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ANTIOXIDANT EFFECTS OF AQUEOUS EXTRACT OF BLACK MUSTARD (*BRASSICA NIGRA)* ON THE STOMACH OF WISTAR RATS INTOXICATED WITH PHENYLHYDRAZINE

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ABSTRACT

Phenylhydrazine is a chemical intermediate utilized in various industries around the world. It has been implicated in the generation of reactive oxygen species in several studies, while Brassica nigra has been reported to possess strong antioxidant activity. As a result, this study evaluated the antioxidant activity of Brassica nigra seed extract on phenylhydrazine-induced oxidative stress in the stomach of Wistar rats. Aqueous seed extract of *Brassica nigra* was prepared in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City and thirty (30) albino male Wistar rats weighing between 180 and 250 g were used during this study. Group A received 1 ml distilled water only, Group B received 10 ml of phenylhydrazine only, Group C received 10 ml of phenylhydrazine and 0.1 ml of oxaliplatin, Group D received 10 ml of phenylhydrazine and a low dose of mustard seed (150 mg/kg body weight), Group E received 10 ml of phenylhydrazine and a moderate dose of mustard seed (300 mg/kg body weight) while Group F received 10 ml of phenylhydrazine and a high dose of mustard seed (600 mg/kg body weight). All administrations were done by gavage and lasted for eight (8) months, after which, the rats were sacrificed, tissues harvested, and appropriate histological and biochemical investigations were carried out. Results showed that phenylhydrazine significantly increased levels of MDA, while simultaneously significantly reducing levels of GPx and SOD. Treatment with *B. nigra* attenuated these biochemical effects while similar positive results were obtained from the histology. This study suggests that *B. nigra* is an important physiological antioxidant that can help to decrease oxidative damage induced by phenylhydrazine in gastric tissues.

KEYWORDS:

INTRODUCTION

Phenylhydrazine is a chemical intermediate utilized mostly in the pharmaceutical, agricultural, and chemical industries around the world. Many edible mushrooms, such as Agaricus biscorpus and Gyromitra esculenta, contain phenylhydrazines, making phenylhydrazine research relevant from an environmental standpoint (Shukla et al., 2012). When exposed to air and light, phenylhydrazine appears as pale-yellow crystals that turn red-brown. It has a melting point of 66°F, at which point it turns into an oily liquid. It has a flash point of 192°F, an autoignition temperature of 345°F, and it is alcohol soluble. The monophenyl derivative of hydrazine is phenylhydrazine and it serves as a xenobiotic (WHO, 2000; NCBI, 2022).

Phenylhydrazine is hazardous when taken orally (LD_{50} 80–188 mg/kg body weight), inhaled, or applied topically. Phenylhydrazine has the potential to irritate the skin and eyes, and there is evidence that it has skin sensitizing properties in people. Exposure to phenylhydrazine may cause red blood cell destruction, potentially leading to anaemia and secondary involvement of other tissues such as the spleen and liver. *In vitro*, phenylhydrazine is mutagenic, and there is some evidence that it may have genotoxic action *in vivo*. Following oral administration, phenylhydrazine is definitely carcinogenic in mice, producing tumors of the vascular system. The mechanism underlying tumor formation is unknown, however a genotoxic component cannot be ruled out. As a result, it is not thought possible to reliably establish a threshold of exposure at which no danger of carcinogenic or genotoxic consequences exists (WHO, 2000; NCBI, 2022).

Phenylhydrazine has been implicated in the generation of reactive oxygen species in several studies (Apaijit et al., 2017; Allahmoradi et al., 2019; Ozcan et al., 2007). Due to Phenylyzine's ability to generate widespread systemic effects, it is considered to be an excellent option for use in experimental toxicity studies seeking for new chemicals that could be utilized to prevent or treat

multiple organ damage or co-morbid conditions. (Henneh et al., 2021).

