Issues in Biological Sciences and Pharmaceutical Research Vol.9(1),pp.1-11, January 2021
Available online at https://www.journalissues.org/IBSPR/
https://doi.org/10.15739/ibspr.21.001
Copyright © 2021 Author(s) retain the copyright of this article
ISSN 2350-1588

Original Research Article

Grape seed and skin extract (GSSE) protects against toxicity induced by garlic in rat brain

Received 22 October, 2020 | Revised 18 December, 2020 | Accepted 22 December, 2020 | Published 4 January, 2021

Sonia Hamlaoui[1*], Yosra Hamdi[1], Ezzedine Aouani[2] and Sana Mezghani[1]

[1]Laboratoire de Neurophysiologie Fonctionnelle et Pathologies, Département des Sciences Biologiques, Faculté des Sciences de Tunis. Rectorat El Manar Tunis, Tunisie.
[2]Laboratoire des Substances Bioactives, Centre de Biotechnologie, Technopole Borj-Cedria, Rectorat de Carthage, Tunisie.

*Corresponding Author E-mail: sonia_hamlaoui@yahoo.fr

Tel: +216 98 96 81 13

Garlic is a medicinal plant with many therapeutic properties. Intraperitoneal injection of garlic induces a harmful stress to health. Grape seed and skin extract (GSSE) have multiple beneficial effects even when used at high dosage. Aim: This work studies the ability of GSSE to correct the harmful effects of intraperitoneal injection of garlic on rat brain. Materials and methods: Rats were intraperitoneally administered with garlic extract (5 g/kg body weight) or GSSE (500 mg/kg body weight) or a combination of garlic and GSSE at the same doses daily for one month. Brain oxidative stress markers and antioxidant status were evaluated. Results: Garlic increased malondialdehyde, carbonyl protein, calcium, magnesium, free iron, nitric oxide and reactive oxygen species (superoxide ion and hydrogen peroxide) levels. However, it decreased antioxidant enzymes as catalase and superoxide dismutase. Garlic also altered brain metabolic enzymes activities by increasing lactate dehydrogenase, xanthine oxidase and lipase but it dropped creatinekinase and acethylcholinesterase. GSSE alone decreased malondialdehyde, carbonyl protein, lactate dehydrogenase, calcium, magnesium and hydrogen peroxide levels but increased antioxidant enzymes activities. GSSE in co-treatment with garlic removed almost all the deleterious effects of garlic. Conclusion: High garlic dose induced a pro-oxidative state in brain via the Fenton reaction between hydrogen peroxide and free iron, and GSSE exerted antioxidant properties.

Keywords: Garlic, grape seed and skin extract, brain, oxidative stress, antioxidant enzymes, intracellular mediators, metabolic enzymes.

INTRODUCTION

Dietary factors play a major role in the metabolic disorders prevention. Garlic has been widely used in traditional medicine and has been extensively studied as a nutritional supplement. It has attracted the attention of modern medical science because of its widespread over the counter use. Garlic exerted wide range effects as antiviral and antibacterial (Bhatwalkar et al., 2019), antitumoral (Wang et al., 2019), and anti-apoptotic (Hassan et al., 2019). Its health effects are mainly attributable to organosulfur compounds (Berginc et al., 2010) and phenols as flavonoids (Shirzad et al., 2011).

Grape seed and skin extract (GSSE) exhibited beneficial health effects including lung (El Ayed et al., 2018), renal (Mokni et al., 2016) and hepatic (Hamlaoui-Gasmi et al., 2012) protection. GSSE is also protective against the toxic side effects linked to the use of antineoplastic drugs as doxorubicin (Mokni et al., 2015), cisplatin (Tian et al., 2018) and bleomycin (Khazri et al., 2016). GSSE is a complex mixture of bioactive components including proantho-cyanidins, flavonoids and stilbenes (Khanal et al., 2009). Flavonoids are highly concentrated in grape seeds and mainly composed of monomeric catechins and flavonols as quercetin (Casazza et al., 2011). Non-flavonoids, highly present in grape skin contained stilbenes

as resveratrol which is at the basis of the French Paradox (Renaud and de Lorgeril, 1992). Pro-antho-cyanidins exert antineoplasic effects by cell cycle arrest and induction of apoptosis (Kaur et al., 2008). GSSE, a potential health food ingredient, has been attributed the Generally Recognized As Safe (GRAS) certification from US FDA. Oxidative stress induced injury to the brain, an organ that has a relatively low level of antioxidant enzymes. In the present work, we investigated the toxic effect of a high garlic dose on brain antioxidant status as well as in GSSE neuroprotection. This study evaluated lipid peroxidation, protein carbonylation, reactive oxygen species (ROS), intracellular mediators, metabolic and antioxidant enzymes activities that could be involved in garlic-induced brain injury.

MATERIALS AND METHODS

Preparation of garlic and grape seed and skin extracts

Garlic (*Allium sativum* L.) was purchased from local market, peeled and grounded during 30 min with an electric mincer (FP3121 Moulinex manufacture) until an aqueous suspension was obtained. It was diluted in double distilled water at 4 g/ml on the basis of the weight of the starting material and centrifuged (Beckman J20, 15 min at 10000 g and 4°C). Supernatant was aliquoted into one ml fractions and stored at -80°C until use.

GSSE was processed from a grape cultivar (Carignan) of *Vitis vinifera* from northern Tunisia. Waste from winemaking was collected from Tardi Cooperative Winery (Ain Ghelal). Seeds and skin were dried and grounded separately with an electric mincer (FP3121 Moulinex) until a fine powder was obtained. Total phenolic content was determined by the Folin-Ciocalteau colorimetric method (Singleton and Rossi, 1965), flavonoids and condensed tannins according to Dewanto et al. (2002) and Sun et al. (1998), respectively. Powder mixture containing grape seed (50%) and skin (50%) was dissolved in 1 mL of 10% ethanol in the dark, vigorously vortexed for 10 min, centrifuged at 10 000 g for 15 min at 4°C for debris elimination and the supernatant containing soluble polyphenols was used.

Animals and treatment

Forty male Wistar rats (200-240 g) from Pasteur Institute of Tunis were used for these experiments in accordance with the local ethic committee of Tunis University and care of animals in conformity with NIH guidelines (National Research Council, 1985). They were provided with food and water and libitum and maintained in animal house at controlled temperature (22 ± 2°C) with a 12h light-dark cycle. Rats were divided into four groups of ten animals each. Group I received ethanol 10% (control), group II aqueous extract of garlic (5 g/kg body weight), group III GSSE (500 mg/kg body weight) and group IV garlic plus GSSE. Animals were daily intraperitoneally (IP) injected during 30 days. Twenty-four hours after the last injection, animals were sacrificed; their brains rapidly excised, weighted and homogenized in phosphate buffer saline pH 7.4 with an ultrathurax T25 homogenisator at a 2ml/g ratio. After centrifugation at 10000 g for 10 min at 4°C, supernatant was used for the determination of lipoperoxidation, carbonylation, antioxidant enzyme activities, Reactive oxygen species (superoxide ion (O_2^-) and hydrogen peroxide (H_2O_2)), metabolic enzymes activities and intracellular mediators (free iron, nitric oxide (NO), calcium and magnesium).

