OPEN ACCESS
Scholars International Journal of Anatomy and Physiology
Abbreviated Key Title: Sch Int J Anat Physiol
ISSN 2616-8618 (Print) |ISSN 2617-345X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Evaluation of the Effects of Extracts of *Laurus nobilis* on some Biochemical Parameters of Wistar Rats

Chibuike Obiandu[1*], Joy O. A. Laz-Okenwa[1], Bright I. Owhorji[1], Abiye Tamuno-Opubo[1], Henrrietta O. Asuzu-Samuel[2]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria
[2]Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Choba, Rivers State, Nigeria

DOI: 10.36348/sijap.2023.v06i04.001 | **Received:** 17.02.2023 | **Accepted:** 03.04.2023 | **Published:** 07.04.2023

***Corresponding author:** Chibuike Obiandu
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Abstract

Laurus nobilis is one of the most popular species belonging to the lauraceae family and it has been reported to possess many physiological properties such as antimicrobial, antifungal and wound healing effects. In this study, effects of ethanolic leaf extract of *Laurus nobilis* on some oxidative stress markers and liver function parameters of female Wistar rats were investigated. Five rats were randomly assigned into each of groups 1-4, of which group one (1) served as control and received distilled water. Groups 2-4 were treated with 100mg/kg bw, 200mg/kg bw and 400mg/kg bw of the ethanolic extract of the leaves of *Laurus nobilis* respectively, for a period of 14 days. The results obtained indicated that the superoxide dismutase and catalase enzyme activities as well as, gluthathione reductase and peroxidase activities were not significantly affected. The malondialdehyde level did not change significantly after two weeks of extract administration. The extract caused no significant ($P<0.05$) alteration in the enzyme **activities** of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), as well as serum concentrations of total protein (TP), albumin (ALB) and total bilirubin. This study have shown that extract of *Laurus nobilis* neither altered hepatic function parameters nor promoted toxic stress in the female Wistar rats.

Keywords: Laurus nobilis, hepatic function parameters, ethanolic extract, Wistar rats.

INTRODUCTION

One of the most important uses of plants is that they serve as a source of food consumed for the purpose of providing energy and promoting the growth of the animal. In recent times, there has been much knowledge shared on the importance of consuming healthy foods which aids in the prevention and treatment of diseases. *Laurus nobilis* (family Lauraceae) is used as a valuable flavouring agent in culinary and food industries. It is grown for diverse purposes at different locations around the world. But it is native to the Mediterranean countries with a high annual rainfall. They are also grown in the subtropics and tropics of East Asia, North and South America. It is grown as a decorative species in Europe, Russia, the USA and cultivated in Turkey, Morocco, Algeria, Spain, France and Mexico etc (Barla *et al*., 2007; Marzouki *et al*., 2009). *Laurus nobilis* has tremendous folklore relevance. Traditionally it is usually applied as treatment for rheumatism, dermatitis, bloating, poor digestion and flatulence. The aqueous extract is used in Turkish folk remedy for hemorrhoids and as a diuretic; also administered as antidote for snakebites and for stomach ache, Baytop (1985); Aqili-Khorasani, (1992); Kilic *et al*., (2004); Gülçin (2006). Several experimental studies carried out with *Laurus nobilis* has documented numerous pharmacological actions of this plant. The findings in some studies showed that, *Laurus nobilis* has antibacterial Ghadiri *et al*., (2014), anticancer (Verdian-Rizi, 2009) and wound healing (Vardapetyan *et al*., 2013) effects. The anti-diabetic effects of this plant was evaluated and it was found to demonstrate antihyperglycemic effects. And so, *Laurus nobilis* had been used for preventing and treating type II diabetes due to its ability to reduce the level of glucose in the blood (Khan *et al*., 2009). Furthermore, it has been

