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Root extracts of *Saussurea costus* as prospective detoxifying food additive against sodium nitrite toxicity in male rats

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ABSTRACT

The goal of this study was to investigate the effects of three different extracts of *Saussurea costus* roots (ethanol, methanol, and water) as a food additive in alleviating the harmful effect of sodium nitrite in rat meals. Thirty-five adult male rats were divided into five groups as follows: control, sodium nitrite ($NaNO_2$; 75 mg/kg BW, single oral dose), *S. costus* 70% ethanol, 70% methanol, and aqueous extracts (300 mg/kg BW), respectively for four weeks followed by a single dose of $NaNO_2$ 24h before decapitation. Results showed that the 70% ethanol extract of *S. costus* has a higher concentration of total phenolic content, total flavonoids, and antioxidant effect than the 70% methanol and water extracts. Rats pretreated with *S. costus* extracts reduced the harmful effects induced by $NaNO_2$ and improved the hematological parameters, liver, and kidney function biomarkers as well as lipid profile as compared to the $NaNO_2$ group. Furthermore, *S. costus* improved the histopathological alterations in the liver and kidney induced by $NaNO_2$ and improved meat sensory evaluation. Conclusively, the 70% ethanol extract of *S. costus* roots is the most effective extract as an antioxidant against the toxicity of sodium nitrite in male rats and might be used safely as a natural additive in the food industry.

1Introduction

Food additives are natural or manufactured compounds that are added to food and are classed as coloring agents, preservatives, flavors, emulsifiers, and stabilizers (Khodjaeva et al., 2021). Food additives must be tested and reviewed for food safety, and sodium nitrite is one of the most common. It works by limiting the growth of *Clostridium botulinum* spores in refrigerated meats by inhibiting the formation of iron-sulfur clusters, which are necessary for energy metabolism (Milkowski et al., 2010). It causes the red color of meat by forming nitrosylating agents, which then react with myoglobin to give the red color (Sindelar and Milkowski, 2012). Furthermore, sodium nitrite can effectively delay the onset of oxidative rancidity by interacting with heme proteins and metal ions and chelating free radicals, effectively interrupting the lipid oxidation cycle that causes rancidity (Sullivan et al., 2012), Animals, including humans, can be harmed by high levels of sodium nitrite. The poisonous effects of sodium nitrite are caused by nitrite reacting with amines and amides in food to form nitrosamines and nitrosamides, which have toxic effects on many organs (Tong et al., 2010). Hepatotoxicity, cancer, nephrotoxicity, inflammation dysregulation, and tissue injury have all been linked to sodium nitrite (Salama et al., 2013).

Antioxidant properties have been discovered in a variety of medicinal plants and plant-derived antioxidants play a key role in decreasing the consequences of oxidative stress. *Saussurea costus*, a perennial, aromatic, and medicinal plant found in the Himalayan region between 2500 and 3500 m in height, belongs to the Asteraceae family (Pandey et al., 2007). Since ancient times, dried roots of *S. costus* (SC) have been used in folk medicine to cure a variety of maladies and disorders, including asthma, dyspepsia, certain bronchitis, ulcer, rheumatism, cough, throat

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Abbreviations

Sodium nitrite: **$NaNO_2$**
Saussurea costus **SC**
S. costus aqueous extract **SCAE**
S. costus aqueous methanol extract **SCME**
S. costus aqueous ethanol extract **SCEE**
Total phenolic content **TPC**
Total flavonoid content **TFC**
Di phenyl picrylhydrazyl **DPPH**
High performance liquid chromatography **HPLC**
Complete blood picture **CBC**
Aspartate transaminase **AST**
Alanine transaminase **ALT**
Alkaline phosphatase **ALP**
High density lipoproteins **HDL-C**
Low density lipoproteins **LDL-C**
Very low density lipoproteins **VLDL**

infections, tuberculosis, stomach difficulties, and many more (Hassan and Masoodi, 2020). The major active ingredients discovered in this plant are terpenes, anthraquinones, alkaloids, and flavonoids (Zahara et al., 2014). Furthermore, roots contain significant components such as costunolide and dehydrocostus lactone with a wide range of biological properties, including antifungal activity (Barrero et al., 2000), anthelmintic, antidiabetic antitumor (Ko et al., 2005), antimicrobial (Khalid et al., 2011), immunostimulant (Kulkarni, 2001), antiulcer (Sutar et al., 2011), anti-inflammatory (Sunkara et al., 2010) and antihepatotoxic (Yaeesh et al., 2010). Also, they inhibit hepatoma Hep3B cells in humans, preventing the generation of hepatitis B antigen (HBsAg). This plant has the potential to be used in the development of powerful antiviral medicines (Zahara et al., 2014). Anti-inflammatory properties of a drug can be used to treat COVID-19. A decrease in the inflammatory cytokynins (TNF-α, IL-1β) would also be effective in the treatment (Attah et al., 2021). Moreover, Deabes et al. (2021) demonstrated that *S. costus* extracts can play a significant role in the defense action against human multi-resistant diseases and can be utilized instead of antibiotics in the treatment of specific infections. So, the present work was designed to investigate the role of *S.costus* in alleviating the harmful effects of sodium nitrite as a food additive.

2. Materials and methods

2.1. Chemicals and plant materials

Sodium nitrite was purchased from El-Gomhouria Co. for Trading Drugs, Chemicals and Medical Supplies. All other chemicals were of analytical grade. The roots of *Saussurea costus* were purchased from a herbal store in Alexandria, Egypt, and were recognized and authenticated as *Saussurea costus* (Falc.) Lipschitz, synonymous with Saussurea lappa C.B. Clarke, belongs to family Asteraceae, Genus; Saussurea DC. by a plant taxonomy specialist at the Department of Botany, Faculty of Science, Alexandria University, Alexandria, Egypt.

2.2. Preparation of plant extracts

2.2.1. Preparation of S. costus aqueous ethanol extract (SCEE)

The roots were dried and reduced to a moderately coarse powder and stored in air-tight containers for further use in the extraction process (Srivastava et al., 2012). The method of herbal preparation has been previously reported by Akhtar et al. (2013). In brief, the raw herb root (200g) was ground into powder, extracted by simple maceration at room temperature with 70% aqueous ethanol, macerated for 72 h, filtered three times, and evaporated using a rotary evaporator to get a crude *S. costus* extract. The semi-solid paste of the root extract was dissolved in bi-distilled water before administration to the experimental animals (Tag et al., 2016).

2.2.2. Preparation of S. costus aqueous methanol extract (SCME)

S. costus methanol extract (SCME) was prepared according to S Alnahdi (2017), 100 g of the plant roots were ground and then extracted using 70% methanol solution. The root extract's supernatant was separated by filtration with filter paper and then condensed into a thick semi-solid paste by evaporation with a rotary evaporator then was dissolved in bi-distilled water before administration to the experimental animals.