Brassica nigra belongs to the botanical family of Brassicaceae. Brassicaceae is the botanical family that includes black mustard. It is a multi-branched, scented, weedy, annual plant that can reach a height of 4 meters. Its seeds are produced in long, thin pods with 10-12 brown or black seeds in per pod. It has little yellow flowers that are rather showy. Protein, selenium, manganese, omega-3 fatty acids, calcium, zinc, essential oils, phosphorus, magnesium, dietary fiber, iron, phytonutrients and vitamins A, B-complex, and C, are all found in mustard seeds (Nielsen and Rios, 2000). Previous experimental studies have demonstrated that Brassica nigra has anti-epileptic activity (Kiasalari et al., 2012; Zargari, 1991), hypoglycemic activity (Anand et al., 2009), anticancer (Tseng et al., 2002), antimicrobial (Gómez de Saravia and Gaylarde, 1998) and antioxidant properties (Sujatha and Srinivas, 1995; Kim et al., 2003). As a result, utilizing malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as indicators, we evaluated the antioxidant activity of Brassica nigra seed extract on phenylhydrazine-induced oxidative stress in the stomach of Wistar rats.

MATERIALS AND METHODS

Experimental rats

Thirty (30) albino male Wistar rats weighing between 180 and 250 g were used. The rats were given a two-week acclimatization period before the administration method began. They were given free access to conventional rat feed and water. The research ethics committee's guidelines for animal treatment at the University of Benin's College of Medicine were espoused and fully implemented.

Collection and identification of plant material

B. nigra seeds were obtained in Benin City, Edo State, Nigeria's Egor Local Government Area. They were identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, and then air-dried for seven (7) days before being ground into powder and weighed on an electrical weighing scale. Extraction was carried out utilizing proven methods (Eze and Akonoafua, 2019).

Preparation of aqueous extracts of *Brassica nigra* stock solution

Preparation of aqueous seed extract of Brassica nigra was conducted in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City. Before being macerated in distilled water in a jar, the seeds were pulverized in a British milling machine. 500 g of powder was soaked in 2 litres of cold distilled water in a conical flask. After twenty-four (24) hours, the solution (a mixture of seed extract powder and distilled water) was filtered with a filter rag and funnel. Before decanting the supernatant, the filtrate was allowed to settle for a time. At 60^{o}C, the supernatant was steamed to dryness in an evaporating dish (Royal Worcester, England) using an H-H Digital Thermometer Water Bath (Mc Donald Scientific International – 22050Hz1.0A). The extracts were kept refrigerated at 4°C in plastic vials until needed.

Experimental Protocol

GROUPS	DOSAGE
GROUP A	Received 1 ml distilled water only for thirty-four (34) weeks
GROUP B	Received 10 ml of phenylhydrazine only
GROUP C	Received 10 ml of phenylhydrazine and 0.1 ml of oxaliplatin
GROUP D	Received 10 ml of phenylhydrazine and a low dose of mustard seed (150 mg/kg body weight)
GROUP E	Received 10 ml of phenylhydrazine and a moderate dose of mustard seed (300 mg/kg body weight)
GROUP F	Received 10 ml of phenylhydrazine and a high dose of mustard seed (600 mg/kg body weight)

All administrations were done by gavage and lasted for eight (8) months.

Administration

The extracts were given using a gavage as an orogastric tube. The rats received special care to avoid any oral or esophageal injuries. Phenylhydrazine was.

Tissue collection, processing and staining, histopathology

The rats were sacrificed and the stomachs were taken at the end of the eight-month study. Blood (5 mL) was collected in EDTA vials for analysis and was immediately sent to the University of Benin Teaching Hospital's Chemical Pathology department for biochemical testing. The stomach tissues were preserved for 24 hours in 10% buffered formalin before being histologically processed and stained with haematoxylin and eosin using standard procedures (Drury et al., 1976). The sections obtained were examined and photomicrographs were taken using a Leica DM750 research microscope with an attached digital camera (Leica CC50). The tissues were photographed digitally at magnifications of x100.

Biochemical assays

Misra and Fridovich (1972) technique was used to measure superoxide dismutase activity, Cohen et al (1970) method for catalase activity, and Flohe and Gunzler (1984) method for glutathione peroxidase activity. The Vershney and Kale (1990) technique was used to measure a lipid peroxidation marker (malondialdehyde, MDA).

