Austin Journal of Pharmacology and Therapeutics

Open Access

Austin Publishing Group

Research Article

Aristolochia longa (Aristolochiaceae) Spice Alleviates Nickel-Induced Oxidative Stress and Biochemical Alterations in Rats

Ibtissam L[1], Samir D[1,2]* and Wiam Z[1]

[1]Department of Cellular and Molecular Biology, Faculty of Natural Science Sand Life, University of El Oued, El-Oued, Algeria

[2]Laboratory of Biodiversity and Application of Biotechnology in the Agricultural Field, University of El Oued, El-Oued, Algeria

***Corresponding author:** Derouiche Samir, Department of Cellular and Molecular Biology, Faculty of Natural Science Sand Life, University of El Oued, El-Oued 39000, Algeria; Laboratory of Biodiversity and Application of Biotechnology in the Agricultural Field, University of El Oued, El-Oued 39000, Algeria

Received: August 18, 2021; **Accepted:** September 03, 2021; **Published:** September 10, 2021

Abstract

The objective of this work is to study the protective effect of *Aristolochia longa* against the variation of biochemical parameters and oxidative stress in rats exposed sub acutely to nickel. This is an experimental study carried out in the laboratory on 15 female Wistar rats divided into three groups (n=5), the first group of which serves as a control, the second group is contaminated with nickel, the third group is contaminated with nickel and treated with *A. longa*. Biochemical and oxidative stress parameters were analyzed on the rats of the various. The aqueous extract of *A. longa* included a variety of bioactive components, including polyphenols, saponins, terpenoids, glycosides, and flavonoids, according to phytochemical screening results, and nickel treatment resulted in a considerable increase in serum Urea, Uric acid, creatinine and GOT, GPT activities. The results obtained also reveal a bioaccumulation of Ni and an oxidative stress in group contaminated with nickel, with an increase in the level of hepatic Ni and a significant augmentation in the Kidney MDA and decrease in the level of GSH and the activity of tissue SOD. Treatment with *A. longa* improves biochemical parameters and reduces tissue nickel levels, with protection of organs against oxidative radical attacks induced by nickel. In conclusion, this study shows that treatment with *A. longa* induces a beneficial effect against the toxicity of nickel at the molecular and tissue level.

Keywords: *Aristoloshia longa*; Nickel; Liver; Kidney; Oxidative stress; Wistar rats

Introduction

Man and his surroundings have been exposed to various harmful heavy metals as a result of the rapid development of technological sciences, industries (chemical and metallic), medicine, and agriculture. Heavy metals including nickel, cadmium, lead, and others build up in the body, causing short- and long-term harmful effects [1]. They have the potential to harm the neurological system, kidneys, liver, and lungs [2]. Nickel comes in different chemical forms, but it only effectively enters cells in the bivalent cationic form (Ni^{2+}) or as nickel chloride or sulfate [3]. Nickel ion can cause direct damage to all cellular components: peroxidation of lipids, proteins and nucleic acids, due to free radical attacks generated by nickel [4]. Nickel ion can cause direct damage to all cellular components: peroxidation of lipids, proteins and nucleic acids, due to free radical attacks generated by nickel [5]. With these radicals or indirectly by producing peptides such as metallothioneins or glutathione [6]. Numerous studies indicate massive production of oxidant species and inhibition of the activities of major antioxidant enzymes due to the cytotoxicity of nickel in a cell can promote excessive cell death or tumor development [7]. Herbal medicine is the art of healing yourself with plants. Currently, many drugs originate from medicinal plants. *Aristolochia longa* L is a plant widely used in traditional medicine in the treatment of cancer and other chronic diseases in several regions of Algeria [8]. In the light of these data, our objective for this study is to evaluate the effectiveness of a therapeutic system based on herbal medicine by *Aristolochia longa* against oxidative stress and toxic effects of nickel in rats.