2.2.3. Preparation of S. costus aqueous extract (SCAE)

S. costus aqueous extract (SCAE) was prepared according to Saleem et al. (2013) and Seddiek et al. (2020). Plant material was cleaned, crushed and 1 kg was the powdered material that was boiled with 5 L of distilled water for 30 min and filtered through filter paper and concentrated into thick semi-solid paste under reduced pressure and lyophilized. The freeze-dried material was weighed (about 35 g), dissolved in water (at a final concentration of 50 mg/ml), and used for this study.

2.3. HPLC analysis

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using the Kromasil C18 column (4.6 mm × 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (85% A) and 15–16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 μl for each of the sample solutions. The column temperature was maintained at 35 °C (Kolaylı et al., 2010). All standards were dissolved in ethanol and injected with the following concentrations as in table Compounds were identified by comparing their retention times and UV–Vis spectra with those of the standards, while their concentrations were calculated depending on the area under the peak of standards.

2.3.1. Determination of polyphenols of S. costus extracts using HPLC

High-Performance Liquid Chromatography (HPLC) was used to quantify the number of phenolic compounds in the three extracts of *S. costus* roots (Croci et al., 2009; Seddiek et al., 2020). Sixteen phenolic standards of phenolic compounds were used gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, syringic acid, pyro catechol, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, taxifolin, cinnamic acid, and kaempferol.

2.4. Determination of total phenolic content (TPC)

The Folin-Ciocalteu technique was used to determine the total phenolic content of the three *S. costus* extracts according to Ainsworth and Gillespie (2007) and El Sohaimy et al. (2015). One gram of Gallic acid was dissolved in 100 ml of methanol to get 1% solution of Gallic acid (10 mg/ml) termed as (standard 1 solution). A standard Gallic acid curve was prepared by several dilutions (5, 10, 20, 50,100,150, and 200 mg/ml) in methanol. 100 μl of each dilution was mixed with 500 μl of water and then with 100 μl of Folin -Ciocalteu reagent and allowed to stand for 6 min. Then, 1 ml of 7% sodium carbonate and 500 μl of distilled water were added to the reaction mixture. The absorbance at 760 nm was recorded versus the prepared blank using a spectrophotometer. The same procedure was repeated with the three extracts of *S. costus* roots. The total phenolic content of each extract was calculated as gallic acid equivalents (mg GAE/g). All the experiments were performed

in triplicate.

2.5. Determination of total flavonoid content (TFC)

The total flavonoid content of the extracts was determined using an aluminium chloride complex-forming assay (Seddiek et al., 2020). Quercetin was used as standard and flavonoid content was determined as quercetin equivalent. Similarly, 1g of Quercetin was dissolved in 100 ml of solvent separately to get 1% solution of Quercetin (10 mg/ml) termed as standard 2 solutions. A calibration curve for quercetin was drawn using the standard 2 quercetin solution with different dilutions (5, 10, 20, 50,100 and 150 mg/ml) prepared in methanol. 100 μl of each of the quercetin dilutions was mixed with 500 μl of distilled water and then with 100 μl of 5% Sodium nitrate and allowed to stand for 6 min. Then 150 μl of 10% Aluminium chloride solution was added and allowed to stand for 5 min after which 200 μl solution of 1M Sodium hydroxide was added sequentially. The absorbance of this reaction mixture was recorded at 510 nm on a spectrophotometer. The same procedure was repeated with the three extracts of *S. costus* roots and total flavonoid content was calculated as quercetin equivalents (mgQE/g). All the procedures were performed in triplicate.

2.6. Determination of DPPH free radical scavenging activity

DPPH scavenging activity was measured by the spectrophotometric method (Hamad et al., 2020). The stable free radical DPPH was used to test the free radical scavenging activity of plant extracts in terms of hydrogen donating or radical scavenging ability (Rakesh and Singh, 2010). The absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid solutions as standards in the concentration range of 50–500 mg/ml were used to establish a standard curve. DPPH radical scavenging activity was expressed as mg AAE/g extract. A fresh 0.002% solution of DPPH was prepared in methanol and its absorbance was recorded at 515 nm. 50 μl of pure extracts were mixed with 3 ml solution of DPPH and allowed to stand in darkness for 15 min. The absorbance was again recorded at 515 nm. The percentage inhibition of DPPH by extracts was calculated by using the following formula: % Inhibition = [(A-B)/A] X 100 Where A is the absorbance of pure DPPH in oxidized form while B is the absorbance of a sample taken after 15 min of reaction with DPPH. A calibration curve of percentage inhibition of ascorbic acid was drawn for concentrations (50, 100, 150, 200,250,300,350,400,450, and 500 mg/ml) to determine the IC_{50} values of each extract that is used to represent the radical-scavenging activity of DPPH. All the experiments were performed in triplicate.

2.7. Experimental design

After the acclimatization period, thirty-five healthy adult male albino Wistar rats were randomly divided into five equal groups each of seven as follows: group one; used as control and received distilled water, group two; treated with sodium nitrite ($NaNO_2$; 75 mg/kg BW) as a single oral dose (Ansari et al., 2017), group three; rats received *S. costus* 70% ethyl extract (SCEE; 300 mg/kg BW) and $NaNO_2$ (75 mg/kg BW), group four received *S. costus* 70% methanol extract (SCME; 300 mg/kg BW) plus $NaNO_2$ (75 mg/kg BW) while group five given *S. costus* aqueous extract (SCAE; 300 mg/kg BW) and $NaNO_2$, respectively (Alnahdi, 2017; Saleem et al., 2013). Doses of *S. costus* different extracts were given orally by gavage for four weeks then received a single dose of sodium nitrite orally by gavage 24h before decapitation. After the end of the treatment period, rats were anesthetized using isoflurane, euthanized, and blood, kidneys, and livers were collected for further analysis. The kidney and liver specimens were collected in formalin for histopathological examination.

2.8. Blood samples

Blood samples were drawn through cardiac puncture and allowed to clot for 30 min at 25 °C before being centrifuged for 15min at 3000 g. Serum was taken and kept at −80 °C until it was utilized in different assays. Other blood samples were placed in EDTA tubes and analyzed using an automated analyzer for the identification of the complete blood count (CBC) (ABX Micros 60 automated hematology analyzer HORIBA ABX DIAGNOSTIC COMPANY, FRANCE).

2.9. Body and organ weights

The body weights were recorded at the start of the treatment period (starting body weight) and at the end of the treatment period (final body weight). The livers and kidneys were dissected and weighed after the associated tissues were cut away. In addition, organ relative weight was reported as g/100 g of body weight.