Statistical analyses

A one-way analysis of variance (ANOVA) was used to compare treatment groups' values to those of the control group. Data were analyzed using the IBM SPSS statistics program Version 25 (SPSS, Inc., Chicago, Illinois, USA). The post-hoc test used was the LSD. P values less than 0.05 were defined as significant.

RESULTS

Table 2: Antioxidant enzyme activities and level of lipid peroxidation of rats.

	Control	Phenylhydrazine only	Phenylhydrazine + oxaliplatin	Phenylhydrazine + 150mg/kg *B. nigra*	Phenylhydrazine + 300mg/kg *B. nigra*	Phenylhydrazine + 600mg/kg *B. nigra*	P-value
MDA (x10-3 mmole/ml)	20.58±0.02	28.99±0.23*	19.77±0.19*	26.18±0.19	24.45±0.19	23.21±0.02	0.012
SOD (U/ml)	7.50±0.01	4.17±0.02*	8.23±0.01	6.88±0.01	6.16±0.03	7.47±0.02	0.042
CAT (U/ml)	233.00±1.25	240.76±0.72*	252.70±0.43*	255.43±0.93*	246.66±0.83*	243.28±0.01*	0.000
GPx (U/ml)	2.40±0.02	1.87±0.01*	3.15±0.02*	2.12±0.01	2.33±0.01	2.73±0.01	0.000

*Significantly different from the control group at P<0.05

The results are mean of five rats in each group ± SEM

Photomicrographs

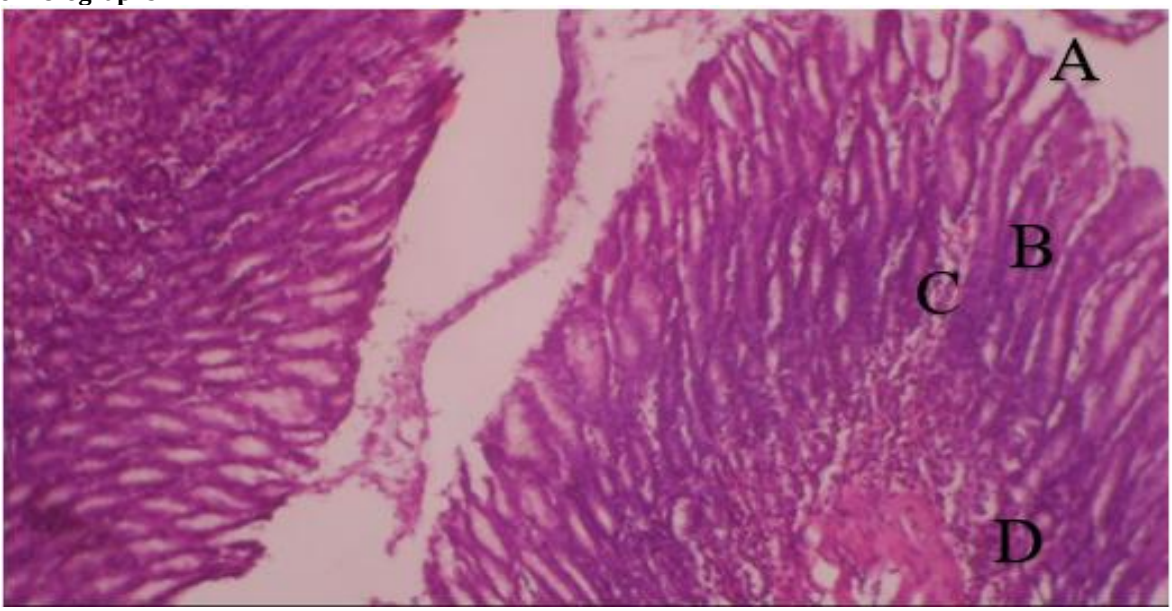

Plate 1: Stomach control composed of A: mucosal lining with pits, B: glands, C: lamina propria and D: muscularis mucosa (H&E x100).