reported to cause simultaneous reductions in the serum level of total cholesterol and LDL-cholesterol while enhancing HDL-cholesterol (Khan *et al*., 2009). Usually, observed actions are due to the total amount and nature of bioactive substances present in the extract. The extract of *Laurus nobilis* possess several biologically active substances such as phenolic acids, flavonoids (Lu *et al*., 2011), terpenoids (Otsuka *et al*., 2008; Liu *et al*., 2009), glycosides and anthocyanins [Luigia & Giuseppe (2005); Verdian-Rizi, (2009). The essential oils extracted from *Laurus nobilis* are reportedly responsible for multiple biological effects of the plant (Esra *et al*., 2007; Ramling *et al.*, 2012). In recent years, great efforts has been directed towards the search for new drugs of plant origin that possess a wide range of pharmacological effects on different functions including the liver. One of the primary functions of the liver is the metabolism of digestible ingredients including foods and food supplements, some drugs and alcohol; some of which may trigger dysfunctions in liver function (Chatterjee *et al*., 2006). Although, the effects of *Laurus nobilis* on chemical induced hepatotoxicity has been carried out (Gasparyan *et al*., 2015); but, studies investigating its physiological potentials (enhancement or reduction) on hepatic functions and some oxidative stress markers in the absence of liver toxicity are scarce. The objectives of this study are to investigate the effects of *Laurus nobilis* on liver function parameters and oxidative stress markers in Wistar rats without pre-induced hepatotoxicity.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Laurus nobilis* were purchased from a local market in Port Harcourt, Rivers State, Nigeria and identified at the herbarium, Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria. The leaves were washed to remove dirt and then chopped to small pieces and air dried for three weeks. The dried forms were blended to fine powder using a Manual blender. The powdered samples were kept in polythene bags and preserved at room temperature.

Preparation of plant extracts:

A total of 500g of powdered sample was soaked in 400ml of ethanol and left to stand for 24 hours to allow for extraction at room temperature. Thereafter, the solution was filtered with a Whattman's filter paper and the filtrate concentrated under reduced pressure using a rotary evaporator. The yield was stored air tight in a refrigerator until required for use.

Animal Models:

Adult female Wistar rats weighing 150 to 180g were used for the study. Animal care and handling conformed to standard guidelines on the use of experimental animal in research (American Physiological society, 2002). Institutional ethical approval was obtained before commencement of study. The Wistar rats were divided into four (4) groups of five (5) rats each. Group one (1) served as control and received distilled water. Groups two (2); three (3) and four (4) were administered with 100mg/kg bw, 200mg/kg bw and 400mg/kg bw of the ethanol extract respectively. The extract was administered orally, once per day for a period of 14 days. The rats were sacrificed under chloroform anaesthesia on day 15 after 24hours of last administered dose.

Collection of Blood

Blood samples were collected through cardiac puncture into appropriate dry sample tubes and allowed to stand for about 15-20 minutes to clot. It was further centrifuged at 3000 rev/min for 5 minutes using a table centrifuge machine. The serum was separated using a pasteur pipette into sterile sample tubes and stored at -4^{0}C until used.

The biochemical analysis of serum for liver enzymes, TP, ALB and total bilirubin was carried out by standard methods reported by Gasparyan *et al*., (2015).

Determination of activities of oxidative stress markers

A laparotomy incision was done to locate and remove the liver. The organ was blotted with tissue paper then cut very thinly with sterile scapel blade and homogenized in ice-cold 0.25M (mol/L) sucrose solution (mass-to-volume ratio of 1:5). The homogenates was centrifuged for 10 minutes at 4000r/minute at 4°C to obtain a clear supernatant. The supernatant was then carefully aspirated with pasteur pipette into sample bottle and stored frozen at -20°C until used for biochemical assays.

The methods adopted in determination of some enzymatic and non enzymatic oxidative stress indicators in this study has been previously documented (Goldberg, 1984; Wasowick *et al.*, 1993; Slaughter and O'Brien, 2000; Vives-Bauza *et al.*, 2007; Condezo-Hyos *et al*., 2013; Peskin and Winterbourn, 2017).

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 23.0 software tool was used for the statistical data processing. Values were expressed as mean±SEM. The differences in the mean values between groups were analyzed using the analysis of variance with post hoc least significant difference and considered statistically significant at $p<0.05$.

RESULT

The result for the study is presented in Tables 1-4.