2.10. Determination of liver function biomarkers

The activities of liver aminotransferases (AST; EC 2.6.1.1,CAT.NO. AS 10 61 (45) and ALT; EC 2.6.1.2, CAT.NO. AL 10 31 (45)) and alkaline phosphatase (ALP; EC 3.1.3.1) CAT. NO. AP 10 20, Albumin (CAT.NO. AB 10 10) as well as total bilirubin concentration (CAT.NO. BR 1111), were measured in rat serum using commercially available kits from Biodiagnostic Company, Egypt.

2.11. Determination of kidney function biomarkers

Serum urea (CAT.NO. UR 21 20), Creatinine (CAT.NO. CR 12 51), and uric acid (CAT.NO. UA 21 20) levels were estimated using commercially available kits from Biodiagnostic Company, Egypt.

2.12. Determination of lipid profile

Lipid profile including total cholesterol (TC) (CAT.NO. CH 12 20), triglycerides (TG) (CAT.NO. TR 20 30), high-density lipoprotein-cholesterol (HDL-C) (CAT.NO. CH 12 30), low-density lipoprotein-cholesterol (LDL-C) (CAT.NO. CH 12 31), and very-low-density lipoprotein-cholesterol (VLDL-C) were estimated utilizing commercial Biodiagnostic kits.

2.13. Histopathological examinations

Ten percent formalin buffered solution was used for fixation of liver and kidney tissues then embedded in paraffin then sections were taken out to examine the histological variations utilizing hematoxylin and eosin stain (Bancroft and Gamble, 2008). After that, slides were examined and photographs were taken using a light microscope (Olympus BX 41, Japan).

2.14. Sensory evaluation

Frozen meat chunks were purchased from the local market in Alexandria, Egypt, Fresh sausage was prepared according to the procedure of Saleh et al. (2021). The size of the meats was decreased by running them through a meat grinder. The minced meat, fat, ice water, functional components (Salt, 18g seasoning 0.1g and 0.1g fresh garlic), and additives (*S. costus* (SC) 70% ethanol extract 0, 3, 5, and 7% (v/w) were then mixed using a bowl cutter). The product was then filled out in natural casings (sheep casings were used) (Plate 3.2 and 3.3) according to packed in polyethylene bags, and then conveyed to freezing storage (Musa et al., 2020). Thus it comprises four different treatments:

(1) Sausages + 0% Control (15 mL distilled water)
(2) Sausages + 3% (v/w) SCEE (3 mL SCEE + 12 mL distilled water)

(3) Sausages + 5% (v/w) SCEE (5 mL SCEE + 10 mL distilled water)
(4) Sausages + 7% (v/w) SCEE (7 mL SCEE + 8 mL distilled water)

The frozen fresh sausages were thawed overnight at 4 °C, covered in aluminium foil, and roasted in an electric oven at 175 °C for 45 min, without salt or spices, to an internal temperature of 75 °C (Musa et al., 2020). Every sample was cut into 2 cm^2 squares and served hot (about 60 °C). In the evaluation process, ten untrained panellists were used. They were instructed to chew on a sample from each treatment and score it for color, flavor, texture, juiciness, and tenderness. Bottled water was served to the panellists to rinse their mouths after scoring each sample to reduce flavor carryovers. The panelists scored each sample on a nine-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely). Overall acceptability was scored on a three-point scale (1 = least acceptable, 2 = more acceptable and 3 = most acceptable) (Bello and Tsado, 2014).

2.15. Statistical analysis

In vitro test for each *S. costus* extract was performed in triplicate and the results were represented as the mean ± standard deviation. Other data from several experimental animal groups (n = 7/group) were given as mean ± standard error of the mean (SEM) and examined with SPSS software (version 22, IBM Co., Armonk, NY). Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test for multiple comparisons. *P*-values of less than 0.05 were deemed significant.

3. Results and discussion

3.1. Phytochemical analysis of polyphenols of different S. costus extracts by HPLC

Sixteen phenolic standards of phenolic compounds were used including gallic acid, chlorogenic acid, catechin, Methyl gallate, caffeic acid Syringic acid, pyro catechol, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, taxifolin, cinnamic acid, and kaempferol. The concentration of standard and different *S. costus* extracts are listed in Table 1. HPLC analysis of different *S. costus* extracts revealed that ethanolic extract gives a high concentration of phenolic compounds especially gallic acid, chlorogenic acid naringenin (7881.15, 3265.11, and 1197.63 μg/g, respectively), then methanol extract gives a concentration of gallic acid, chlorogenic acid and naringenin (5619.53,3129.66, and1643.42 μg/g, respectively) and the lowest concentration obtained by aqueous extract with a concentration of gallic acid, chlorogenic acid and naringenin (3057.65, 1188.15, and 449.25 μg/g, respectively). The present results are in agreement with Gheraibia et al. (2020) they found that the total flavonoids and polyphenols content in the ethanolic extract of *Costus speciosus* was the highest followed by the methanolic then the aqueous extracts and they were increased by increasing the extraction time. Also, Lee et al. (2020) founds that HPLC pattern analysis of *Aucklandia lappa* extracts were able to identify that costunolide was eluted at 22.62 min and dehydrocotus lactone was eluted at 21.11 min. Also, *Aucklandia lappa* extracts show a difference in the content of the two representative compounds (costunolide and dehydrocostus lactone) according to the solvent.

Table 1
HPLC analysis of the total polyphenols of different *S. costus* extracts.

Polyphenols	Concentration(μg/g)			
	Standards	SCEE	SCME	SCAE
Gallic acid	16.8	7881.15	5619.53	3057.65
Chlorogenic acid	28	3265.11	3129.66	1188.15
Catechin	67.5	0.00	0.00	0.00
Methyl gallate	10.2	81.74	29.57	11.39
Coffeic acid	18	59.77	68.63	48.85
Syringic acid	17.2	51.71	61.80	0.00
Pyro catechol	29.2	0.00	0.00	0.00
Rutin	61	0.00	0.00	0.00
Ellagic acid	34.3	71.85	81.91	0.00
Coumaric acid	13.2	0.00	0.00	0.00
Vanillin	12.9	115.05	125.49	65.18
Ferulic acid	12.4	194.62	220.85	119.66
Naringenin	15	1197.63	1643.42	449.25
Taxifolin	13.2	99.03	149.44	0.00
Cinnamic acid	5.8	198.16	16.04	0.00
Kaempferol	12	0.00	0.00	0.00

3.2. Total phenolic content (TPC) of different S. costus extracts

Phenolic compounds are important as antioxidant and antimicrobial agents that have many benefits for preventing several diseases and promoting human health (Tungmunnithum et al., 2018). TPCs of different *S. costus* roots extracts ranged from 53.5 to 88.17 mgGAE/g extract. Ethanol extract showed the highest TPC among the three extracts followed by methanol and aqueous extract (Table 2). These results are in agreement with several authors such as Ahmed et al. (2016); Al Kattan and Al Sheikh (2011) and Saleem et al. (2013). Furthermore, Tachakittirungrod et al. (2007) suggested that the values of the phenolic compounds differ according to the extracts' properties as well as the polarity of the solvents during the extraction process.