Brain oxidation

Lipoperoxidation was determined by malondialdehyde (MDA) measurement according to the double heating method (Draper and Hadley, 1990). Briefly, an aliquot of the homogenate was mixed with 2, 6,-di-tert-butyl-4-hydroxy-toluene - acetic acid (BHT/TCA) solution containing 1% (w/v) BHT dissolved in 20% TCA (w/v) and centrifuged at 4000g for 15 min at 4°C. Supernatant was blended with 0.6 N HCl and 120 mmol L^{-1} thiobarbituric acid in a 26 mmol L^{-1} Tris buffer. The mixture wash eated at 80°C for 10 min, cooled, and absorbance measured at 532 nm. MDA was determinate using the absorbance coefficient of the MDA-TBA complex: $1.56.10^5 cm^{-1} mmol\ L^{-1}$.

Oxidative damage to brain proteins was evaluated by quantifying protein carbonylation in brain homogenates according to Levine et al. (1990). After proteins precipitation with 20% TCA and centrifugation at 11000 g during 3 min at 4°C (Beckman J20), pellet was dissolved in 10 mM DNPH-containing buffer. After 3 washings with ethanol-ethylacetate (1:1), pellet was dissolved in 20 mM potassium phosphate (pH 2.3) containing 6 M guanidine HCl and absorbance measured at 366 nm using the molar extinction coefficient of 22000 $M^{-1}cm^{-1}$. Results were expressed as nmol carbonyl residues/mg protein.

Antioxidant enzymes activities

All spectrophotometric analyses of antioxidant enzymes activities were performed with a SmartSpec 3000 BIORAD UV-visible spectrophotometer (Germany). Catalase (CAT) activity was assayed by measuring the initial rate of H_2O_2 disappearance at 240 nm (Aebi, 1984). The reaction mixture contained 33 mM (1000 µl) H_2O_2 in 50 mM (1995 µl) phosphate buffer pH 7.0 and 5µl of brain extract. CAT activity was calculated using the extinction coefficient of 40 $mM^{-1}cm^{-1}$ for H_2O_2 (Aebi, 1984).

Peroxidase (POD) activity was measured at 25°C using guaiacol as hydrogen donor as described by Chance and Maehly, 1955. The reaction mixture contained 9 mM (25 µl) guaiacol, 19 mM (100 µl) H_2O_2 in 50 mM (870µl) phosphate buffer pH 7 and 5 µl of brain extract in 1 ml final volume. The reaction was initiated by the addition of H_2O_2 and monitored by measuring the increase in absorbance at 470 nm each 30 s during 3 min. Peroxidase activity was expressed as nmol of guaiacol oxidized per min.

Superoxide dismutase (SOD) activity was determined by using modified epinephrine assay (Misra and Fridovich, 1972). At alkaline pH, superoxide anion (O_2^-) causes the auto-oxidation of epinephrine to adenochrome. One unit of SOD is defined as the amount of extract that inhibits the rate of adenochrome formation by 50%. Brain extract was added in 2 ml reaction mixture containing 10 µl bovine catalase (0.4 U/µl), 20 µl epinephrine (5 mg/ml) and 62.5 mM sodium carbonate/sodium bicarbonate buffer pH 10.2. Changes in absorbance were recorded at 480 nm. Characterization of SOD isoforms was performed using KCN (3 mmol) as a Cu/Zn inhibitor or H_2O_2 (3 mmol), which affects both Cu/Zn and Fe-SOD, whereas Mn-SOD is insensitive to both inhibitors.

Intracellular mediators

Brain free iron was determined according to Leardi et al. (1998) using a commercially available kit from Biomaghreb, Tunisia. Briefly, at acidic pH 4.8 all Fe^{3+} is released from transferrine; ascorbic acid reduced Fe^{3+} to Fe^{2+}, which constituted with ferrozine a colorful complex measurable at 560 nm.

NO was measured by quantification of nitrite and nitrate, determined colorimetrically using a commercially available kit from Roche Diagnostics France (Katrina et al., 2001).

Brain ionizable calcium was determined according to Stern and Lewis (1957) using a commercially available kit from Biomaghreb, Tunisia. At basic pH calcium constituted with cresolphtalein a purple colourful complex measurable at 570 nm. Briefly, brain extract was added to reaction mixture containing 2-amino-2-methyl 1-propanol buffer (500 mmol/L), cresolphtalein (0.62 mmol/L) and hydroxy-8 quinoleine (69 mmol/L). Incubation was carried out at room temperature during 5 min assuming the complex was stable during 1 hour.

Magnesium level was determined using a commercially available kit from Biomaghreb, Tunisia (Olusanya et al., 2015). Magnesium forms a purple colored complex when treated with calmagite in alkaline solution, measurable at 520 nm.

Brain enzymology

Acetylcholinesterase (AChE) activity was measured using a spectrophotometric method as described by Ellman et al. (1961). The enzymatic activity of AChE is expressed in nmol/min/mg of protein.

Creatine kinase activity was determined using a commercially available kit from Biomaghreb (Tunisia) according to Benini et al. (2015).

Lactate dehydrogenase (LDH) activity was assayed spectrophotometrically according to Howell et al. (1979) using a commercial kit from BioMaghreb Tunisia. Briefly, in the presence of pyruvate and $NADH/H^+$, LDH produces lactate and NAD^+. LDH activity was measured following the decrease in NADH which absorbed at 340 nm and expressed as follows: ΔDO/min * 8095.

Lipase activity was evaluated by the hydrolysis of laurate spectrophotometrically. Briefly, aliquots from brain homogenates were mixed with a Tris HCl buffer (pH = 8.5) dissolved in laurate and incubated for 5 minutes at 37°C. After addition of EDTA, substrates were centrifuged at 10 000 g for 5 minutes at 4° C and read at 412 nm against the blank in a UV–visible spectrophotometer using an extinction coefficient for the MDA-TBA complex of 18.3 $M^{-1}cm^{-1}$ (Stoytcheva et al., 2012).

Xanthine oxidase activity was assayed by measuring the initial rate of xanthine/NAD^+ disappearance at 295 nm for 2 minutes. Briefly, 20µl xanthine/NAD^+ were added in 380µl phosphate buffer (pH 7.4) and xanthine oxidase activity was calculated using the extinction coefficient of $3{,}84.10^3$ $mM^{-1}\,cm^{-1}$ for xanthine/NAD^+ (Hall et al., 2014).

ROS determination

H_2O_2 level was determined enzymatically (Suzuki et al., 1980) using a commercially available kit from Biomaghreb, (Tunisia). Briefly, in the presence of POD, H_2O_2 reacts with 4-amino-antipyrine and phenol to give a red-colored quinoeimine that absorbs at 505 nm. Results are expressed as mmol H_2O_2/mg protein.

The intracellular production of superoxide anion (O_2^-) was detected with dihydroethidium (DHE; Invitrogen/Life Technologies), which is a nonfluorescent compound that can diffuse through cell membranes and rapidly oxidized in hydroethidine (HE) under the action of O_2^- (Rothe and Valet, 1990). DHE was initially prepared at 10 mM in dimethyl sulfoxide (DMSO) and used at a 2 µM final concentration. Samples were incubated with DHE for 15 min at 37°C. Fluorescence intensity (λEx Max= 488 nm; λEm Max= 575 nm) was measured with a FL800TBI fluorescence microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

Statistical analysis

Data were analyzed by unpaired Student's t-tests or 1-way analysis of variance (ANOVA) and expressed as means ± S.E.M. All statistical tests were 2-tailed, and P <0.05 was considered significant.

(*) indicated significance for G or GSSE *versus* control and § for GSSE/G *versus* G.

(**) and (§§) indicated P <0.01.