Materials and Methods

Plant materials and preparation of aqueous extract

Aristolochia longa roots wood was taken from a local market in El-Oued by herbalists. The vegetable materials were rinsed in water and then dried for 48 to 92 hours at room temperature. Crushed into a powder, then kept at room temperature until needed. About 10g of *A. longa* root powder was soaked in 100ml of distilled water and stored at room temperature for 24 hours in the dark. Following that, it was filtered using filter paper. After extraction, the water was evaporated using a rotary evaporator, and the sample was then dried thoroughly at 40°C. For future analysis, the extract was weighed and stored in a refrigerator at 4°C [9].

Animals and handling

Experiments and Animals Female albino rats, 8 weeks old and weighing around 190g, were procured from the Pasteur Institute's animal home in Algeria. The animals were given free access to a regular feed as well as distilled water. Rats were separated into three groups of 5 rats each after the adaption phase and kept in the animal house of the El-Oued University. Unless otherwise stated, standard rat food and water were supplied ad libitum during the tests. Animals were acclimated for two weeks under the identical lab conditions of photoperiod (12 hours of light/12 hours of darkness), relative

Citation: Ibtissam L, Samir D and Wiam Z. *Aristolochia longa* (Aristolochiaceae) Spice Alleviates Nickel-Induced Oxidative Stress and Biochemical Alterations in Rats. Austin J Pharmacol Ther. 2021; 9(6).1151.

humidity 65.3 percent, and room temperature 23 ± 2 C°.

Experimental design

The experimental methods were carried out in accordance with the National Institute of Health's Animal Care Guidelines and were authorized by our institution's Ethics Committee. Over the course of 20 days, the experiment was carried out. The animals were divided into three experimental groups of five animals each after a period of adaptation:

Group 1 (Control group): Animals served as normal control.

Group 2 (Ni): Rats exposed to nickel (20mg / kg rat weight) for 20 days.

Group 3 (Ni + Ar): Rats exposed to nickel and treated with Aristolochia Longa powder for 20 days.

Aristolochia longa (roots powder) were added to the feed (at a dose 5% of diet).

Preparation of serum and tissue samples

Rats were fasted for 16 hours after receiving *Aristolochia longa* therapy, anaesthetized with chloroform by inhalation, beheaded, and blood was placed into non-heparinized tubes for serum biochemical examination. Blood was centrifuged at 3000rpm for 10 minutes to obtain serum, which was then rapidly frozen at 20°C until used. Liver and kidney samples were quickly excised, weighed, and rinsed in ice-cold saline (0.9 percent NaCl (w/v) before being treated for oxidative stress marker analysis.

Phytochemical screening

In our study, the methods descripted by Zebidi et al. [10] were used to identify the different phytochemicals in extracts such as flavonoids, alkaloids, saponins, tannins, terpenoids and glycosides.

Determination of total phenolic, flavonoids content and antioxidant activity

The Folin-Ciocalteu technique was used to determine the polyphenols. Slinkard and Singleton [11] were the first to describe this strategy. Lin and Tang [12] proposed a method for determining the total flavonoid content of the aqueous extracts of *Aristolochia longa*. Burits and Bucar [13] proposed a method for determining *in vitro* antioxidant activity by evaluating the scavenging power of the DPPH (1,1-diphenyl-2-picryhydrazyl) radical.

Determination of tissue Nickel concentration

Dry calcination of liver is done for 6 hours in a muffle furnace at 600°C. An acid attack of 3ml pure nitric acid dissolves the ash produced (HNO_3). In a 20ml flask, the liquid is filtered through filter paper and finished with demineralized water to reach its ultimate volume. Nickel standards are made from a 1000-ppm stock solution for the nickel assay.

Biochemical parameters analysis

Urea, uric acid, creatinine, serum protein in serum were determined using the commercial kit from Spinreact, Spain (ref: urea-20141, uric acid-20091, creatinine-20151, total protein-1001291). And for enzymes TGO and TGP are also measured by the use of commercial kits (Spinreat, ref: GOT-20042, GPT-20046).

Measurement of oxidative stress markers

Liver Malondialdehyde (MDA) and kidney was measured according to the method described by Yagi et al. [14]. The concentration of reduced Glutathione (GSH) was performed with the method described by Weckbecker, [15] and superoxide was measured by the method of Beauchamp [16].