3.3. Total flavonoid content (TFC) of different S. costus extracts

Flavonoids are polyphenolic natural substances with antibacterial and antioxidant properties that aid in the prevention of diseases such as Alzheimer's, cancer, atherosclerosis, etc. (Castañeda-Ovando et al., 2009). The TFC of various *S. costus root* extracts ranged from 98.83 to 102.91 mg CE/g extract. The highest TFC value of SC was achieved using ethanol extract and the lowest value obtained using an aqueous one (Table 2) and these results are consistent with the findings of many authors as Ahmed et al. (2016); Al Kattan and Al Sheikh (2011) and Saleem et al. (2013). and agreement with Gheraibia et al. (2020) who found that the 70% ethanolic extract had the highest Antioxidant and radical scavenging properties of the costus extracts because it had the highest total flavonoids and polyphenols content, followed by the 70% methanolic and then the aqueous extracts.

3.4. The antioxidant activity of Saussurea costus by DPPH

The DPPH is an important parameter to evaluate the antioxidant activity of an extract (Silva et al., 2005). The antioxidant activity findings were consistent with the TPC and TFC results (Table 2), where a positive correlation between total phenolic content and antioxidant activity was observed. In comparison to methanol and water extracts, ethanol extract has stronger antioxidant activity; revealed that polarity changes in solvents can affect the composition of extracts and, as a result, antioxidant activity (Gheraibia et al., 2020). Furthermore, the amount of plant secondary metabolites is affected by a variety of factors including genes, environment, storage, and handling, resulting in phenolic content differences amongst herbal sources (Balasundram

Table 2
Phytochemical analysis of *S. costus* extracts.

SCAE	SCME	SCEE	Test
53.50 ± 4.57^{b}	57.13 ± 3.49^{b}	88.17 ± 4.35^{a}	TPCs (mg GAE/g)
98.60 ± 9.40^{a}	101.31 ± 8.78^{a}	103.75 ± 9.97^{a}	TFCs (mg QE/g)
57.80 ± 5.72^{b}	74.60 ± 6.54^{a}	82.37 ± 8.21^{a}	DPPH (mg AAE/g)

Values are expressed as means ± SD (each test was triplicate). Mean values within a row not sharing common superscript letters were significantly different, $p < 0.05$.

et al., 2006). The variation between these results and those reported previously may be due to the difference in the reactivity of the extracts because of their chemical complexity (El-Far et al., 2018). Moreover, the polarity differences and the functional groups position in the active secondary metabolites which have potent antioxidant activities (Ahmed et al., 2016). Furthermore, the antioxidant activity is probably due to the occurrence of specific phytochemicals which may be served as a donor of electrons (Yu et al., 2002). All the studied extracts showed good antioxidant activity but less than that of ascorbic acid due to their high proton donating ability.

3.5. Hematological results

Results in Table 3 indicated a significant decrease in total erythrocyte counts (RBCs), platelets counts, total leukocyte counts (WBCs), and a non-significant decrease in hemoglobin (Hb), hematocrit (Ht) in rats administered with a single dose of sodium nitrite as compared with control. While, the pre-treatment of rats with aqueous, methanol and ethanol extract of *S. costus* leads to a significant increase in platelets counts and a non-significant increase in Hb, Ht, and WBCs. While RBCs showed a significant increase with ethanol and aqueous extracts while a non-significant increase with methanol extract when compared to the $NaNO_2$ group. Ethanolic extract was found to be the best one and this results in agreement with Abdel-Baky (2019) who found that $NaNO_2$ induced a significant decrease in the percentages of RBCs count, Hb content, PCV, MCH, MCHC, platelets, and WBCs, and the toxic action of sodium nitrite on bone marrow, spleen, and liver may be responsible for the decrease in RBCs count and Hb content (Aita and Mohammed, 2014) or may be due to sodium nitrite's direct interaction with Hb, in which it oxidizes the ferrous ion of oxyhemoglobin (oxy Hb) molecules to the ferric state, resulting in met Hb, which causes cell death (Ibrahim et al., 2009). The decrease in WBCs count after treatment with sodium nitrite may be due to the failure of the hematopoietic tissues to produce new WBCs (Tan et al., 1992). In agreement, *S. costus* extracts administration successfully mitigated the toxic impact of deltamethrin (DM) insecticide on hematological parameters, and markedly modulated deviations in hemogram parameters (RBCs, Hb, and WBCs) (Alnahdi et al., 2017).

3.6. Body and organs weights

The results listed in Table 4 revealed that administration of rats with sodium nitrite affect body weight, BWG, and some organs weight, and the pretreatment with costus extract improved the toxic effect of sodium nitrite and there are no significant changes between all the three extract aqueous, methanol and ethanol extract of *S. costus* but the best result is for the ethanolic extract. In agreement with Ahmed (2017), the aqueous extracts of costus caused considerable improvements in essential physiological processes as well as weight loss. Also, Krishna Dutta Tejaswi et al. (2018) founds that the ethanol extract of *S. costus* causes a significant reduction in the weight of the liver compared to toxicant groups.

Table 3
Hematological parameters in different groups.

Parameters	Control	$NaNO_2$	SCEE + $NaNO_2$	SCME + $NaNO_2$	SCAE + $NaNO_2$
RBCs (10^6 cells/μL)	6.29 ± 0.226^{a}	5.3 ± 0.188^{b}	6.21 ± 0.232^{a}	5.82 ± 0.211^{ab}	6.08 ± 0.22^{a}
Hb (g/dl)	11.61 ± 0.393^{a}	11.14 ± 0.358^{a}	11.59 ± 0.373^{a}	11.4 ± 0.382^{a}	11.5 ± 0.366^{a}
Ht(%)	34.57 ± 1.22^{a}	33.0 ± 1.13^{a}	35.03 ± 1.25^{a}	34.14 ± 1.23^{a}	34.66 ± 1.21^{a}
Platetlets (10^3 cells/μL)	832 ± 27.04^{a}	603 ± 16.64^{d}	770 ± 25.76^{c}	685 ± 22.77^{bc}	724 ± 26.98^{bc}
WBCs (10^3 cells/μL)	12.66 ± 0.312^{a}	11.01 ± 0.39^{b}	11.93 ± 0.387^{ab}	11.06 ± 0.372^{b}	11.34 ± 0.377^{b}

Values are expressed as means ± SE; n = 7 for each treatment group. Mean values within a row not sharing common superscript letters were significantly different, $p < 0.05$. Statistically significant variations are compared as follows: $NaNO_2$ group is compared vs control while SCEE + $NaNO_2$, SCME + $NaNO_2$, and SCAE + $NaNO_2$ are compared to $NaNO_2$ group.