RESULTS

Brain oxidation

We reported in Figure 1 the effect of garlic and GSSE either alone or in combination on brain lipoperoxidation (Figure 1a) and carbonylation (figure 1b). Garlic increased brain MDA by 40% and carbonyl protein by 92%. GSSE *per se* decrease MDA level by almost 75% but has no effect on carbonylation. GSSE also counteracted garlic-induced

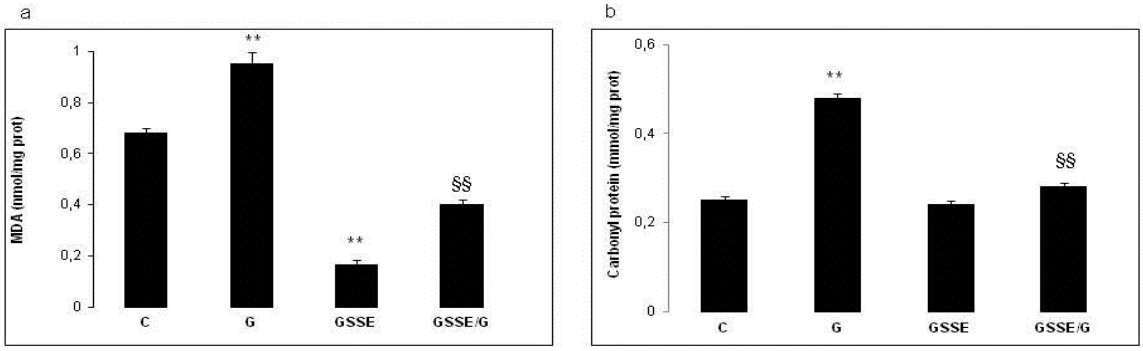

Figure 1 : Effect of garlic and GSSE on brain lipoperoxidation and carbonylation. 10% ethanol (C), garlic (G), grape seed and skin extract (GSSE) or garlic plus GSSE (GSSE/G) were administered to rats for 1 month and brain lipoperoxidation (a) and carbonylation (b) were determined. Results are expressed by mean ± SEM (n=10) and assays done in triplicate.

* indicated $p < 0.05$ versus C. § $p < 0.05$ compared to garlic (G).
** indicated $p < 0.01$ versus C. §§ $p < 0.01$ compared to garlic (G).

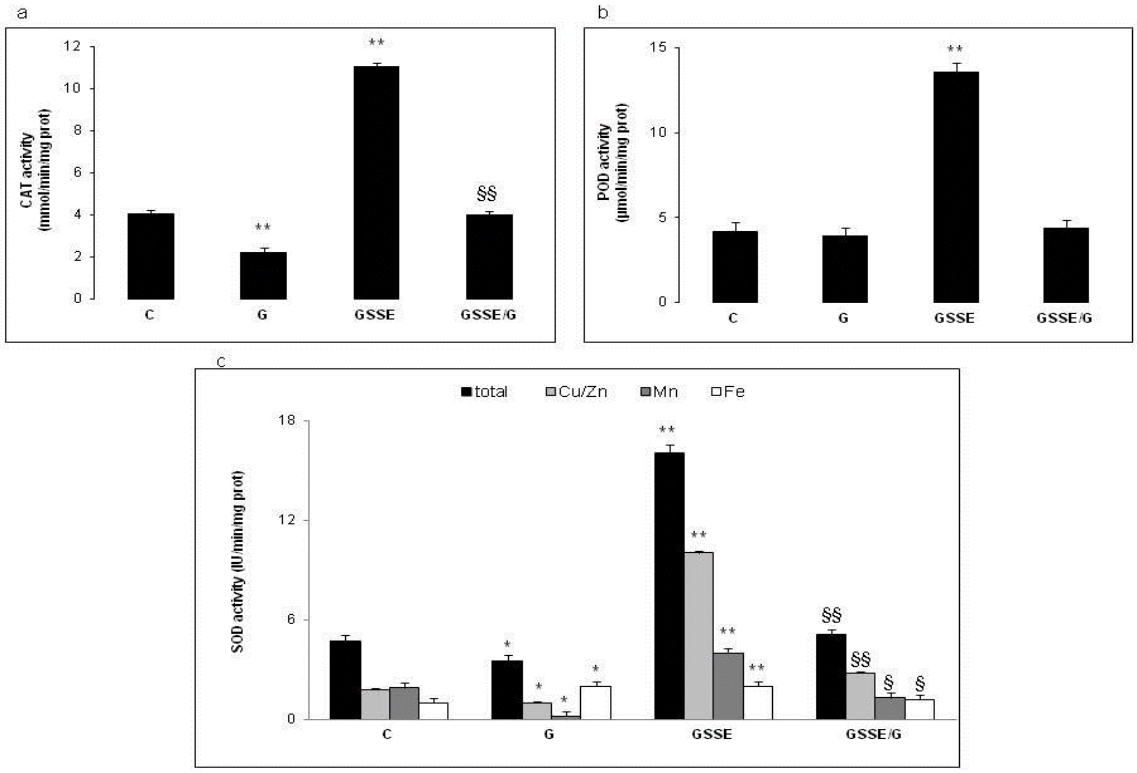

Figure 2: Effect of garlic and GSSE on brain antioxidant enzyme activities.

Rats were daily administered with 10% ethanol (C), garlic (G), grape seed and skin extract (GSSE) or garlic plus GSSE (GSSE/G) for one month and brain catalase (a), peroxidase (b) and superoxide dismutase (c) activities determined.Results are expressed by mean ± SEM (n=10) and assays done in triplicate.
* indicated $p < 0.05$ versus C. § $p < 0.05$ compared to garlic (G).
** indicated $p < 0.01$ versus C. §§ $p < 0.01$ compared to garlic (G).

deleterious effect on carbonylation to near control level and decreased MDA level by 60%.

Brain antioxidant enzyme activities

We further evaluated the effect of garlic and GSSE on brain antioxidant enzyme activities (Figure 2). Garlic decreased CAT (Figure 2a) and SOD (Figure 2c) activities mainly the Cu/Zn and Fe-isoforms but has no effect on POD (Figure 2b). GSSE alone increased CAT, POD and SOD activities mostly mainly the Cu/Zn isoform. However, GSSE abrogated all garlic-induced increase in antioxidant

