PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 26 (4) 661-666 April 2022

Ameliorative Effects of Aqueous Extract of *Brassica nigra* on Phenylhydrazine-Induced Liver Toxicity in Wistar Rats

***[1]OBAYUWANA, E; [2]OBAYUWANA, MO**

*[*1]Department of Anatomy, School of Basic Medical sciences, College of Medical sciences, University of Benin, Benin City, Nigeria.*
[2]Department of Physiology, School of Basic Medical sciences, College of Medical sciences, University of Benin, Benin City, Nigeria.
**Corresponding Author Email: obayuwanaedobor24@gmail.com*

ABSTRACT: *Brassica nigra* has been reported to possess antioxidant activity, anti-inflammatory activity, anti-epileptic activity, antidiabetic activity, anthelminthic activity, nephroprotective activity and hepatoprotective activity. In experimental models, phenylhydrazine been used to cause reproductive toxicity, nephrotoxicity, hepatotoxicity, hyperbilirubinemia and haemolytic anaemia. This study therefore aimed to investigate the effects of *B. nigra* on phenylhydrazine-induced liver toxicity through analysis of liver enzymes and histoarchitecture of the liver. Thirty (30) adult Wistar rats weighing between 180 and 200 grams were utilized in this investigation. They were bred in the Animal House of the Department of Anatomy, University of Benin, and were randomly assigned into six (6) groups of five (5) animals. Group A received 1 ml distilled water, Group B received phenylhydrazine only, Group C received phenylhydrazine and 0.1 ml of oxaliplatin, Group D received phenylhydrazine and 150 mg/kg body weight of *B. nigra*, Group E received phenylhydrazine and 300 mg/kg body weight of *B. nigra* while Group received phenylhydrazine and 600 mg/kg body weight of *B. nigra.* The study lasted for a period of eight (8) weeks. Significant elevations ($P<0.05$) in the mean concentrations of liver enzymes (ALP, AST, ALT) and total protein were recorded after the administration of phenylhydrazine while the treatment with different concentrations of *B. nigra* induced a reversal in those parameters to levels comparable with the control. In conclusion, *B. nigra* administration lowers a number of the harmful effects of *in vivo* phenylhydrazine administration in the liver of Wistar rats, according to the findings in this study.

DOI: https://dx.doi.org/10.4314/jasem.v26i4.14

Impact factor: http://sjifactor.com/passport.php?id=21082

Google Analytics: https://www.ajol.info/stats/bdf07303d34706088ffffbc8a92c9c1491b12470

Dates: Received: 05 March 2022; Revised: 13 April 2022; Accepted: 27 April 2022

Keywords: *Brassica nigra*, Phenylhydrazine, Liver, Wistar rats.

People have been searching for medications in nature to treat their ailments since ancient times. The use of medicinal plants began instinctively, just as it does with animals (Stojanoski, 1999; Petrovska, 2012). Since there was insufficient information at the time, neither about the causes of the ailments nor about which plant and how it could be used as a cure, everything was based on experience. As the reasons for the use of various medicinal plants for the treatment of specific ailments were identified over time, the use of medicinal plants shifted away from an empiric framework and toward one based on explicatory facts. Plants have been the source of treatment and prophylaxis until the invention of iatrochemistry in the 16th century (Kelly, 2009; Petrovska, 2012). A Sumerian clay slab from Nagpur, estimated to be five thousand (5000) years old, contains the oldest recorded documentation of medicinal herbs' use in medication manufacture. It contained twelve (12) drug-preparation instructions pertaining to over two-hundred and fifty (250) different plants, some of which were alkaloid-rich, notably: poppy, henbane, and mandrake (Kelly, 2009; Petrovska, 2012). Only a limited number of medicinal plants that are employed in both Western and non-Western medical systems have gotten a lot of attention and use in recent years (Briskin, 2000). In present day Germany, there are an estimated 600 to 700 plant-based medicines, which are prescribed by nearly 70% of German doctors, and herbal medicines are actually recommended more frequently than conventional pharmaceuticals. The claims of a number of components in these plant-based medications have been proven to be true, while others have been found to be ineffective or more harmful than prescribed equivalents (Schulz *et al*., 2001). The plant - *Brassica*