Statistics analysis

Mean data values are presented, with their standard deviations (mean ± SD). The Student's t-test was used to do all statistical comparisons, and statistical significance was determined as $P < 0.05$.

Results

Results of phytochemical analysis

Results showed the existence of alkaloids, glycosides, phenol compounds, tannins, flavonoids, Terpenoids and saponins in the extract plants (Table 1). These compounds are antioxidant activities, which could play a major role in the capture of free radicals induced by heavy metal such as nickel.

Quantification of phytochemical compounds

Total phenolic and flavonoid compounds were expressed in terms of Gallic acid equivalent (mg GA eq/g dry extract) and of Quercetin equivalent (mg Q eq/g dry extract) respectively, using the following equations based on the calibration curve: Y=0.0045x+0.09, R2= 0.9925 for phenolic compounds and Y=0.0096x+0.0521, R2= 0.994 for flavonoids compounds (Table 2).

Antioxidant (DPPH) test

Table 3 presented the results of the antioxidant power of the extracts tested show that the percentage inhibition of the aqueous extract of *A. longa* at a concentration of 12.23mg/ml, which is calculated by the equation y=1.6181x + 30.757 with R2=0.9882, obtained by a curve of percentage inhibition as a function of the concentrations of the extract of *A. longa*. Likewise, the percentage inhibition of ascorbic

Table 1: Phytochemical composition of aqueous extracts of rhizome *Aristolochia longa*.

Phytochemical	*A. longa*
Total phenols	+
Flavonoids	+
Tannins	+
Alcaloids	+
Saponines	+
Terpenoids	+
Glycosids	+

+Presence of phytochemicals.

Table 2: Phenolic and flavonoids content from *A. longa* aqueous extract.

Phytochemical	*A. longa*
Total phenolics (mg GA eq/g dry extract)	9.20 ± 0.05
Total flavonoids (mg Q eq/g dry extract)	2.17 ± 0.04

Table 3: IC_{50} value of aqueous extract of *A. longa* and ascorbic acid.

Sample	IC_{50} (µg/ml)
Aristoloshia longa	12.23 ± 0,42
Ascorbic acid	2.89 ± 0,09

Table 4: Mean initial Body weight, Weight gains relative liver weight of control and experimental rats (n=5).

Parameter	Control	Nickel	Nickel + Ar
Initial Body Weight (g)	194.80 ± 6.26	167.80 ± 16.4	185.60 ± 8.01
Weight Gains (g/d/rat)	0.05 ± 0.08	-1.03 ± 0.30*	-0.16 ± 0.01*a
Relative Liver Weight	2.92 ± 0.16	2.56 ± 0.26**	2.68 ± 0.19Ns
Relative Kidney Weight	0.58 ± 0.01	0.70 ± 0.05*	0.40 ± 0.02b

Values are mean ± SEM; n: number of observations. *p <0.05, **p <0.01: Significantly different from control group; ap <0.05, bp <0.01: Significantly different from Ni group.

Table 5: Biochemical markers Levels in control and experimental group (n=5).

Parameter	Control	Nickel	Nickel + Ar
Liver Ni (µg/g)	6.98 ± 0.54	10.35 ± 1.59*	7.26 ± 1.29NSb
Serum Urea (g/l)	0.48 ± 0.05	0.67 ± 0.17**	0.55 ± 0.17c
Serum Creatinine (mg/l)	7.42 ± 0.91	7.91 ± 0.69*	7.85 ± 0.81NS
Serum Uric Acid (mg/l)	18.07 ± 1.09	29.73 ± 0.99*	15.55 ± 0.34c
Serum Total Proteins (g/l)	62.23 ± 0.56	71.46 ± 0.88***	82.34 ± 0.76***a
Serum GOT (U/l)	93.00 ± 23.10	185.20 ± 14.30***	130.86 ± 1.54***c
Serum GPT (U/l)	38.61 ± 8.13	54.07 ± 6.63**	36.09 ± 2.74c

Values are mean ± SEM; n: number of observations. *p <0.05, **p <0.01, ***p <0.001: Significantly different from control group; ap <0.05, bp <0.01: Significantly different from Ni group.

acid at 2.89mg/ml, which is calculated by the equation y=0, 0.6232x + 48.111 with R2=0.9607, obtained by a percentage curve d 'inhibition as a function of ascorbic acid concentrations.