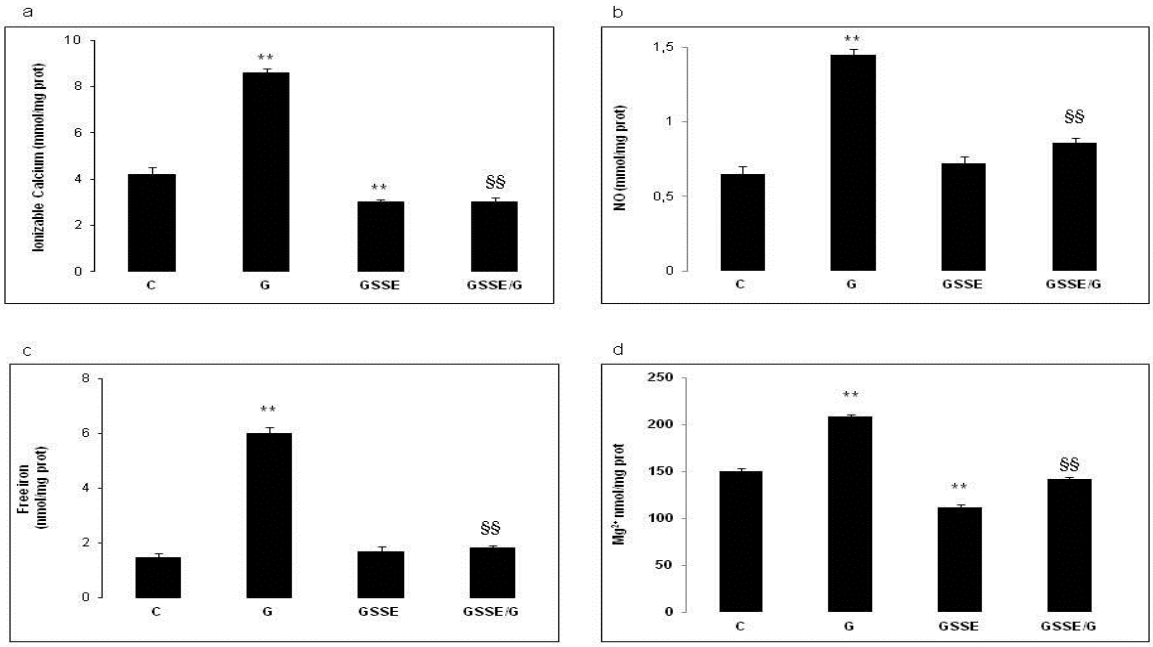

Figure 3: Effect of GSSE on garlic-induced intracellular mediators.

Rats were daily administered with 10% ethanol (C), garlic (G), grape seed and skin extract (GSSE) or garlic plus GSSE (GSSE/G) for one month and brain calcium (a), NO (b), free iron (c) and magnesium (d) levels determined. Results are expressed as means ± SEM (n=10)and assays done in triplicate.
* indicated $p < 0.05$ versus C. § $p < 0.05$ compared to garlic (G).
** $p<0.01$ compared to control (C). §§ $p<0.01$ compared to garlic (G).

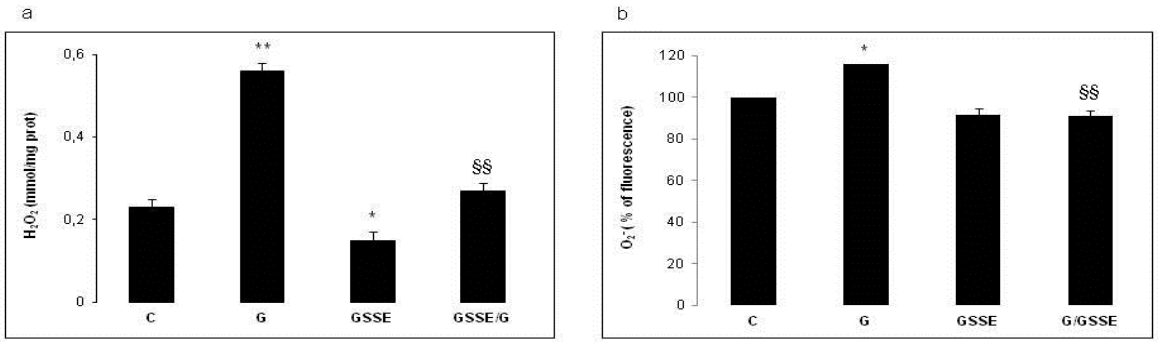

Figure 4: Effect of GSSE on garlic-induced ROS.

Rats were daily administered with 10% ethanol (C), garlic (G), grape seed and skin extract (GSSE) or garlic plus GSSE (GSSE/G) for one month and H_2O_2 (a) and O_2^- (b) levels determined. Results are expressed as means ± SEM (n=10)and assays done in triplicate.
* indicated $p < 0.05$ versus C. § $p < 0.05$ compared to garlic (G).
** $p<0.01$ compared to control (C). §§ $p<0.01$ compared to garlic (G).

enzyme activities to near control level.

Brain intracellular mediators

We next sought to determine the putative involvement of intracellular mediators in garlic and GSSE mode of action (Figure 3). Garlic clearly increased brain calcium by 100% (Figure 3a), NO by 123% (Figure 3b), free iron by more than 300% (Figure 3c) and Mg^{2+} by 40% (Figure 3d). GSSE *per se* decreased ionizable calcium and Mg^{2+} by almost 25% but has no effect on free iron and NO. Co-treatment with GSSE counteracted the effect of garlic on these parameters still control and even to lower level regarding calcium and magnesium.

Brain ROS level

Figure 4 showed that garlic increased H_2O_2 and O_2^- by almost 150% (Figure 4a) and 16% (Figure 4b) respectively. GSSE *per se* decreased H_2O_2 by 35% and O_2^- by almost 10%.

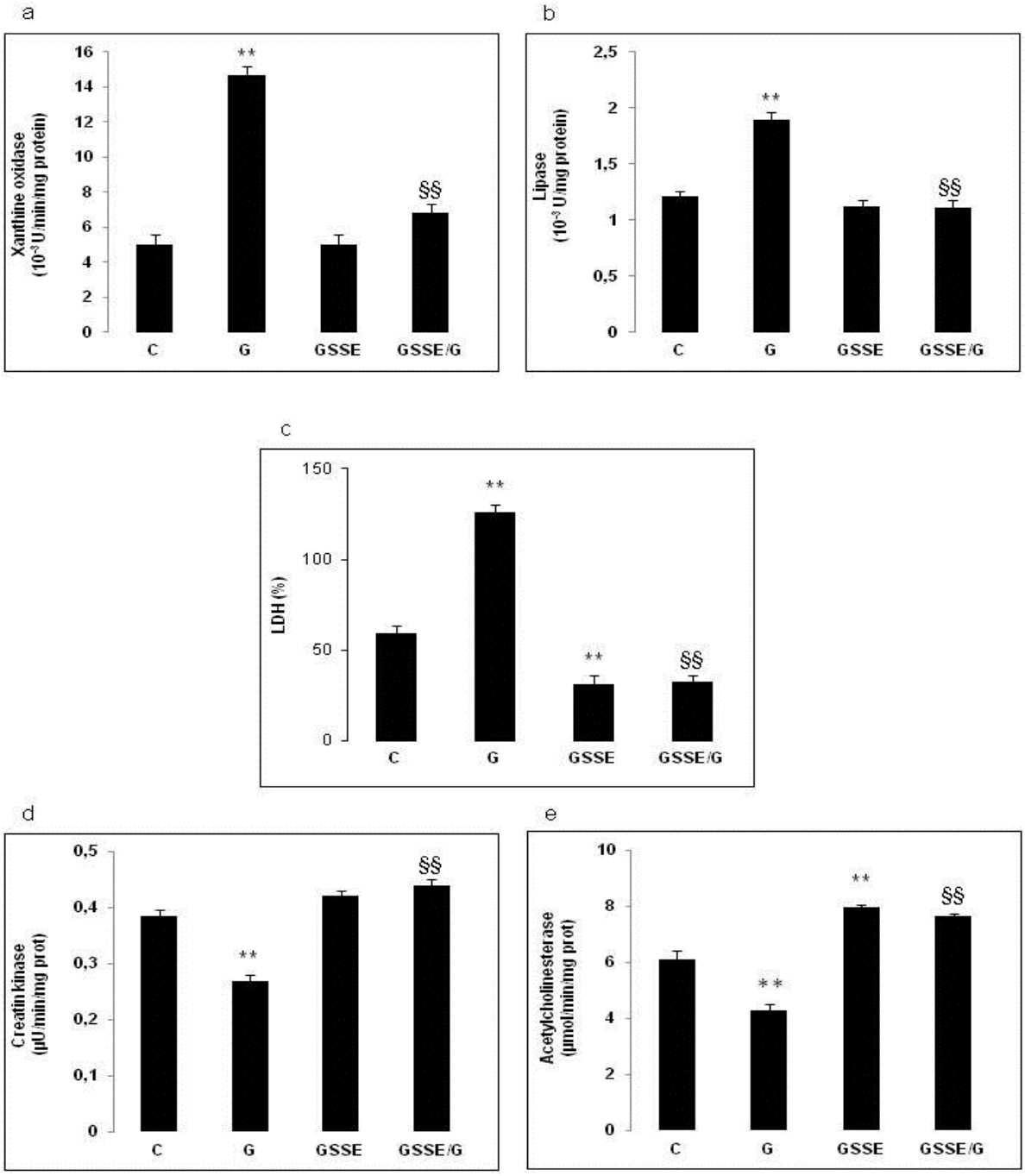

Figure 5. Effect of GSSE on garlic-induced metabolic enzymes.