**Corresponding Author Email: obayuwanaedobor24@gmail.com*

nigra - widely known as black mustard, is extensively cultivated and consumed. It is an annual, fragrant weedy plant that is grown globally and belongs to the Brassicaceae family. *Brassica nigra* is also utilized as a source of green vegetable, salad crop, oilseed crop, green manure, fodder crop and industrial oil, in addition to being a spice (Thomas *et al*., 2012; Kumar *et al*., 2013; Lakwani *et al*., 2021). It originated originally from Eurasia, but it has now spread around the world (Young-Mathews, 2012). *B. nigra* has been reported to possess antioxidant activity (Rajamurugan *et al*., 2012), anti-inflammatory activity (Vinyas *et al*., 2012), anti-epileptic activity (Praveen *et al*., 2013), antidiabetic activity (Anand *et al*., 2007), hepatoprotective activity (Rajamurugan *et al*., 2012), nephroprotective activity (Rajamurugan *et al*., 2012), anthelminthic activity (Upwar *et al*., 2011), amongst others. Herman Emil Fisher discovered phenylhydrazine (PHZ) in 1875, and it was first used as an antipyretic medication (Shukla *et al*., 2012). In experimental models, it has been used to cause reproductive toxicity (Karimipour *et al*., 2018), nephrotoxicity (Anbara *et al*., 2018), hepatotoxicity via liver glutathione depletion and lipid peroxidation (Valenzuela *et al*., 1987), hyperbilirubinemia (jaundice) (Nawaz *et al*., 2016), and haemolytic anaemia (Diallo *et al*., 2008). Phenylhydrazine's potential to cause extensive systemic effects makes it a suitable agent in experimental toxicity investigations looking for novel compounds that may be used to prevent or treat multiple organ damage or co-morbid diseases. It is presently mostly utilized as a chemical intermediary in the pharmaceutical industry, and its toxicity in numerous organs is well-known (Henneh *et al*., 2021). This study therefore aimed to investigate the effects of *Brassica nigra* on phenylhydrazine-induced liver toxicity.

MATERIALS AND METHOD

Plant material: The seeds of *B. nigra* were collected in Egor Local Government Area of Benin City, Edo State, Nigeria. They were recognized in the Herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, and air-dried for seven (7) days before being ground into powder and measured with an electrical weighing scale. Extraction was done using tried and true procedures (Eze and Akonoafua, 2019).

Experimental animals: In this investigation, thirty (30) adult Wistar rats, randomly assigned into six (6) groups of five (5) animals were employed as experimental animals. At the start of the study, they weighed between 180 and 200 grams. The animals were acquired and placed in washed and disinfected conventional cages at the Animal House of the University of Benin's Department of Anatomy. The rats were allowed to acclimate for two (2) weeks prior to the start of treatment. For the course of the experiment, all animals were given food (livestock's growers marsh made by Top Feed limited, Sapele, Delta State, Nigeria) and water *ad libitum*.

Induction of hepatotoxicity: Hepatotoxicity was induced in the experimental animals using previously established methods of Henneh *et al* (2021) where the animals were treated with 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours, orally.

GROUPS	DOSAGE
GROUP A	Received 1 ml distilled water only for eight (8) weeks
GROUP B	Received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours
GROUP C	Received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours and 0.1 ml of Oxaliplatin for eight (8) weeks
GROUP D	Received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours and a low dose of mustard seed (150 mg/kg body weight) for eight (8) weeks
GROUP E	Received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours and a moderate dose of mustard seed (300 mg/kg body weight) for eight (8) weeks
GROUP F	Received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours and a high dose of mustard seed (600 mg/kg body weight) for eight (8) weeks

Tissue collection, processing and staining: The rats were sedated with chloroform and sacrificed after eight (8) weeks. Every rat was sacrificed by cutting a midline incision in the ventral abdominal wall. Each rat's liver was promptly taken. Serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), conjugated bilirubin, and total bilirubin were all measured using blood drawn directly from the abdominal aorta. The liver tissues were fixed in 10% buffered formalin for 24 hours before being processed histologically and stained with haematoxylin and eosin. Staining of tissues was done by established methods (Drury *et al.,* 1976).

Photomicrography: A histopathologist used a Leica DM750 research microscope with an attached digital camera (Leica CC50) to view the H&E stained slides of the liver. The tissues were photographed digitally at magnifications of x100.