Table 4
Organs and body weights in different groups.

Parameters (g)	Control	$NaNO_2$	SCEE + $NaNO_2$	SCME + $NaNO_2$	SCAE + $NaNO_2$
Initial body weigh	159 ± 1.84^{a}	159 ± 2.08^{a}	155 ± 1.90^{a}	154 ± 2.15^{a}	**159 ± 1.52^{a}**
Final body weight	196 ± 2.94^{b}	186 ± 2.75^{b}	214 ± 4.09^{a}	209 ± 4.45^{a}	**214 ± 3.87^{a}**
Body weight gain	37.0 ± 3.70^{b}	28.0 ± 4.04^{b}	59.0 ± 3.57^{a}	55.0 ± 4.67^{a}	**55.0 ± 4.54^{a}**
Liver weight	4.04 ± 0.147^{c}	5.44 ± 0.141^{a}	4.60 ± 0.126^{b}	4.65 ± 0.153^{b}	**4.58 ± 0.122^{b}**
Relative liver weight	2.06 ± 0.080^{b}	2.90 ± 0.088^{a}	2.16 ± 0.057^{b}	2.27 ± 0.101^{b}	**2.13 ± 0.069^{b}**
Kidney weight	1.34 ± 0.037^{a}	1.07 ± 0.042^{b}	1.35 ± 0.067^{a}	1.31 ± 0.043^{a}	**1.32 ± 0.047^{a}**
Relative kidney weight	**0.69 ± 0.023^{a}**	**0.57 ± 0.025^{b}**	**0.64 ± 0.038^{ab}**	**0.64 ± 0.015^{ab}**	**0.61 ± 0.021^{ab}**

Values are expressed as means ± SE; n = 7 for each treatment group. Mean values within a row not sharing common superscript letters were significantly different, $p < 0.05$. Statistically significant variations are compared as follows: $NaNO_2$ group is compared vs control while SCEE + $NaNO_2$, SCME + $NaNO_2$, and SCAE + $NaNO_2$ are compared to $NaNO_2$ group.

3.7. Liver function biomarkers

Rats treated with a single dosage of sodium nitrite showed a significant increase of liver function indices (ALT, AST, ALP, and total bilirubin) and a significant drop in serum albumin when compared to the control group. Rats supplemented with different *S. costus* roots extracts before sodium nitrite treatment resulted in a significant decrease in ALT, AST, ALP, and total bilirubin as well as a significant increase in serum albumin, indicating that the ethanol extract is the best one for alleviating the toxic effect of sodium nitrite over methanol and aqueous extract (Table 5). The observed changes in hepatic biomarkers could be attributable to lipid peroxidation, which disrupts the integrity of cellular membranes, allowing cytoplasmic enzymes to leak into the circulation following hepatocellular injury (El-Demerdash et al., 2021; Jebur et al.,

Table 5
Liver and kidney function biomarkers in different groups.

Parameters	Control	$NaNO_2$	SCEE + $NaNO_2$	SCME + $NaNO_2$	SCAE + $NaNO_2$
AST (U/L)	144 ± 5.37^{b}	174 ± 6.45^{a}	157 ± 5.52^{ab}	167 ± 6.22^{a}	165 ± 6.03^{a}
ALT (U/L)	36.6 ± 0.89^{c}	51.27 ± 1.46^{a}	44.96 ± 1.15^{b}	49.63 ± 1.8^{ab}	48.64 ± 1.7^{ab}
ALP (U/L)	74.49 ± 2.79^{b}	94.51 ± 3.53^{a}	78.44 ± 2.93^{b}	83.01 ± 3.13^{b}	79.74 ± 2.87^{b}
ALb (g/dl)	3.62 ± 0.124^{a}	3.08 ± 0.115^{b}	3.47 ± 0.116^{a}	3.72 ± 0.128^{a}	3.58 ± 0.116^{a}
Bilirubin (mg/dl)	0.404 ± 0.012^{d}	0.694 ± 0.017^{a}	0.503 ± 0.02^{c}	0.71 ± 0.024^{a}	0.607 ± 0.023^{b}
Creatinine (mg/dl)	0.62 ± 0.022^{d}	1.129 ± 0.043^{a}	0.784 ± 0.026^{c}	0.91 ± 0.033^{b}	0.853 ± 0.031^{bc}
Urea (mg/dl)	39.27 ± 1.45^{b}	51.31 ± 1.93^{a}	46.7 ± 1.63^{a}	49.49 ± 1.87^{a}	48.31 ± 1.77^{a}
Uric acid (mg/dl)	1.35 ± 0.044^{b}	1.85 ± 0.061^{a}	1.61 ± 0.040^{a}	1.77 ± 0.060^{a}	1.73 ± 0.061^{a}

Values are expressed as means ± SE; n = 7 for each treatment group. Mean values within a row not sharing common superscript letters were significantly different, $p < 0.05$. Statistically significant variations are compared as follows: $NaNO_2$ group is compared vs control while SCEE + $NaNO_2$, SCME + $NaNO_2$, and SCAE + $NaNO_2$ are compared to $NaNO_2$ group.

2022). In parallel, several authors concluded that sodium nitrite exposure caused changes in enzymes activity since they have the potency to induce hepatic injury and physiological impairments (El-Nabarawy et al., 2020; Adewale et al., 2019). Also, the toxic effects of nitroso compounds generated in the acidic environment of the stomach induced severe hepatic necrosis which is responsible for the disturbance in the balance of liver enzymes activity (Özen et al., 2014). Protein is also one of the most sensitive cell components to free radical damage. Excessive protein loss via nephrosis, or induction of proteolytic enzymes activity or degradation, were the main causes of the observed drop in protein content, In addition, a change in normal liver function could be linked to the significant drop in albumin level in sodium nitrite-treated rats (Bhushan et al., 2013). Furthermore, an increase in total bilirubin may be caused by decreased liver output or occlusion of the bile ducts as a result of liver cell failure. On the other hand, several authors reported that oral administration of *S. costus* extracts was able to reduce lipid peroxidation and improve liver function markers because of its wide range of phenolic components and natural products observed here. The increase in radical scavenging activity of *S. costus* in this study was most likely due to its significant components chlorogenic acid, naringenin, and gallic acid, respectively (Ahmed, 2017; Alnahdi et al., 2017; Krishna Dutta Tejaswi et al., 2018). so, *S costus* has a hepatoprotective effect.