Rats were daily administered with 10% ethanol (C), garlic (G), grape seed and skin extract (GSSE) or garlic plus GSSE (GSSE/G) for one month. Xanthine oxidase (a), lipase (b), LDH (c), creatin kinase (d) and acetylcholinesterase (e) activities were determined. Results are expressed as means ± SEM (n=10)and assays done in triplicate.

** $p<0.01$ compared to control (C). §§ $p<0.01$ compared to garlic (G).

GSSE co-treatment backed them to near control level.

Brain metabolic enzymes activities

We also determined the impact of garlic administration on brain metabolic enzymes activities (Figure 5). Garlic highly increased the activity of xanthine oxidase (Figure 5a), lipase (Figure 5b) and LDH (Figure 5c) by almost 190%, 60% and 110% respectively. However, Garlic decreased both creatinekinase (Figure 5d) and acetylcholinesterase activities (Figure 5e) by 30%. GSSE *per se* decreased significantly LDH by 50% but increased

acetylcholinesterase by 30%. GSSE counteracted all garlic-induced deleterious effect on metabolic enzyme activities to near control level.

DISCUSSION

This present study reported the toxic effect of intraperitonneally high garlic dose on rat brain as well as the protection offered by GSSE. Data showed that high garlic doses induced an oxidative stress status into rat brain, characterized by an increase in lipid and protein oxidation and a drop in antioxidant defense. Garlic-induced brain toxicity was also reflected by an elevation of ROS (H_2O_2 and O_2^-) and also a concomitant increase in intracellular mediators levels as Ca^{2+}, Fe, NO and Mg^{2+} that could lead to structural injury and brain dysfunction.

MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA, commonly known as a marker of oxidative stress. In fact, it is well known that MDA and carbonyl proteins increase affected the fluidity of the membrane lipid bilayer which are correlated to pathological or stress conditions including aging (Rizvi et al., 2007). Neuronal cells are highly susceptible to oxidative damage because of the high polyunsaturated fatty acid content of their membranes.

Brain oxidative stress induced by garlic is also characterized by decreased antioxidant enzymes activities as CAT, POD and SOD allowing high amount of ROS. In fact, mitochondria are the primary source of ROS, which renders them vulnerable to oxidative damage and induce mitochondrial dysfunction which could be responsible for neuronal death (Chang and Yu, 2010). The present study demonstrates that garlic-induced an increase in brain ROS production notably O_2^- which can be converted into H_2O_2 by SOD leading to the increase of intracellular H_2O_2 levels. This observation could explain the increase in H_2O_2 levels obtained in brain garlic group. Garlic could also exert brain toxicity by increasing H_2O_2 which in turn increases free iron and calcium. This later has been previously shown to be linked to iron transport across the hepatocyte plasma membrane by a mechanism dependant on membrane fluidity (Nilsen, 1991).

Garlic increased simultaneously free iron, O_2^- and H_2O_2 in brain, inducing to the harmful hydroxyl radical synthesis via the Fenton reaction and the Haber-Weiss cycle, which ultimately led to neuronal alteration or death (Wang et al., 2008). It had be shown that the increase of free iron is linked with the opening of the blood brain barrier subsequent to the loss in iron-mediated tight junction proteins and to cerebral endothelial cells degeneration (Won et al., 2011). Ding et al. (2011) described that the blood brain barrier opening was linked to the high level of ferritin and low ferroportin provoked by the increased expression of hepcidin into injured brain. This increase in neuronal free iron could also been attributed to DADS which induced the degradation of the ferritin's light chain (Thomas et al., 2002). SAC, which be haved as a metal chelator, is known to interacte with iron to prevent its redox cycling and by this way alleviated lipid peroxidation (Dairam et al., 2008). Furthermore both iron deficiency and iron excess can lead to cellular dysfunction, maintaining iron homeostasis is crucial (Andrews, 1999).

Our results demonstrate that garlic also increased intracellular mediators as NO. In normal conditions, there is a balance between the production of NO and other mediators and their destruction by antioxidant systems (Freitas et al., 2005). So, overproduction of NO observed in garlic group suggest that there is a decrease in the antioxidant system. This hypothesis is supported by our data showing the decrease of antioxidant enzymes activities as CAT and SOD in the garlic group.

Further, increased brain NO level, induced by garlic, is associated with Ca^{2+} elevation which could alter several pathways. In fact, accumulation of Ca^{2+} could induce NADPH oxidase or nitric oxide synthetase activation, resulting in the production of superoxide anion and NO respectively (Lu and Thompson, 2012). It is well known that NO can generate radical nitrogen species such as peroxynitrite implicated in various molecular disorders inducing inhibition of ATP production or demyelination (Van der Veen and Roberts, 1999).

We next investigated whether garlic-induced oxidative stress was associated with alteration of ionic concentration in the brain. In fact, cellular energy production processes are composed of many Mg^{++} dependent enzymatic reactions (Yamanaka et al., 2016) and recently, it has been found that Mg^{++} acts by trapping free radicals to reduce oxidative stress (Zheltova et al., 2016). In agreement with these reports, we showed that garlic increases brain Mg^{++} concentration which may be responsible for oxidative stress generation and neuronal hyperexcitability. Our data are in concordance with other study which demonstrated that Mg^{++} plays a major role in brain excitability (Isaev et al., 2012) and that Mg^{++} homeostasis dysregulation is involved in brain injury (Toffa et al., 2018). Moreover, disturbance of transition metals homeostasis as depletion or excess causes severe brain damage and lead to vascular type dementia. In fact, an excess in metals level was observed in Alzheimer or Parkinson Diseases or aging (Shcheglovitov et al., 2012) which is in concordance with our results showing anincrease in the rate of Mg^{++} in animals that received garlic alone.

Regarding to metabolic enzymes, our study showed that garlic altered their activities. In fact, garlic induced an increase in LDH activity, a Zn dependent enzyme implicated in glucose metabolism and energy production as well as an elevation in the activity of lipase. Garlic also elevated xanthine oxidase activity, an iron containing enzyme that generates reactive oxygen species. However our data showed that garlic induced a drop of creatinekinase and acetylcholinesterase activities in brain.