Statistical analyses: The IBM SPSS statistics program (Statistical Package for Social Science) Version 25 (SPSS, inc., Chicago, Illinois, USA) was used to analyze all of the data and generate appropriate statistical values. The treatment groups' values were compared to those of the control group using a one-way analysis of variance (ANOVA). P values of less than 0.05 were deemed significant. As a post-hoc test, LSD was utilized.

RESULTS AND DISCUSSION

Plate 1 is a photomicrograph of the liver of the control group at x100 magnification. It shows normal histoarchitecture: normal hepatocytes, normal sinusoids, bile duct and portal vein, and devoid of Kupffer cells. Plate 2 is a photomicrograph of the liver of the group given phenylhydrazine only, at x100 magnification. It shows vascular ulceration, periportal infiltrates of inflammatory cells, zonal necrosis and Kupffer cell activation. Plate 3 is a photomicrograph of the liver of the group given phenylhydrazine + oxaliplatin, at x100 magnification. It shows normal hepatocytes and mild periportal infiltrates of inflammatory cells. Plate 4 shows a photomicrograph of the liver of the group that received phenylhydrazine + 150 mg *Brassica nigra* aqueous extract.

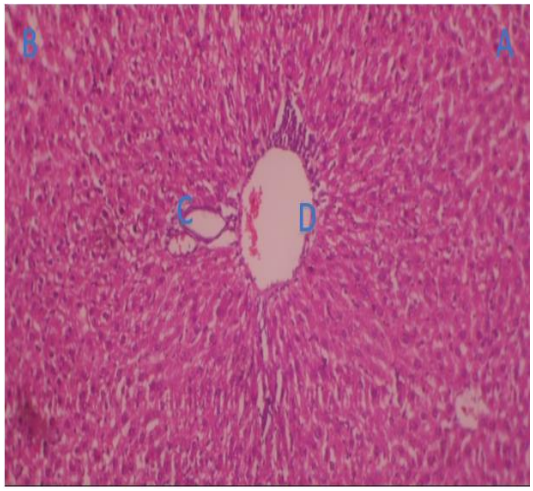

Plate 1: Liver control composed of A: hepatocytes, B: sinusoids, C; bile duct and D: portal vein (H&E x100)

It reveals Kupffer cell activation, vascular congestion, and inflammatory cell periportal mobility. Plate 5 shows a photomicrograph of the liver of the group that received phenylhydrazine + 300 mg *Brassica nigra* aqueous extract. It depicts activated Kupffer cells as well as normal hepatocytes. Plate 6 shows a photomicrograph of the liver of the group that received phenylhydrazine + 600 mg *Brassica nigra* aqueous extract. Hepatocytes and vascular architecture are normal, and Kupffer cells are active. In this study, the ameliorative effects of *Brassica nigra* against phenylhydrazine-induced toxicity in liver of exposed Wistar rats were examined, and these effects were compared with the control group, in the presence of a well-known standard drug, oxaliplatin.

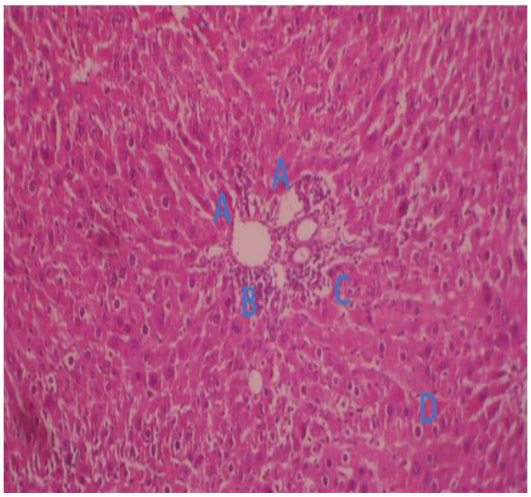

Plate 2: Rat given Phenylhydrazine only showing A: vascular ulceration, B: periportal infiltrates of inflammatory cells, C: zonal necrosis and D: Kupffer cell activation (H&E x100)