3.8. Kidney function biomarkers

Sodium nitrite administration induced urea, creatinine, and uric acid levels significantly as compared to control. While the existence of different *S costus* extracts with $NaNO_2$ maintained all the measured parameters closer to the normal values especially the ethanolic extract (Table 5). In parallel, the increase in renal function biomarkers was observed by many authors (El-Nabarawy et al., 2020). Serum uric acid levels could be affected by various drugs by influencing the uric acid net reabsorption in the nephron (Reyes, 2003). Besides, urea, a major nitrogen-containing end product of protein metabolism, was increased in the blood of $NaNO_2$-treated rats. These results correlated with the increased protein catabolism in mammals and/or with the conversion of ammonia to urea as a result of increased synthesis of the arginase enzyme involved in urea production (Hooper et al., 1998). The observed improvement of kidney function biomarkers is attributed to the antioxidant activity of *S. costus* extracts which are rich in bioactive components (Ayaz, 2017).

3.9. Lipid profile in rat serum

A significant elevation in total cholesterol, TG, LDL-C and VLDL-C levels ($P < 0.05$) were observed in rats received sodium nitrite, while HDL-C level was decreased. On one hand, in rats treated with different *S. costus* roots extracts with sodium nitrite, a significant reduction in total cholesterol, TG, LDL-C, and VLDL-C levels, and significant induction in HDL-C as compared to the $NaNO_2$ group was detected and the ethanol extract is the best one which alleviates sodium nitrite toxicity more than methanol and water extract (Table 6). Lipids are thought to be among the most sensitive biomolecules in terms of reactive oxygen species (ROS) susceptibility. In particular, unsaturated fatty acids, which are located in cellular membranes, tissues, and blood are prone to ROS attack induced by sodium nitrite. The significant elevation in cholesterol level after $NaNO_2$ administration and this might be attributed to increased cholesterol synthesis in the liver, loss of membrane integrity and block of the liver bile ducts causing reduction or discontinuation of its secretion to the duodenum (Kalender et al., 2005; Sarin et al., 1997). Previous studies reported disturbance in lipids profile in serum of experimental animals (El-Demerdash et al., 2020; Helal et al., 2020) due to xenobiotics accumulation in the liver leading to alterations in lipid metabolism and consequently escalation in cholesterol blood level (Whihelm et al., 1996). The observed increase in triglycerides might be due to the inhibition of lipase activity in the liver and plasma

Table 6
Lipid profile in different groups.

Parameters (mg/dl)	Control	$NaNO_2$	SCEE + $NaNO_2$	SCME + $NaNO_2$	SCAE + $NaNO_2$
Total Cholesterol	118 ± 2.67^{c}	147.4 ± 2.92^{a}	129 ± 2.59^{b}	136.6 ± 2.4^{ab}	136.1 ± 2.8^{ab}
Triglycerides	147 ± 5.47^{c}	178 ± 6.57^{a}	147 ± 5.47^{c}	168 ± 6.28 ^{ab}	157 ± 5.7 ^{bc}
HDL-C	54.9 ± 1.01^{a}	36.1 ± 1.32^{b}	49.9 ± 1.01^{d}	43.1 ± 0.82^{c}	38.5 ± 1.52^{b}
LDL-C	33.8 ± 1.19^{c}	75.8 ± 3.29^{a}	49.8 ± 2.98^{b}	59.8 ± 2.73^{ab}	66.2 ± 4.13^{ad}
VLDL-C	29 ± 1.09^{b}	36 ± 1.31^{a}	29 ± 1.09^{b}	34 ± 1.265^{bc}	31 ± 1.14^{bc}

Values are expressed as means ± SE; n = 7 for each treatment group. Mean values within a row not sharing common superscript letters were Significantly different, $p < 0.05$. Statistically significant variations are compared as follows: $NaNO_2$ group is compared vs control while SCEE + $NaNO_2$, SCME + $NaNO_2$, and SCAE + $NaNO_2$ are compared to $NaNO_2$ group.

lipoproteins (Goldberg et al., 1982). HDL-C is fundamentally synthesized in the cells of the liver and intestine so, it has an essential role in cholesterol outflow from tissues and loads it back to the liver for removal as bile acids (Shakoori et al., 1988). It has been established that the reduction in HDL-C levels is accompanied by a high risk for coronary artery disease. The observed improvement in lipid profile especially the ethanolic extract agreed with several authors as Ahmed (2017) and Krishna Dutta Tejaswi et al. (2018) as the protective role of *S. costus* roots might be attributed to its high content of active ingredients that have potent antioxidant and anti-inflammatory activities (Ahmed et al., 2016).

3.10. Histological observations in rat liver

Histopathological examination of control liver sections exhibited normal architecture. In contrast, liver sections in sodium nitrite-treated rats showed portal tract dilatation surrounded by fibrotic and necrotic cells. However, rats pretreated with *S. costus* extract showed detectable recovery in the liver architecture and improved the toxic action of sodium nitrite on liver cells and no significant changes between the improvement action of aqueous, ethanol and methanol extract of *S. costus* roots (Fig. 1 and Table 7). Similar observations exhibited fatty liver, increased fat droplet deposition, and decreased cellular density and distribution as well as morphological alterations in the $NaNO_2$ treated group (El-Nabarawy et al., 2020). Ras pretreated *S. costus* extracts then treated with $NaNO_2$ showed some improvement in the kidney histopathological structure and this might be due to its active constituents.

3.11. Histopathological observation in rat kidney

Light microscopic evaluation of the renal cortex of rat kidney tissue from control (G1) revealed the normal histological architecture of the glomeruli and renal tubules. In contrast, renal cortex sections of sodium nitrite group (G2) microscopic examination revealed necrotic glomeruli and marked dilatation of renal tubules and interstitial tissue. On the other hand, pre-treatment with *S. costus* extract (G3, G4, G5) improved the toxic action of sodium nitrite on liver cells, and no significant changes between the improvement actions of the aqueous, ethanol, and methanol extracts are observed (Fig. 2 and Table 8). Histopathological studies of kidney sections of $NaNO_2$ intoxicated rats showed variable abnormal morphological changes in the liver and renal tissues, pathological changes, and abnormalities in glomeruli and some parts of the urinary tubules. This is due to the oxidative toxicity caused by $NaNO_2$ which may have led to severe alterations in Malpighian corpuscles and loss of their distinctive configuration. Additionally, renal tubules with wide lumen, degenerated epithelium, and marked congestion in the