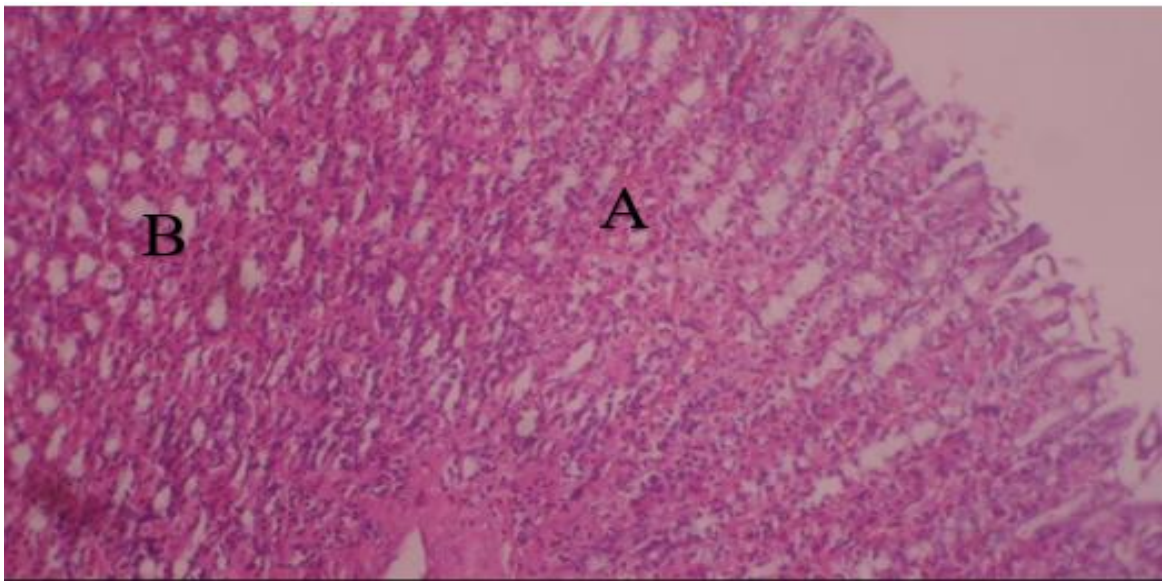

Plate 2: Rat given phenylhydrazine only, showing A: mucosal congestion and B: oedema (H&E x100)

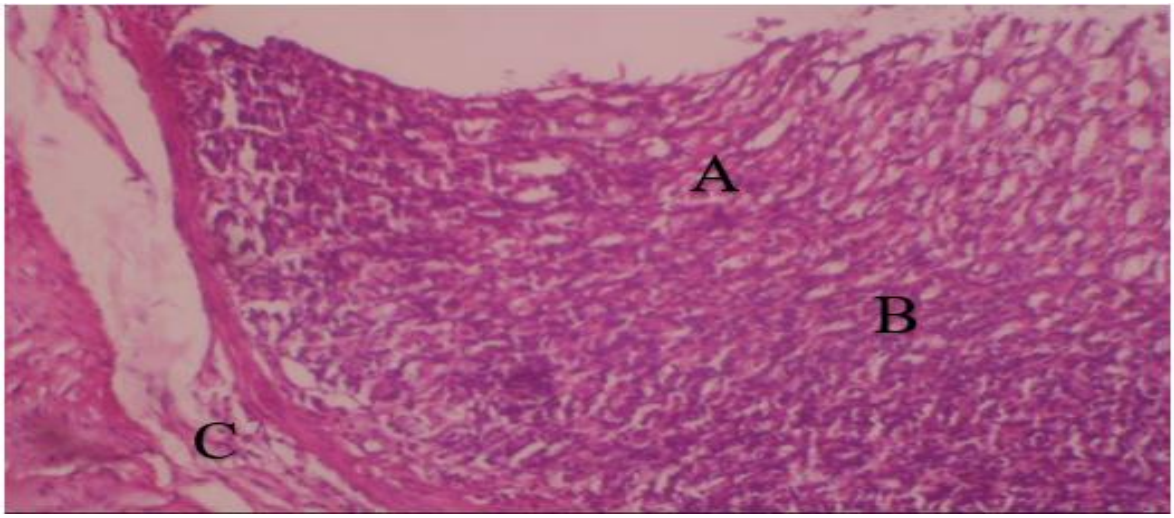

Plate 3: Rat given phenylhydrazine + oxaliplatin, showing A: mucosal congestion, B: mucosal and C: submucosal mobilization of eosinophils (H&E x100).