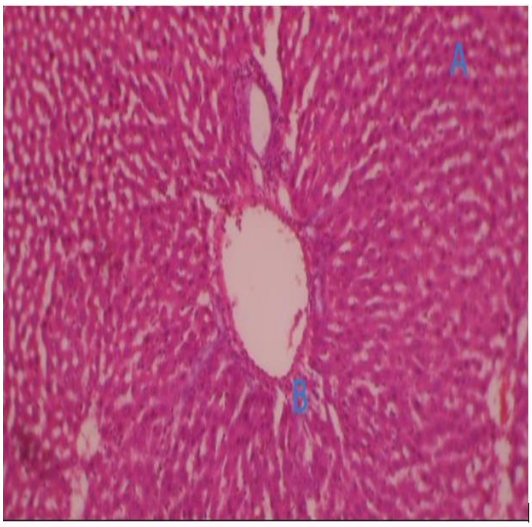

Plate 3: Rat given Phenylhydrazine + oxaloplatin, showing A: normal hepatocytes and B: mild periportal infiltrates of inflammatory cells (H&E x100)

Examination of phenylhydrazine's toxicity was measured by the results of the liver function test and histopathology, since liver function tests are sensitive indicators of liver cell injury (Pratt and Kaplan, 2000). The liver, the key organ involved in numerous metabolic functions (production and secretion of bile, production of fibrinogen, prothrombin, heparin and sulfuric acid ester, and conversion of sugar into glycogen) plays a central role in the detoxification process while facing the threat of maximum exposure

to xenobiotics and their metabolic by-products (Meyer and Kulkarni, 2001; Nadeem *et al*., 1997).

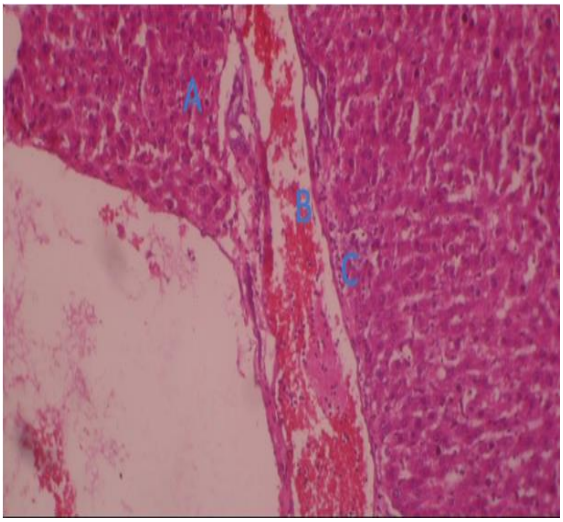

Plate 4: Rat given Phenylhydrazine + 150mg extract, showing A: Kupffer cell activation, B: vascular congestion and C: periportal mobilization of inflammatory cells (H&E x100)

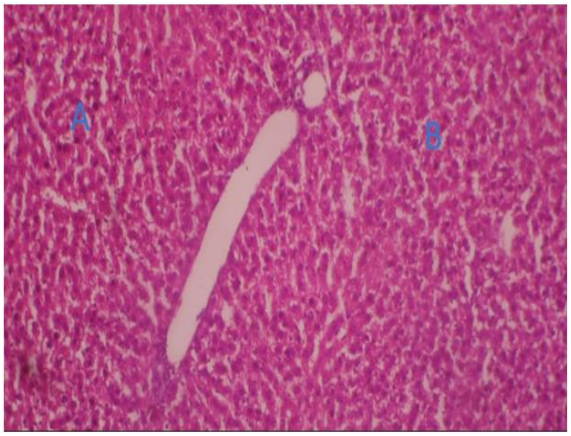

Plate 5: Rat given Phenylhydrazine + 300mg extract, showing A: Kupffer cell activation and B: normal hepatocytes (H&E x100)

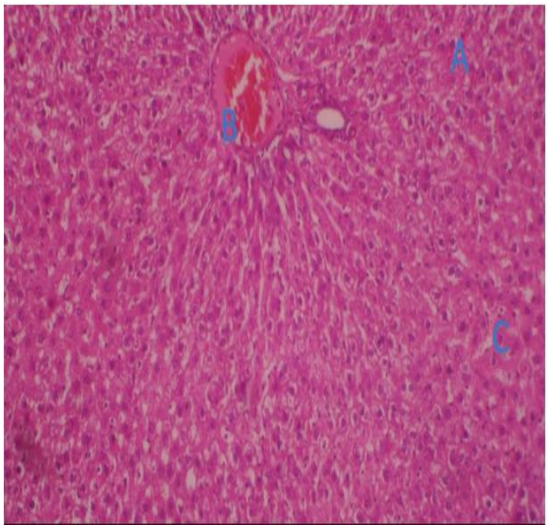

Plate 6: Rat given Phenylhydrazine + 600mg extract, showing A: normal hepatocytes, B: normal vascular architecture and C: Kupffer cell activation (H&E x100)

