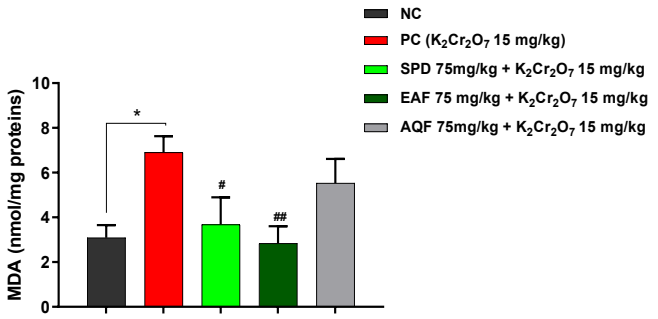

Figure 5: Effect of SPD and its fractions on kidney homogenates MDA levels

SD rats of both sexes were divided into five groups, pretreated with SPD and its fractions for 7 days, and received on the 8th day s.c: NaCl 9 g/L (Normal Control, NC); $K_2Cr_2O_7$ (15 mg/kg) alone (Positive Control, PC); SPD 75 mg/kg + $K_2Cr_2O_7$ (15 mg/kg); ethyl acetate fraction (EAF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg; and aqueous fraction (AQF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg. 48 h post-injection, MDA levels were measured in kidney homogenates. The values are expressed as Means ± SEM, n = 8. * P<0.05: PC vs NC. # P<0.05: SPD 75 mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC; ## P<0.01: EAF 75 mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC.

Glutathione (GSH) levels

Animals treated with K2Cr2O7 alone showed a significant decrease in the level of renal glutathione (P<0.01) compared to NC rats (Figure 6). Administration of SPD 75 mg/kg and EAF 75 mg/kg restored the glutathione level in the kidneys, despite the presence of potassium dichromate (+38.80%, P<0.001, and +33.92%, P<0.001, respectively, compared to PC rats).

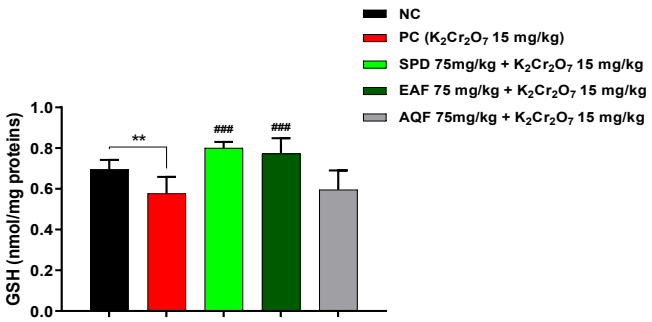

Figure 6: Effect of SPD and its fractions on kidney homogenates GSH levels

SD rats of both sexes were divided into five groups, pretreated with SPD and its fractions for 7 days, and received on the 8th day s.c: NaCl 9 g/L (Normal Control, NC); $K_2Cr_2O_7$ (15 mg/kg) alone (Positive Control, PC); SPD 75 mg/kg + $K_2Cr_2O_7$ (15 mg/kg); ethyl acetate fraction (EAF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg; and aqueous fraction (AQF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg. 48 h post-injection, GSH levels were measured in kidney homogenates. The values are expressed in Means ± SEM, n = 8. * P<0.05: PC vs NC. # P<0.05: SPD 75mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC; ## P<0.01: EAF 75 mg/kg + $K_2Cr_2O_7$ 15 mg/kg vs PC.

Histopathological observations

Figure 7 shows the normal architecture of glomeruli and tubules in animals of NC group (A), SPD 75 mg/kg treated animal group (C), and EAF 75 mg/kg treated rats (D). The structure of the proximal tubules appears typical, with numerous brush border membranes present. Glomeruli and distal tubules also exhibited normal structures (Figure 7 A, C, D). Conversely, rats treated with potassium dichromate alone (Figure 7 B) and those treated with AQF (Figure 7 E) exhibited important structure alterations. Samples of these animals (n = 5) presented few foci of tissue necrosis with infiltration of lymphocytic inflammatory elements and well-marked tubular atrophy. The glomerular damage was mostly observed in rats treated with $K_2Cr_2O_7$ alone.