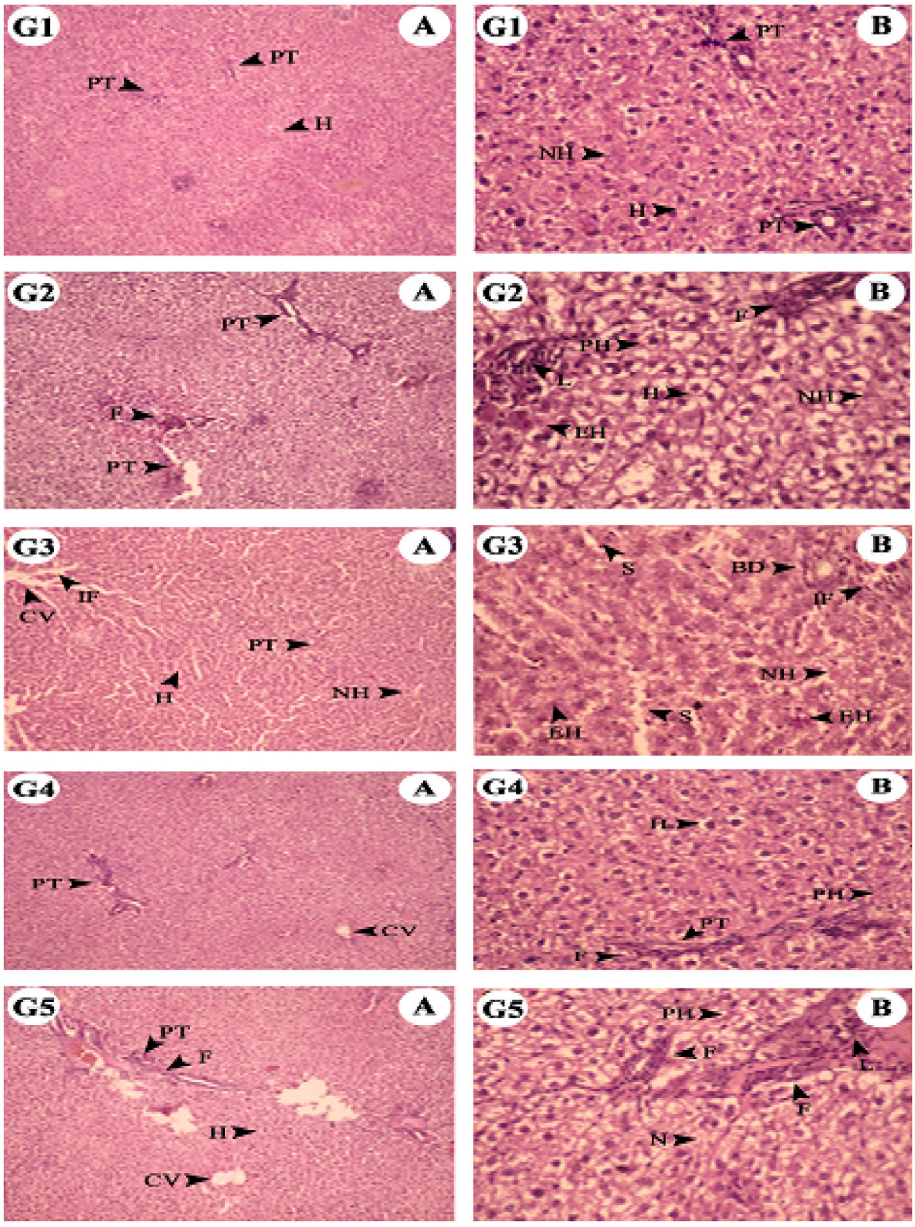

Fig. 1. Photomicrographs of rat liver sections, group1: Control, group 2: sodium nitrite, group 3: Ethanol extract of SC and sodium nitrite, group 4: Methanol extract of SC and sodium nitrite and group 5: Aqueous extract of SC and sodium nitrite, (CV: central vein, PT: portal tract, F: fibrotic cells, L: lymphocyte, H: hepatocyte, EH: eosinophilic hepatocytes,BD: bile duct, NH: necrotic hepatocyte, AM: mitotic hepatocytes, PH: pyknotic hepatocytes, IF: infiltrating lymphocytes, N: necrotic cells), (A) the left power field (H&E stain, 100Xmagn.) and (B) the right, high power of the same filed (H&E stain, 400Xmagn.).

Table 7
The scores of histological alterations of liver in different groups.

Groups / parameters	Control	$NaNO_2$	SCEE/ $NaNO_2$	SCME/ $NaNO_2$	SCAE/ $NaNO_2$
CV dil.	–	+	+	+	–
PT dil.	–	+	+	+	+
Fibrosis	Few	++	–	+	+
Necrosis	Few	+	Few	–	+
Regenerative H.	–	–	+	+	+

renal blood vessels were appeared (El-Nabarawy et al., 2020). In agreement, *S. costus* improved the alteration induced by $NaNO_2$ in kidney architecture due to its bioactive components that have powerful antioxidant activity (Ayaz, 2017); Gheraibia et al. (2020); Hassan and Masoodi (2020). The kidneys of animals treated with S lappa extract only revealed normal glomeruli with a tuft of blood capillaries surrounded by Bowman's capsule, suggesting that early administration of 70% methanol extract of S lappa (300 mg/kg BW) for 28 days may protect renal tissue from oxidative damage caused by the toxic effects of deltamethrin pesticide.

3.12. Sensory evaluation

The results in Table 9 show that the mean color scores for control and treated samples did not differ significantly. The 5% SC treated sample had significantly higher mean flavors scores than the other treatments. The decreased flavors scores of 7% SC treated samples in our study could be attributed to Saussurea costus delivering too much costus flavor at higher concentrations. Treatment with *Saussurea costus* improved the juiciness scores compared to the control. All *Saussurea costus*-treated samples had a substantial increase in texture and tendernesswhen compared by control and the 5 %and 7% treated samples had significantly higher mean scores than the 3% treated group. The difference between the 5% and 7% treated samples was not significant. Samples showed a significant improvement in overall acceptability scores. There was no change between the 5% and 7% treated samples.