Herbal medicines play a major role in the treatment of liver disorders. A number of medicinal plants and their formulations are widely used for the treatment of these disorders (Kalantari *et al*., 2018; Liu *et al*., 2018). The majority of the time, serum enzymes are employed to assess hepatic dysfunction. According to Awad *et al* (1998), cell injury is strongly link to enzyme leakage. As a result, an increase in these enzymes could be linked to liver dysfunction, disturbance in their production, and alterations in the permeability of the liver. According to Meyer and Kulkarni (2001), alanine aminotransferase (ALT) is produced by liver cells and is the most sensitive marker for liver cell death. Any sort of hepatic cell injury might induce ALT to leak into the bloodstream and raise serum levels.

Table 2: Levels of liver function enzymes

	ALP (µ/L)	**ALT (µ/L)**	**AST (µ/L)**	**Total bilirubin (mg/dl)**
Control	95.33±1.67	31.67±2.91	36.67±5.93	0.10±0.00
Phenylhydrazine only	128.00±7.23*	43.67±1.20*	54.33±2.60*	0.19±0.02*
Phenylhydrazine + Oxaliplatin	97.33±2.33	26.00±2.89	28.33±1.45	0.13±0.03
Phenylhydrazine + 150mg/kg *Brassica nigra*	117.00±10.50*	36.33±1.76	39.33±0.67	0.11±0.03
Phenylhydrazine + 300mg/kg *Brassica nigra*	95.00±14.73	35.00±2.65	37.00±1.53	0.10±0.00
Phenylhydrazine + 600mg/kg *Brassica nigra*	94.33±8.09	33.33±0.67	37.67±1.33	0.10±0.00
P- value	0.011	0.047	0.042	0.022

**Significantly different from the control group at P<0.05; the results are mean of five rats in each group ± SEM*

In the biochemical investigation recorded in this present study, there were significant increases in the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TB), after the administration of phenylhydrazine. These results give us the confirmation that the animals treated with 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours suffered liver toxicity. Phenylhydrazine intoxication leads to haemolysis resulting in severe hepatic and splenic iron overload causing a number of pathophysiological changes such as fatty liver and hepatocyte necrosis (Karbownik *et al*., 2000). The current study is in agreement with previous studies on

the hepatotoxicity of phenylhydrazine (Karbownik *et al*., 2000; Anbara *et al*., 2015).The treatment of the experimental animals with 150 mg/kg body weight of *Brassica nigra*, 300 mg/kg body weight of *Brassica nigra* and 600 mg/kg body weight of *Brassica nigra* caused a restoration in the previously elevated serum levels of the liver enzymes, to levels comparable with the control group, with the highest dose of the extract – 600 mg/kg – being the most effective. Histologically, the liver of the animals that received phenylhydrazine showed hepatic degenerations. The implication of these results is that the hepatocyte's membrane integrity was compromised by the administration received, while a return to normalcy after the administration of *Brassica nigra* is indicative of its ameliorative potency. Phytochemical analysis of *Brassica nigra* from other studies have shown that the plant contains numerous biologically active compounds, such as flavonoids, phenylpropanoid derivatives, indole alkaloids, sterol glucosides, sulphur-containing compounds, glucosinolates, phenanthrene derivative, isothiocyanate and anthocyanins (Romani *et al*., 2006; Wu *et al*., 2013; Rafatullah *et al*., 2006) and it is opined that these are responsible for its ameliorative ability, especially as alkaloids have been shown to produce hepatoprotective effects in an animal study (Vijayan *et al*., 2003).

Conclusion: Brassica nigra administration lowers a number of the harmful effects of *in vivo* phenylhydrazine administration in the liver of Wistar rats, according to the findings in this study.

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