Initial body weight, body weight gain and relative liver weight

In comparison to the control rats, nickel treatment at a dose of 20mg/kg body weight induce a decrease (p <0.05) in body weight and an increase (p <0.05) in Relative Kidney weight. The animals who were given rhizome *A. longa* powder demonstrated a partial reversal of this alteration (Table 4).

Hepatic nickel and Biochemical parameters levels

The content of nickel in the liver of nickel-contaminated rats increased significantly (p <0.05), as shown in Table 5 of our findings. The treatment of *A. longa* powder to nickel-exposed rats, on the other hand, considerably reduced the levels of lead in the liver (p <0.01). Our findings reveal a significant increase (p <0.001) in urea, uric acid and total protein (p <0.001) in the nickel-contaminated group when compared to controls. Our findings in Table 5 show a very significant (p <0.001) and extremely significant (p <0.01) increase in transaminase (TGO, TGP) activity in the nickel-contaminated group when compared to controls. On the other hand, we found a significant reduction in urea levels in the *A. longa* treatment groups (p <0.01 and p <0.05) and serum uric acid content (p<0.001), as well as a very substantial drop in TGO (p <0.001) and TGP. However, no difference in serum creatinine has been seen as compared to the Nickel group.

Oxidative stress parameters levels

Table 6 shows that rats in the nickel group had a substantial rise (p <0.05) in lipid peroxidation in the kidney and a significant decrease (p <0.05) in GSH concentration and SOD activity in the liver and kidney when compared to the control group. In contrast, our findings demonstrate that MDA levels in the kidney are significantly lower (P <0.01), whereas GSH concentration and SOD activity in the kidney and liver are significantly higher in *A. longua* groups compared to Ni groups.

Table 6: Oxidative stress markers levels of control and experimental groups (n=5).

Parameter		Control	Nickel	Nickel + Ar
MDA (µmol/mg pro)	Liver	2.54 ± 0.04	2.38 ± 0.33Ns	1.58 ± 0.27**a
	Kidney	1.79 ± 0.03	2.19 ± 0.14*	1.797 ± 0.01b
GSH (nmol/mg pro)	Liver	1.19 ± 0.27	0.77 ± 0.23*	1.52 ± 0.15*
	Kidney	0.10 ± 0.01	0.04 ± 0.01***	0.08 ± 0.01**
SOD (UI/mg pro)	Liver	7.25 ± 0.13	3.13 ± 0.64*	4.52 ± 1.61*
	Kidney	0.22 ± 0.01	0.17 ± 0.02**	0.23 ± 0.02*c

Values are mean ± SEM; n: number of observations. *p <0.05, **p <0.01, ***p <0.001: Significantly different from control group; ap <0.05, bp <0.01: Significantly different from Ni group.