As biological membranes are integral to living cells and are largely composed of phospholipids, lipases play important roles in cell biology. In fact, increase in lipase

levels would induce an elevation in damage on cell membranes lipids, which provoke an increase in cell death by membrane lysis. Furthermore, oxidative stress and the associated damage of cellular lipids and proteins contribute to a decline of cellular functions and apoptosis in various cell types, including brain cells (Hamdi et al., 2015). Indeed, a recent study showed that lead administration induced an elevation of plasmatic lipase activity associated with an increase of hepatic oxidative stress (Soussi et al., 2018). Moreover, lipase and other degradative enzymes which in turn promote the conversion of xanthine dehydrogenase to xanthine oxidase, initiating the production of reactive oxygen species as superoxide, are likely implicated in the initiation and progression of brain oxidative stress (Ozkol et al., 2016). Xanthine oxidase is used as a marker of the oxidant system and in agreement with previous reports (Arhan et al., 2011; Maciejczyk et al., 2018) our work showed an increase in xanthine oxidase level in garlic group. Besides, regarding to acetylcholinesterase activity, our results correlated with a recent study which showed that pesticide chlorpyrifos induced decrease in acetylcholinesterase associated with an increase in brain oxidative stress parameters (Adedara et al., 2018).

Another relevant effect of garlic into the brain is its ability to induce a fall in the activity of metallo-enzymes as CAT and SOD. Concerning SOD, we noted that garlic decreased the Cu/Zn and Mn-SOD isoforms, however it induced a rise of Fe-SOD, which is in line with the burst in free iron. This result is in favor of a putative exchange between iron and Mn within the mitochondria, leading to the prominence of the prooxidant Fe-SOD isoform, which ultimately elevates the oxidative stress status within the brain (Yamakura and Kawasaki, 2010).

Certainly, the most important result obtained from the current studyis the protection offered by GSSE against garlic-induced brain oxidative stress. Our data fully corroborated recent works in the field about the antioxidant and protective role offered by polyphenols against certain malignancies (Shrotriya et al., 2015 ; Reddivari et al., 2016) or toxicity (Althali et al., 2019). To our knowledge our report is the first one showing that GSSE is protective against garlic-induced oxidative stress in rat brain. Our data showed that treatment with GSSE during one month reduced lipids and proteins oxidation and ROS level. GSSE also counteracted brain intracellular mediators elevation as calcium, free iron, NO and magnesium and all garlic disturbances on metabolic enzymes activities. Moreover, protective role of GSSE against high dose garlic toxic effects are represented by its repercussions on antioxidant enzymes as GSSE upregulated CAT, POD and SOD activities mainly the Cu/Zn and Mn isoforms although decreasing brain free iron. In addition we can't exclude an effect of garlic as well as of GSSE on transition metals as Cu or Zn accumulation into neuronal cells. As garlic induced in the same time free iron accumulation and an increase in Fe-SOD activity, it would be interesting to correlate the observed increase in Cu/Zn-SOD induced by GSSE with a hypothetical accumulation of Cu and Zn into brain cells.

Our results are supported by others recent studies showing the protective effect of GSSE against many disturbances as diabete (Irak et al., 2018), malathion neurotoxic and genotoxic effects (Abdel-Salam et al., 2018) or testicular and thyroid dysfunction (Hasona, 2018). Our data are also in line with Tyagi et al. (2019) who showed that grape seed extract procyanidin mix and its active constituent procyanidin B2 3,3″-di-O-gallate have the potential to target cancer stem cells in prostate tumor. In addition, our study fully agree with some recent works in the field demonstrating that grape seed proanthocyanidin extract inhibited arsenic-induced hepatotoxicity (Xu et al., 2019), reduced human esophageal squamous cancerous cell line via the NF-κB signaling pathway (Guo et al., 2018) and that GSSE polyphenols protect against zearalenone toxicity in highly endemic areas (Althali et al., 2019).

In our case, GSSE was used at a high dosage (500 mg/kg body weight) which is near the optimal concentration previously precognized (Hebbar et al., 2005). Our data indicated that this dose is safe and denied of oxidative effect as shown by GSSE reduction of MDA and H_2O_2 level and also by GSSE-induced elevation of antioxidant enzymes activities. This current work confirmed our previous data on the safety of GSSE in long and at the same term experiments (Hamlaoui et al., 2012a). This dose is yet still lower as GSSE was also tested at wide-ranging doses, reaching 4 g/kg body weight with no sign of toxicity, corresponding to a daily consumption of 280 g GSSE for a human adult, that was previously shown to exert antioxidative and protective properties in various experimental settings as prevention against doxorubicin (Hamlaoui et al., 2012a), lung toxicity (Khazri et al., 2016) or after heart ischemia (Mokni et al., 2012).

A possible mechanism by which GSSE exerts its beneficial effect on rat brain is its ability to chelate free iron and scavenge H_2O_2. In fact, H_2O_2 is able to induce dual roles in both survival and cell death pathways, largely depending on its concentration and also on the cell type. In this respect, one mechanism by which H_2O_2 exerts its effect in several species including rat involves H_2O_2 induction of SOD activity and as observed in the present study concerning the Cu/Zn and Mn isoforms.

We do not yet know which kind of GSSE containing-polyphenol is at the basis of such protection. Our previously works showed that GSSE counteracted garlic-induced oxidative stress in liver (Hamlaoui-Gasmi et al., 2012), red blood cells (Hamlaoui et al., 2012b) and kidney (Hamlaoui et al., 2012c) with mainly enhancing the antioxidant defenses. Our present data are in favor of a synergism between the numerous GSSE-containing polyphenols rather than the specific effect of a single compound. In conclusion, high garlic dose induced brain toxicity owing to its pro-oxidative properties and GSSE exerted protective effects partly by its anti-oxidative action.

Conflict of Interests

The authors declare that there is no conflict of interests

regarding the publication of the paper.

REFERENCES

Abdel-Salam OME, Galal AF, Hassanane MM, Salem LM, Nada SA, Morsy FA (2018). Grape Seed Extract Alone or Combined with Atropine in Treatment of Malathion Induced Neuro- and Genotoxicity. J. Nanosci. Nanotechnol. 18(1): 564-575.

Adedara I, Owoeye O, Awogbindin IO, Ajayi BO, Rocha JBT, Farombi EO (2018). Diphenyl diselenide abrogates brain oxidative injury and neurobehavioural deficits associated with pesticide chlorpyrifos exposure in rats. Chem. Biol. Interact. 296: 105-116.

Aebi H (1984). Catalase in vitro. Methods Enzymol. 105: 121-126.

Althali NJ, Hassan AM, Abdel-Wahhab MA (2019). Effect of grape seed extract on maternal toxicity and *in utero* development in mice treated with zearalenone. Environ. Sci. Pollut. Res. 26: 5990-5999.

Andrews NC. Disorders of iron metabolism (1999). N. Engl. J. Med. 341: 1986-1995.

Arhan E, Serdaroglu A, Ozturk B, Ozturk HS, Ozcelik A, Kurt N, Kutsal E, Sevinc N (2011). Effects of epilepsy and antiepileptic drugs on nitric oxide, lipid peroxidation and xanthine oxidase system in children with idiopathic epilepsy. Seizure. 20: 138-142.

Benini R, Nunes PRP, Orsatti CL, Portari GV, Orsatti FL (2015). Influence of sex on cytokines, heat shock protein and oxidative stress markers in response to an acute total body resistance exercise protocol. J. Ex. Sc. and Fitn. 13: 1-7.

Berginc K, Milisav I, Kristl A (2010). Garlic flavonoids and organosulfur compounds: Impact on the hepatic pharmacokinetics of saquinavir and darunavir. Drug. Metab. Pharmacokinet. 25: 521-530.