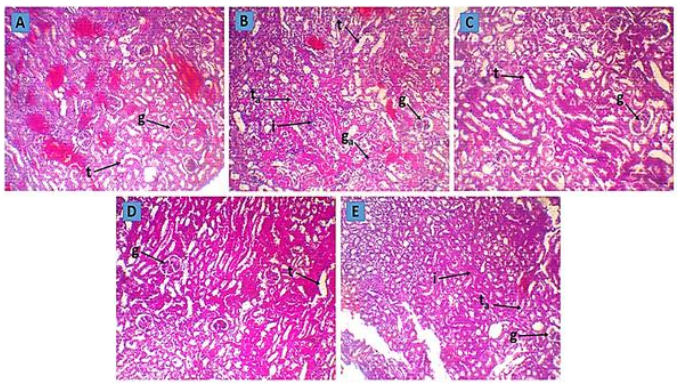

Figure 7: Histopathological analysis of rats' kidneys paraffin sections stained with hematoxylin and eosin (HE)

Abbreviations: A: Normal control (NC); B: Positive control (PC, $K_2Cr_2O_7$ alone); C: SPD 75 mg/kg + $K_2Cr_2O_7$ (15 mg/kg); D: ethyl acetate fraction (EAF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg; E: aqueous fraction (AQF 75 mg/kg) + $K_2Cr_2O_7$ 15 mg/kg. g: normal glomeruli; ga: abnormal glomeruli; t: normal tubules; ta: abnormal tubules; i: inflammatory infiltrates.

DISCUSSION

SPD nephroprotective capacity was evaluated using a reference nephrotoxic compound, potassium dichromate. $K_2Cr_2O_7$ in single-dose subcutaneous (s.c.) injection is a powerful oxidizing agent capable of inducing nephrotic lesions in the acute phase, as validated by numerous studies in rats [17,21-24]. Therefore, it is on a well-established model of nephrotoxicity that this work was performed. Fatima et al [25] showed that dichromate acts mainly on the proximal convoluted tubule, leading to an elevation of markers of renal injury (creatinine and urea) and hepatic toxicity (AST and ALT), among others. In our study, SPD and its organic fraction were able to reverse the detrimental effects of dichromates on the kidneys of SD rats. Creatinine level in particular, an excellent marker of the efficiency of renal function, increased significantly in rats treated only with dichromate, therefore reflecting acute renal damage. Oral administration of SPD (75 mg/kg) and its organic fraction (EAF 75 mg/kg) protected the kidneys by significantly reducing creatinine levels. The organic fraction, in particular, was more active than the whole alga powder preparation at the same dose, probably due to the high concentration of phenolic compounds in this fraction. It is well known that ethyl acetate, a moderately polar compound, concentrates the phenolic compounds of plant extracts more than water when used for liquid-liquid separation in the presence of water [26-28]. Our observation was in line with the important antioxidant capacity of phenolic compounds, well documented [29], and confirmed by the significant reduction of lipid peroxidation (MDA) and the rise of glutathione levels in kidney homogenates. Dietary polyphenols mainly act through free radical chelation processes [30], then reducing their deleterious effects on cellular components. Excessive intracellular accumulation of free radicals, reactive oxygen species (ROS) in particular, afforded by potassium dichromate in our model, can cause damage to lipids, proteins, DNA, and carbohydrates [31]. The leading products of these cellular component alterations, such as MDA, are considered strong biomarkers of cellular oxidative status [32]. In the present study, the modulation of the MDA marker reinforces the hypothesis of nephroprotection via antioxidant mechanisms. As dichromates are powerful oxidizing agents, the GSH level depletion observed in PC rats may then be linked to their excessive consumption in the process of reducing free radicals produced by $K_2Cr_2O_7$. SPD and its active fraction EAF treatments interestingly corrected oxidative damages, bringing back GSH levels almost to normal.

Histopathological studies confirm the biochemical modulation observed in the levels of liver and kidney markers. SPD and EAF strongly reduced the oxidative stress caused by chromium by counteracting histological alterations. All these observations allow us to conclude that SPD, in addition to its antidiabetic properties, should be used for the management of patients suffering from diabetic complications such as diabetic nephropathy.

CONCLUSION

The persistence of hyperglycemia during diabetes leads to several complications, including, among others, nephropathy. SPD protected kidneys against potassium dichromate-induced nephrotoxicity. The organic fraction in particular, rich in phenolic compounds, essentially points towards antioxidant mechanisms, which are opposed to the pro-oxidant potassium dichromate. This spirulina strain produced locally appears to be an adaptable solution for populations of low-income countries such as Benin in the management of type 2 diabetes and its complications. The important outcomes of the study should be mentioned in this section.

Acknowledgments

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Ethical considerations

All experimental protocols were conducted in accordance with ethical guidelines approved by the Institutional Animal Ethics Committee, University of Lomé, Togo (approval no. 012/2021/CB-FDS-UL).

Conflict of interest

The authors declared no conflict of interest.

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