Discussion

In search of new biologically active compounds, new herbal agents could be used to enhance the antioxidant of standard drug therapy. For this study, we are evaluating the effects of Aristoloshia longa against disturbance and damage induced by nickel. Regarding the DPPH antioxidant test, according to the recorded results, the aqueous extracts of *A. longa* have moderate antioxidant power. The polyphenols contained in the extract of this plant are probably responsible for the antioxidant activity and have anti-radical activities of these extracts [17]. The reduction in body weight is used as an indicator of the deterioration of the general health of the rat. This effect can also be explained by the action of pollutants on the transport of nutrients (amino acids, glucose and essential minerals such as zinc, magnesium, iron) by the blood and consequently, they can induce a poor assimilation of food by the body [18]. Regarding the relative weights of the organs, we notice a hypotrophy hepatic; this is explained by the intense accumulation of this metal in these target organs [19]. This increase in relative organ weight may be due to nickel-induced necrosis [20]. Treatment by aqueous extract of *A. longa* has shown improvement in relative organ weight. This result is in agreement with the study by Derouiche et al. [21]. *A. longa* is probably due to the three phytochemicals identified in this plant as flavonoids (folavonol, flavones and/or flavonoids glycosides) exerting a nickel chelating effect which decreases their toxic effect on these organs and therefore decreases the relative weight. The concentration of nickel in liver tissue increased following administration of nickel to normal rats. Because the liver is the main target of environmental and occupational toxicity and the main site of detoxification [22]. Nickel enters all organs and accumulates mainly in the liver [23]. They can cause certain morphological transformations in many cellular systems [24]. On the other hand, administration of *A. longa* reduced the level of nickel in liver tissue in favor of a detoxification process. Due to their specific chemical structure, flavonoids can chelate metal ions and form complexes [25]. Causes a regulatory action on hepatic functions. Our study of renal function showed increased serum urea, creatinine and uric acid levels in rats treated with nickel. The high serum urea concentration can be explained by the catabolism of protein compounds in the body under the action of glucocorticoid hormones, which play an important role in this process, where proteins can be broken down into amino acids and then urea. Nickel

accumulates in the kidneys, where it induces lesions in the glomeruli and proteinuria, which testifies to the onset of transient renal failure, with reduced glomerular filtration [26]. Nickel poisoning caused a significant increase in the activities of TGP, TGO. The liver constitutes an important target for nickel, which leads to hepatic necrosis and the release of aminotransferases (TGO and TGP) which clearly explains the hepatotoxic effect of nickel, which is, entered cells and crossed the nuclear membrane [27]. The usage of rats by *A. longa* dramatically recovered enzymatic activity and renal biomarkers in serum or tissues compared to nickel-exposed rats, implying that these plants reduce hepatic damage caused by nickel. This is related to Aristolochia's ability to reduce the formation of free radicals and protect against oxidative stress. Aristolochia species' anti-inflammatory properties are most likely due to a direct interaction between aristolochic acid and phospholipid A2 derivatives [28]. The major component of *Aristolochia longa* is aristolochic acid. Furthermore, phospholipase A2 is an enzyme that catalyzes the hydrolysis of arachidonic acid in the membrane, resulting in the generation of local inflammatory mediators Prostaglandins (PG), leukotrienes, and thromboxanes are examples. Arachidonic acid is a critical biological intermediary that is transformed into a variety of biologically active eicosanoids [29]. In our study, nickel poisoning induce oxidative stress by increasing lipid peroxidation and decreasing levels of antioxidant defense systems GSH and SOD in the liver and Kidney the results show a significant decrease in hepatic and kidney glutathione level in rats contaminated with nickel. This explained by the harmful effects of the action of nickel in the body and the induction of the formation of Reactive Oxygen Species (ROS) and the increase in lipid peroxidation in cells. radical oxidation process in which polyunsaturated fatty acids in the cell membrane [18] break down to give, among others, highly reactive lipid hydroperoxides (H_2O_2), a hydroxyl radical ($OH^{\cdot}$) and malondialdehyde can inactivate the enzyme [30]. Reduced SOD activity in the present study can lead to the generation of a peroxy radical, O_2^{-}, associated with the inactivation of SOD. The remarkable improvement in oxidative stress parameters after treatment with *A. Longa* this fact by the phenolic groups of polyphenols can accept an electron to form the phenoxyl radicals relatively stable, which disrupt the chain oxidation reactions of cellular components [31]. Phenolic compounds (in particular flavonoids) are able to modify the kinetics of peroxidation by modifying the order of lipid filling [32]. These phenolic compounds express the antioxidant properties by; direct trapping of reactive oxygen species, suppression of ROS formation by inhibiting some enzymes or chelating metal ions involved in their production [33].

Conclusion

This study's findings imply that *A. longa* rhizome powder therapy reduces nickel-induced liver damage and nickel buildup. *A. longa* could be a truly functional food that helps to promote health by lowering nickel toxicity thanks to its antioxidant properties.

Acknowledgement

This work was supported by the research project D01N01UN390120190001 funded by the ministry of higher education, Algeria and by Directorate general for Scientific Research and Technological Development.

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