Bhatwalkar SB, Gound SS, Mondal R, Srivastava RK, Anupam R (2019). Anti-biofilm and antibacterial activity of *Allium sativum* against drug resistant shiga-toxin producing *Escherichia coli* (STEC) isolates from patient samples and food sources. Ind. J. Microbiol. 59(2): 171-179.

Casazza A, Aliakbarian B, Perego P (2011). Recovery of phenolic compounds from grape seeds: effect of extraction time and solid-liquid ratio. Nat. Prod. Res. 25: 1751-1761.

Chance B, Maehly AC (1955). Assay of catalases and peroxidases. Methods Enzymol. 2: 764-817.

Chang SJ, Yu BC (2010). Mitochondrial matters of the brain: mitochondrial dysfunction and oxidative status in epilepsy. J. of Bioenerg. and Biomemb. 42: 457-459.

Dairam A, Fogel R, Daya S, Limson JL (2008). Antioxidant and iron binding properties of curcumin, capsaicin, and Sallyl cysteine reduce oxidative stress in rat brain homogenate. J. Agric. Food Chem. 56: 3350-3356.

Dewanto V, Wu X, Adom KK, Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50: 3010-3014.

Ding H, Yan C, Shi H, Zhao Y, Chang S, Yu P, Wu W, Zhao C, Chang Y, Duan X (2011). Hepcidin is involved in iron regulation in the ischemic brain. PLoS ONE. 6: e25324.

Draper HH, Hadley M (1990). Malondialdehyde determination as index of lipidperoxidation. Methods Enzymol. 186: 421-431.

El Ayed M, Kadri S, Mabrouk M, Aouani E, Elkahoui S (2018). Protective effect of grape seed and skin extract against high-fat diet-induced dyshomeostasis of energetic metabolism in rat lung. Lipids Health Dis.17(1): 109.

Ellman G, Courtney K, Andres V, Feather-Stone R (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88-95.

Freitas RM, Vasconcelos SM, Souza FC, Viana GS, Fonteles MM (2005). Oxidative stress in the hippocampus after pilocarpine-induced status epilepticus in wistar rats. FEBS J. 272: 1307-1312.

Guo F, Hu Y, Niu Q, Li Y, Ding Y, Ma R, Wang X, Li S, Xie J (2018). Grape Seed Proanthocyanidin Extract Inhibits Human Esophageal Squamous Cancerous Cell Line ECA109 via the NF-κB Signaling Pathway. Mediators Inflamm. 3403972. doi:10.1155/2018/3403972

Hall J, Reschke S, Cao H, Leimkühler S, Hille R (2014). The reductive half-reaction of xanthine dehydrogenase from rhodobacter capsulatus the role of glu232 in catalysis. J. of Biol. Chem. 289: 32121-32130.

Hamdi Y, Kaddour H, Vaudry D, Leprince J, Zarrouk A, Hammami M, Vaudry H, Tonon MC, Amri M, Masmoudi-Kouki O (2015). Octadecaneuropeptide ODN prevents hydrogen peroxide-induced oxidative damage of biomolecules in cultured rat astrocytes. Peptides. 71: 56-65.

Hamlaoui S, Mokni M, Limam N, Carrier A, Limam F, Amri M, Marzouki L, Aouani E (2012a). Grape seed and skin extract protects against acute chemotherapy toxicity induced by doxorubicin in rat red blood cells and plasma. Bangl. J. Pharmacol. 7: 54-62.

Hamlaoui S, Samti O, Mokni M, Limam N, Carrier A, Limam F, Amri M, Aouani E, Marzouki L (2012c). Protective effect of grape seed and skin extract on high dosage garlic-induced renal oxidative stress. Bangl. J. Pharmacol. 7: 77-86

Hamlaoui S, Mokni M, Limam N, Zouaoui K, Ben Rayana MC, Carrier A, Limam F, Amri M, Marzouki L, Aouani E (2012b). Protective effect of grape seed and skin extract on garlic-induced erythrocyte oxidative stress. J. Physiol. Pharmacol. 63(4): 381-388.

Hamlaoui-Gasmi S, Mokni M, Limam N, N'guessan P, Carrier A, Limam F, Amri M, Aouani E, Marzouki L (2012). Grape seed and skin extract mitigates garlic-induced oxidative stress in rat liver. Can. J. Physiol. Pharmacol. 90: 547-556.

Hasona NA (2018). Grape seed extract attenuates dexamethasone-induced testicular and thyroid dysfunction in male albino rats. Androl. 50(5): e13002. doi: 10.1111/and.13002.

Hassan E, Kahilo K, Kamal T, El-Neweshy M, Hassan M (2019). Protective effect of diallyl sulfide against lead-mediated oxidative damage, apoptosis and down-regulation of CYP19 gene expression in rat testes. Life Sci. 226: 193-201.

Hebbar V, Shen G, Hu R, Kim BR, Chen C, Korytko PJ, Crowell JA, Levine BS, Kong AN (2005). Toxicogenomics of resveratrol in rat liver. Life Sci.76: 2299-2314.

Howell B, Mc Cune S, Schaffer R (1979). Lactate-to-pyruvate or pyruvate-to-lactate assay for lactate dehydrogenase: a re-examination. Clinical Chemis. 25: 269-272.

Irak K, Yildirim S, Mert H, Mert N (2018). Grape seed extract effects on serum amylase levels and immune-histochemical alterations in Streptozotocin-induced diabetic rats. Cellule Mol. Biol. 64 (4): 92-97.

Isaev D, Ivanchick G, Khmyz V, Isaeva E, Savrasova A, Krishtal O, Holmes GL, Maximyuk O (2012). Surface charge impact in low-magnesium model of seizure in rat hippocampus. J. Neurophysiol.107: 417-423.

Katrina MM, Michael GE, David AW (2001). A rapid, simple spectrophotometrically method for simultaneous detection of nitrate and nitrite. Biol. Chem.5: 62-71.

Kaur M, Mandair R, Agarwal R, Agarwal C (2008). Grape seed extract induces cell cycle arrest and apoptosis in human colon carcinoma cells. Nutr. Cancer. 60: 2-11.

Khanal RC, Howard LR, Prior RL (2009). Procyanidin content of grape seed and pomace, and total anthocyanin content of grape pomace as affected by extrusion processing. J. Food Sci. 74: 174-182.

Khazri O, Charradi K, Limam F, El May MV, Aouani E (2016). Grape seed and skin extract protects against bleomycin-induced oxidative stress in rat lung. Biomed. and Pharmacother. 81: 242-249.

Leardi A, Caraglia M, Selleri C, Pepe S, Pizzi C, Notaro R, Fabbrocini A, De Lorenzo S, Musicò M, Abbruzzese A, Bianco AR, Tagliaferri P (1998). Desferioxamine increases iron depletion and apoptosis induced by ara-C of human myeloid leukaemic cells. Brit. J. Haematol. 102: 746-752.

Levine LR, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER (1990). Determination of carbonyl content in oxidatively modified proteins. Method Enzymol. 186: 464-478.

Lu C, Thompson CB (2012). Metabolic regulation of epigenetics. Cell Metab. 16: 9-17.

Maciejczyk M, Żebrowska E, Zalewska A, Chabowski A (2018). Redox balance, antioxidant defense and oxidative damage in the hypothalamus and cerebral cortex of rats with high fat diet-induced insulin resistance. Oxid. Med. Cell Longev .6: 6940515.

Misra HP, Fridovich I (1972). The role of superoxide anion in autoxidation of epinephrine and a simple assay for SOD. J. Biol. Chem. 247: 3170-3175.