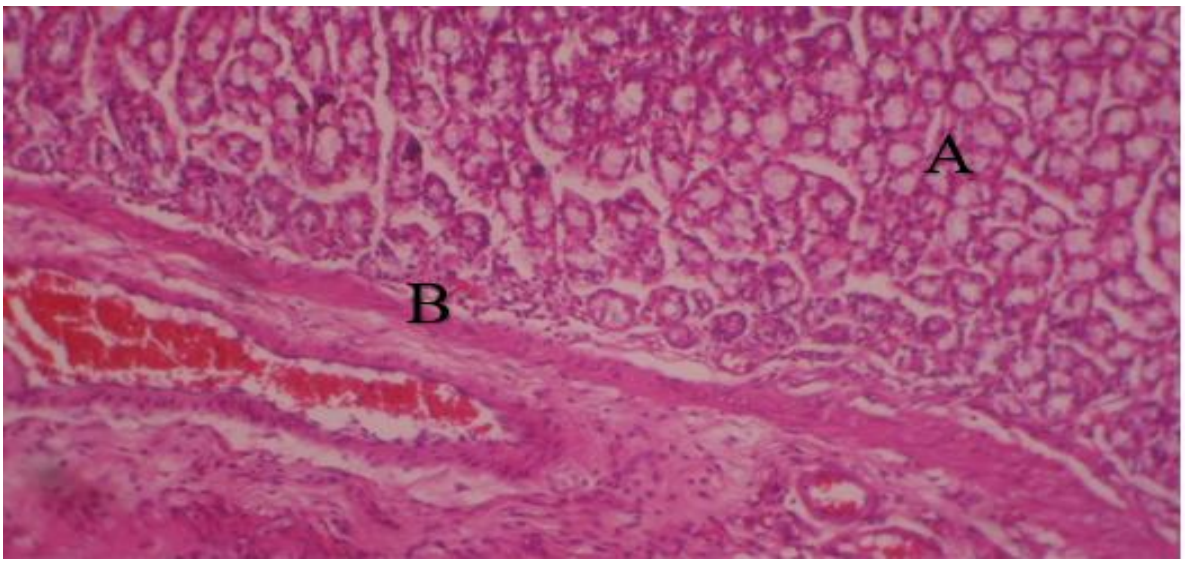

Plate 4: Rat given phenylhydrazine + 150mg extract, showing A: mucosal congestion and B: mucosal mobilization of cells of the immune system (H&E x100).