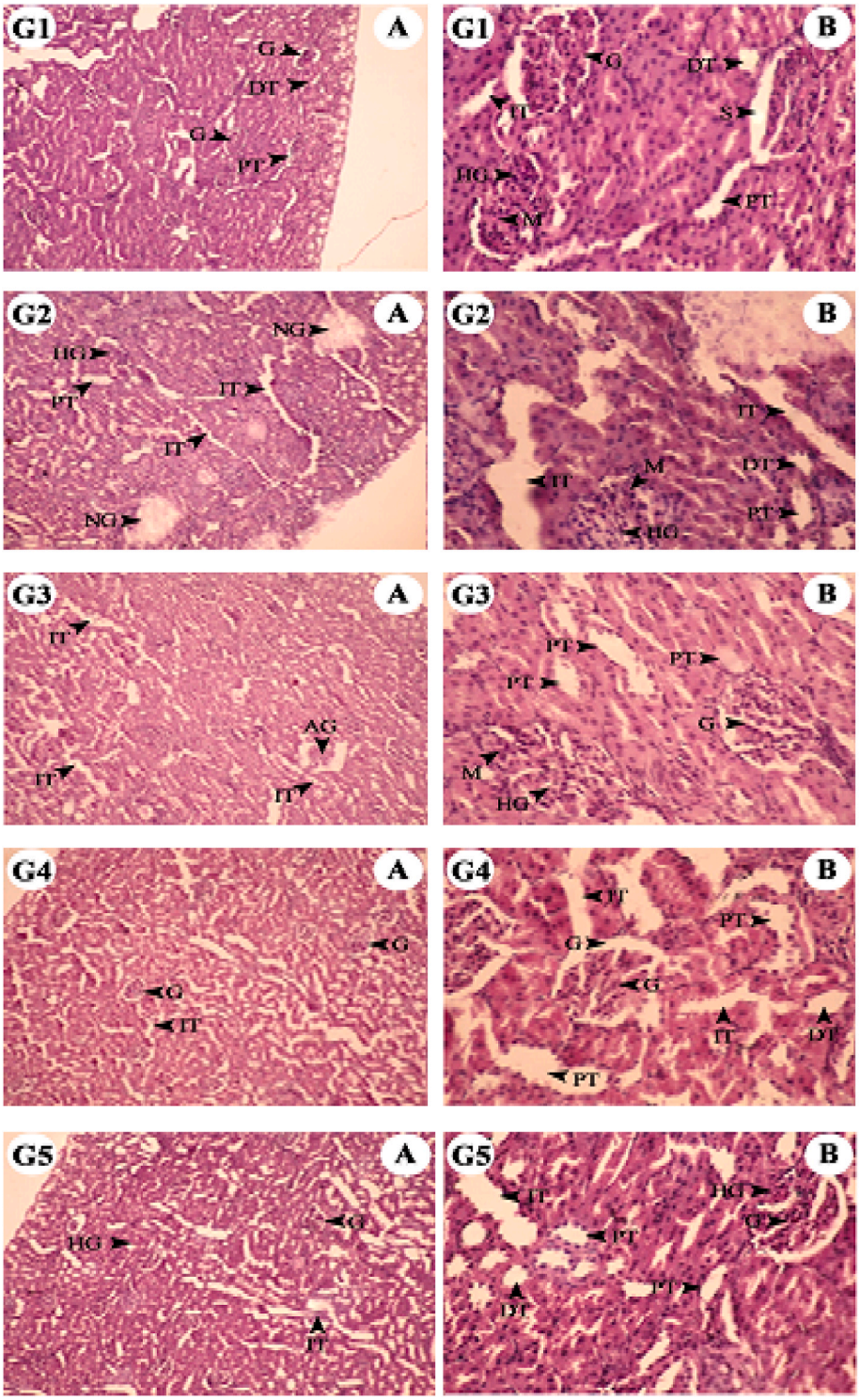

Fig. 2. Photomicrographs of rat kidney sections, group1: Control, group 2: sodium nitrite, group 3: Ethanol extract of SC and sodium nitrite, group 4: Methanol extract of SC and sodium nitrite and group 5: Aqueous extract of SC and sodium nitrite. (IT: interstitial tissue, G: normal glomeruli, HG: hyperemic one, M: mesangial cells, DT: distal tubule, PT: proximal tubule, AG: atrophied glomeruli, S: minimal urinary space), (A) the left power field (H&E stain, 100Xmagn.) and (B) the right, high power of the same filed (H&E stain, 400Xmagn.).

Plant extracts are becoming more popular in the food industry as a result of their antibacterial and antioxidant properties, which help to prevent off-flavors and improve color stability in ready-to-eat (RTE) meat products. They are good candidates to replace synthetic compounds, which are often thought to have toxicological and carcinogenic effects, due to their natural origins (Nikmaram et al., 2018).

4. Conclusion

In conclusion, sodium nitrite induced pronounced hazardous effects in several physio-metabolic functions including body weight, blood, and tissue parameters. Estimation of some hematological, genetics, and biochemical parameters could be used as bioindicators for the harmful effect of sodium nitrite exposure. Pretreatment of different extracts of *S. costus* extract minimized and alleviated its hazardous effects on most of the tested parameters and this may be attributed to the vital role of *S. costus* as food additive especially the ethanol extract of *S. costus* and effective against the sodium nitrate toxicity. Additinally, it can be added with meat to improve the sensory evaluation of meat and detoxifing any hazord effect resulted from sodium nitrate.

Table 8
The scores of histological alterations of kidney in different groups.

Groups / Parameters	Control	$NaNO_2$	SCEE/ $NaNO_2$	SCME/ $NaNO_2$	SCAE/ $NaNO_2$
Glomeruli Atrophy and necrosis	-	++	**few**	-	-
Renal tubule Dilation	**Few**	++	+	++	++
Interstitial tissues dilation	-	++	+	++	++

Table 9
Effect of different concentrations of *S. costus* ethanol extract (SC) on sensory evaluation of frozen meat sausages.

Sensory attributes	(Control) 0% SCEE	3% SCEE	5% SCEE	7% SCEE
Color	7.5 ± 0.12^{a}	7.57 ± 0.13^{a}	7.7 ± 0.17^{ab}	7.5 ± 0.13^{a}
Flavor	7.3 ± 0.13^{a}	7.53 ± 0.09^{a}	8.0 ± 0.15^{b}	7.56 ± 0.14^{a}
Texture	7.13 ± 0.13^{a}	7.4 ± 0.16^{b}	7.7 ± 0.21^{c}	7.89 ± 0.15^{c}
Juiciness	7.3 ± 0.18^{a}	7.39 ± 0.16^{ab}	7.56 ± 0.12^{ab}	7.73 ± 0.16^{b}
Tenderness	6.94 ± 0.14^{a}	7.4 ± 0.16^{b}	7.81 ± 0.16^{c}	7.84 ± 0.14^{c}
Overall acceptability	1.6 ± 0.09^{d}	1.84 ± 0.08^{b}	2.17 ± 0.08^{ab}	2.5 ± 0.10^{a}

Values are expressed as means ± SE; n = 10 for each treatment group. Mean values within a row not sharing common superscript letters were significantly different, $p < 0.05$. Statistically significant variations are compared as follows: 3% SCEE, 5% SCEE, and 7% SCEE are compared to control group.

Ethics approval

Approved by the Ethical Committee on Animal Experimentation of the Institutional Animal Care and Use Committee (ALEXU-IACUC) at Alexandria University, Egypt. The ethical Approval number is (AU14-210126-2-3).

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Availability of data

The original contributions presented in the study are included in the article.

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Samy E. Elshaer: Funding acquisition, Methodology, Software, Data curation, Writing – original draft, Writing – review & editing. **Gamal M. Hamad:** Conceptualization, Methodology, Data curation, Funding acquisition, Software, Writing – original draft, Writing – review & editing. **Elsayed E. Hafez:** Conceptualization, Methodology, Data curation, Funding acquisition, Writing – original draft, Writing – review and editing. **Hoda H. Baghdadi:** Supervision, Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Fatma M. El-Demerdash:** Supervision, Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Jesus Simal-Gandara:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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