Mokni M, Hamlaoui S, Kadri S, Limam F, Amri M, Marzouki L, Aouani E (2015). Efficacy of grape seed and skin extract against doxorubicin-induced oxidative stress in rat liver. Pak. J. Pharm. Sci. 28(6): 1971-1978.

Mokni M, Hamlaoui S, Kadri S, Limam L, Amri M, Marzouki L, Aouani E (2016). Grape seed and skin extract protects kidney from doxorubicin-induced oxidative injury. Pak. J. Pharm. Sci. 29 (3): 961-968.

Mokni M, Hamlaoui-Guesmi S, Amri M, Marzouki L, Limam F, Aouani E (2012). Grape seed and skin extract protects against acute chemotherapy toxicity induced by doxorubicin in rat heart. Cardiovasc. Toxicol. DOI 10.1007/s12012-012-9155-1.

National Research Council (1985). Guide for the care and the use of laboratoryanimals. National institute of health, Bethesda. 20: 85-123.

Nilsen T (1991). Effects of calcium on hepatocyte iron uptake from transferrin, iron-pyrophosphate and iron-ascorbate. Biochim. Biophys. Acta. 1095: 39-45.

Olusanya A, Oguntayo AO, Sambo A (2015). Serum levels of calcium and magnesium in pre-eclamptic-eclamptic patients in a tertiary institution. Intern. J. of Gynecol. and Obstetrics. 9: 101-110.

Ozkol H, Bulut G, Balahoroglu R, Tuluce Y, Ozkol HU (2017). Protective Effects of Selenium, N-Acetylcysteine and Vitamin E Against Acute Ethanol Intoxication in Rats. Biol. Trace Elem. Res. 175(1): 177-185.

Reddivari L, Charepalli V, Radhakrishnan S, Vadde R, Elias RJ, Lambert JD, Vanamala JK (2016). Grape compounds suppress colon cancer stem cells in vitro and in a rodent model of colon carcinogenesis. BMC Complement Altern Med.16: 278.

Renaud S, De Lorgeril M (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet. 339(8808): 1523-1526.

Rizvi SI, Maurya PK (2007). Markers of oxidative stress in erythrocytes during aging in humans. Ann. NY Acad. Sci.1100: 373-382.

Rothe G, Valet G (1990). Flow cytometric analysis of respiratory burst activity in phagocytes with hydroethidine and 2',7'-dichlorofluorescin. J. Leukocyte Biol. 47: 440-448.

Shcheglovitov A, Vitko I, Lazarenko RM, Orestes P, Todorovic SM, Perez-Reyes E (2012). Molecular and biophysical basis of glutamate and trace metal modulation of voltage-gated Ca(v)2.3 calcium channels. J. Gen. Physiol. 139: 219-234.

Shirzad H, Taji F, Rafieian-Kopaei M (2011). Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. J. Med. Food. 14: 969-974.

Shrotriya S, Tyagi A, Deep G, Orlicky DJ, Wisell J, Wang XJ, Sclafani RA, Agarwal R, Agarwal C (2015). Grape seed extract and resveratrol prevent 4-nitroquinoline 1-oxide induced oral tumorigenesis in mice by modulating AMPK activation and associated biological responses. Mol. Carcinog. 54(4): 291-300.

Singleton V, Rossi J (1965). Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagent. Am. J. Enol. Vitic. 16: 144-158.

Soussi A, Gargouri M, El Feki A (2018). Potential immunomodulatory and antioxidant effects of walnut Juglans regia vegetable oil against lead-mediated hepatic

damage and their interaction with lipase activity in rats. Environ. Toxicol. 33: 1261-1271.

Stern J, Lewis WH (1957). The colorimetric estimation of calcium in serum with ocresolphthalein complexone. Clin. Chim. Acta. 2: 576-580.

Stoytcheva M, Montero G, Zlatev R, Leon JA, Gochev V (2012). Analytical methods for lipases activity determination: a review. Current Analytical Chem. 8: 400-407.

Sun B, Ricardo-Da-Silva J, Spranger M (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agric. Food Chem. 46: 4267-4274.

Suzuki H, Kakinuma K, Shimizu M, Sakamoto H, Shimada K (1980). H_2O_2 release from filtration leukapheresis-procured leukocytes. Vox Sang. 263: 258-263.

Thomas M, Zhang P, Noordine ML, Vaugelade P, Chaumontet C, Duee PH (2002). Diallyl disulfide increases rat h-ferritin, Lferritin and transferring receptor genes *in vitro* in hepatic cells and *in vivo* in liver. J. Nutr. 132: 3638-3641.

Tian M, Liu F, Liu H, Zhang Q, Li L, Hou X, Zhao J, Li S, Chang X, Sun Y (2018). Grape seed procyanidins extract attenuates Cisplatin-induced oxidative stress and testosterone synthase inhibition in rat testes. Syst. Biol. Reprod. Med. 64(4): 246-259.

Toffa DH, Kpadonou C, Gams Massi D, Ouedraogo M, Sow AD, Ndiaye M, Samb A (2018). Le magnésium et le calcium réduisent la sévérité des troubles de la mémoire spatiale pour le modèle kaïnique d'épilepsie mésiale temporale chez la souris. Can. J. Physiol. Pharmacol. 96: 1132-1144.

Tyagi A, Kumar S, Raina K, Wempe MF, Maroni PD, Agarwal R, Agarwal C (2019). Differential effect of grape seed extract and its active constituent procyanidin B2 3,3″-di-O-gallate against prostate cancer stem cells. Mol. Carcinog. 58(7): 1105-1117.

Van Der Veen RC, Roberts LJ (1999). Contrasting roles for nitric oxide and peroxynitrite in the peroxidation of myelin lipids. J. Neuroimmunol. 95: 1-7.

Wang J, Si L, Wang G, Bai Z, Li W (2019). Increased Sulfiredoxin Expression in Gastric Cancer Cells May Be a Molecular Target of the Anticancer Component Diallyl Trisulfide. Biomed. Res. Int. 4636804. doi.org/10.1155/2019/4636804

Wang Y, Han R, Liang Z, Wu J, Zhang X, Gu Z, Qin Z (2008). An autophagic mechanism is involved in apoptotic death of rat striatal neurons induced by the non-N-methyl-D-aspartate receptor agonist kainic acid. Autophagy. 4: 214-226.

Won SM, Lee JH, Park UJ, Gwag J, Gwag BJ, Lee YB (2011). Iron mediates endothelial cell damage and blood-brain barrier opening in the hippocampus after transient forebrain ischemia in rats. Exp. Mol. Med. 43: 121-128.

Xu M, Niu Q, Hu Y, Feng G, Wang H, Li S (2019). Proanthocyanidins Antagonize Arsenic-Induced Oxidative Damage and Promote Arsenic Methylation through Activation of the Nrf2 Signaling Pathway. Oxid. Med. Cell Longev. 8549035. doi.org/10.1155/2019/8549035

Yamakura F, Kawasaki H (2010). Post-translational modifications of superoxide dismutase. Biochim. Biophys. Acta. 1804: 318-325.

Yamanaka R, Tabata S, Shindo Y, Hotta K, Suzuki K, Soga T (2016). Mitochondrial Mg^{2+} homeostasis decides cellular energy metabolism and vulnerability to stress. Nature Publishing Group. 1-12.

Zheltova AA, Kharitonova MV, Lezhitsa IN, Spasov AA (2016). Magnesium Deficiency and Oxidative Stress: An Update. BioMed. 6 (4): 8-14.