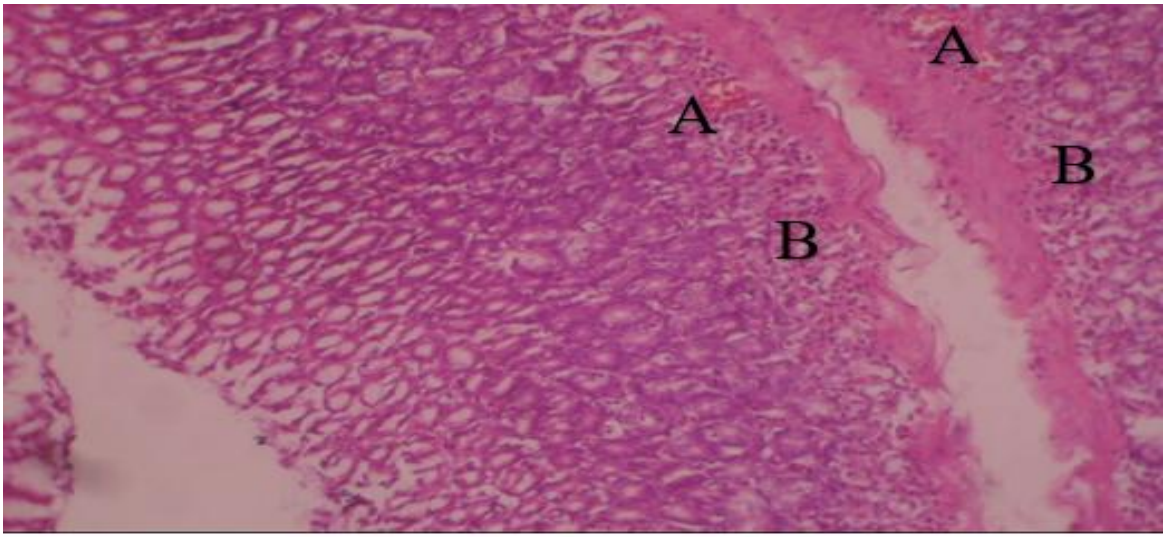

Plate 5: Rat given phenylhydrazine + 300mg extract, showing A: active mucosal congestion and B: mobilization of cells of the immune system (H&E x100).

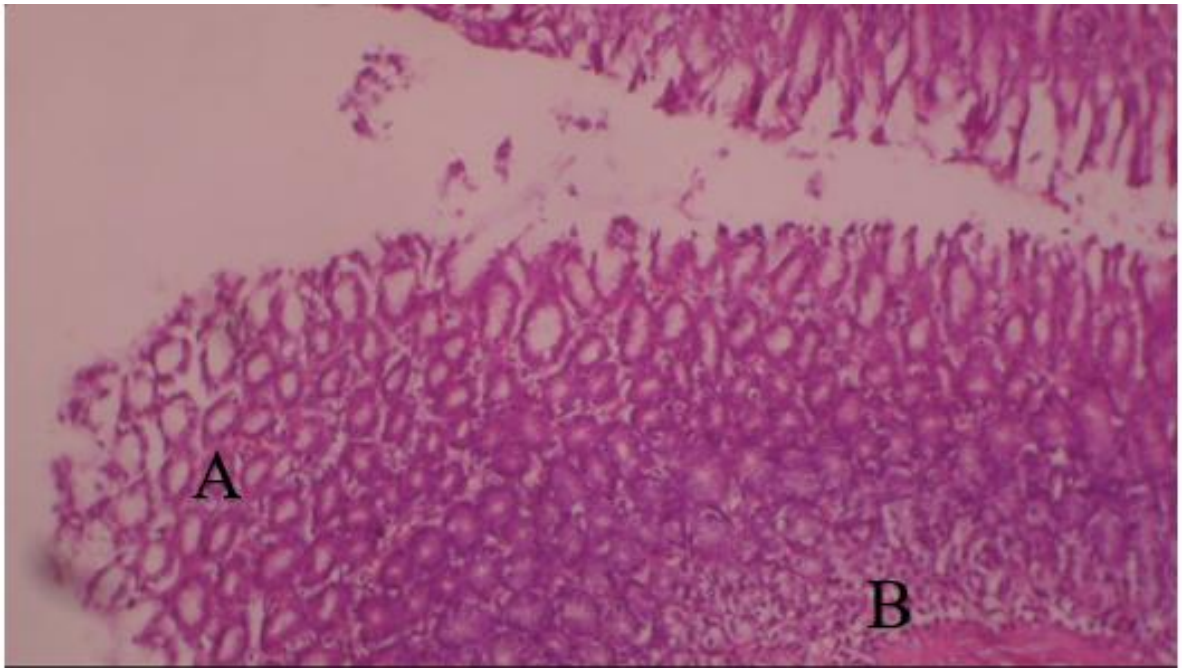

Plate 6: Rat given phenylhydrazine + 600mg extract, showing A: mucosal congestion and B: mobilization of Cells of the immune system (H&E x100).

DISCUSSION

Numerous studies have emphasized the nutritional and physiological advantages of antioxidants, as well as the inverse relationship between them and free radicals. Several health issues, including atherosclerosis, diabetes, cancer, neurological diseases, cardiovascular illnesses, and other chronic ailments, have been linked to free radicals or reactive species as a result of oxidative stress (Giustarini et al., 2009).

Oxidative stress is characterized as an increase in the production of reactive oxygen species (ROS) and/or a decrease in antioxidant defense, both of which can harm biological macromolecules. During the univalent reduction of oxygen to water, reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and hydrogen peroxide are produced. ROS are required for various biological activities, including intracellular differentiation and cell development, growth arrest, apoptosis, immunity, and defense against pathogens, and their production is normally modest. Endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) can protect against ROS damage (Mates et al., 1999; Kurutas et al., 2005).

The group administered with phenylhydrazine had higher levels of malondialdehyde (MDA) in the gastric tissues, indicating that phenylhydrazine may have induced oxidative stress. When Brassica nigra was given with phenylhydrazine, the levels were reduced, as shown

in Table 2, demonstrating that Brassica nigra had a protective effect against phenylhydrazine-induced oxidative damage. MDA has been utilized as a biomarker of lipid peroxidation in plasma in previous investigations. MDA levels were greater in rats given phenylhydrazine compared to the control group, according to Aloke et al (2021). MDA levels in renal and hepatic tissue homogenates were similarly greater in the phenylhydrazine-treated group than in the control group, according to Karbownik et al (2000).

Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase play a critical but often overlooked role in biological systems' antioxidant defense against free radical assault (Ighodaro and Akinloye, 2018). The first detoxifying enzyme and most effective antioxidant in the cell is superoxide dismutase (SOD). It's a crucial endogenous antioxidant enzyme that's part of the body's initial line of defense against reactive oxygen species (ROS). It catalyses the dismutation of two molecules of superoxide anion (O_2) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), reducing the danger of the superoxide anion (Gill and Tuteja, 2010). CAT is an antioxidant enzyme that is found in practically all biological tissues that use oxygen. The enzyme catalyzes the breakdown or reduction of hydrogen peroxide (H2O2) to water and molecular oxygen using either iron or manganese as a cofactor, completing the detoxification process started by SOD (Chelikani et al., 2004). SOD exhibits an antioxidant effect by capturing and the superoxide radical (O^{-}_2) and transforming it into water and hydrogen peroxide (H_2O_2) (Halliwell, 1994). In our study, GPx and SOD activities were decreased in the plasma. These results showed that phenylhydrazine may cause oxidative stress by consuming antioxidant enzymes, as overproduction of free radicals generated during infection may lead to the low levels of antioxidant enzymes

Brassica plants are known to possess antioxidant properties due to the presence of antioxidant phytochemicals mainly the polyphenols, flavonoids, and ascorbic acid. Most of these phytochemicals present act as antioxidants due to their hydrogen-donating and reducing abilities. Polyphenols are the phytochemicals which act as metal ion chelators and interfere with oxidation reactions including lipid peroxidation by donating the proton to free radicals. Phenoxy radicals are relatively stable to stop the oxidation chain reaction. therefore, they stop the initiation of new oxidation chain reaction and stop the initiation of new oxidation chain reaction and terminate the propagation routs by capturing free radicals (Mandal et al., 2010). Flavonoids possess metal ion chelating and free radical scavenging potential (Chawla et al., 1998). This indicates that bioactive phytoconstituents found in B. nigra are likely to have an important role as antioxidative agents.

This study is a demonstration of the gastroprotective property of Brassica nigra against phenylhydrazine-induced gastric damage. Brassica nigra was given at doses of 150, 300 and 600 mg/kg and markedly reduced the gastric damage induced by phenylhydrazine in a dose-related manner.

CONCLUSION

This study suggests that B. nigra is an important physiological antioxidant that can help to decrease oxidative damage induced by phenylhydrazine in gastric tissues. B. nigra appears to be an effective antioxidant even at the low administered dose of 150 mg/kg, bringing the previously depleted levels of the antioxidant enzymes almost towards normal levels seen in controls.

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