Table 1: Mean levels of liver enzymes

Groups (mg/kg)	ALT	AST	ALP
Control	6.61±0.67	16.64±1.40	17.10±1.55
100	6.57±0.62	18.30±2.05	16.87±1.41
200	8.03±0.49	16.52±1.14	16.53±1.73
400	7.76±0.56	17.38±1.59	17.63±1.81

Values presented as Mean±SEM. n=5. P-value= <0.05

Table 2: Mean levels of some biochemical parameters

Groups (mg/kg)	TP	ALB	Total bilirubin
Control	66.47±2.55	41.00±0.71	3.75±0.50
100	63.53±2.98	39.00±0.71	3.90±0.47
200	63.91±3.09	40.80±0.86	4.39±0.31
400	64.21±2.87	42.84±0.77	4.14±0.28

Values presented as Mean±SEM. n=5. P-value= <0.05

Table 3: Mean activities of some enzymatic oxidative stress markers

Groups (mg/kg)	GSH	GPx	CAT
Control	1.60±0.19	0.07±0.00	3.79±0.24
100	1.76±0.20	0.06±0.00	3.60±0.20
200	1.42±0.20	0.07±0.01	4.00±0.25
400	1.41±0.19	0.07±0.01	3.80±0.23

Values presented as Mean±SEM. n=5. P-value= <0.05

Table 4: Mean superoxide dismutase activity and malondialdehyde level

Groups (mg/kg)	SOD	MDA
Control	0.38±0.01	0.45±0.02
100	0.41±0.02	0.48±0.02
200	0.39±0.02	0.45±0.02
400	0.40±0.02	0.44±0.02

Values presented as Mean±SEM. n=5. P-value= <0.05

DISCUSSION

The liver is an organ involved in many metabolic functions. One of the primary and main functions of liver is aiding metabolism of digestible substances such as food and food supplements, most medicines and alcohol. The effects of *Laurus nobilis* on liver function and oxidative stress was investigated in this study. This investigation was based on the hypothesis that the extracts may improve hepatic enzyme activities and other measurable functional parameters of liver function and some antioxidant enzyme system and lipid peroxidation. There was no significant ($p<0.05$) change in the activities of superoxide dismutase, catalase, gluthathione reductase and peroxidase as well as malondialdehyde level. These observations were made when the value of enzyme activities obtained in the test groups were compared to control. The changes in superoxide dismutase and catalase enzyme activities implies that extracts of *Laurus nobilis* did not promote toxic stress and the generation of free radicals and reactive oxygen species in the experimental animal models. The dangerous superoxide radicals are converted to hydrogen peroxide by superoxide dismutase while catalase converts hydrogen peroxide to harmless water and oxygen (Omage *et al*., 2011). These radicals are involved in diverse reactions that precipitate oxidative damage to DNA, proteins, and membrane lipids. The glutathione peroxidase-1 enzymatically reduces hydrogen peroxide to water to limit its harmful effects. The non significant changes in these enzyme activities shows that the extract did not precipitate increased cellular stress. The level of malondialdehyde was not significantly ($p<0.05$) altered in the extract treated groups. The malondialdehyde is a by-product of lipid peroxidation. Reactive oxygen species generated spontaneously in cells during metabolism degrade polyunsaturated lipids leading to the production of malondialdehyde; which serves as a biomarker in the measurement of the level of oxidative stress in an organism (Del *et al*., 2005).

The liver contains several enzymes such as Alanine transaminase (ALT), Aspartate transaminase (AST) and alkaline phosphatase (ALP). The enzymes are found in very low concentrations in serum with increased levels occurring in liver injury and disorders. Depending on the severity of damage, liver cells release quantities of enzyme-markers approximate to cellular damage including cytoplasmic alanine and aspartate aminotransferase etc into the blood (Singh *et al*., 2011).

The results from this study indicated that, there was no significant ($P<0.05$) change in the activities of ALT, AST and ALP following administration of 100, 200 and 400 mg/kg bw of ethanolic leaf extract of *Laurus nobilis.* This shows that, the extract did not negatively interfere with hepatic functions nor caused any toxic effect to hepatic cellular metabolism. ALT is an endogenous enzyme belonging to transferases group and useful in diagnosis of liver damage; it contains aminotransferase subgroup which catalyzes the reaction between L-alanine and 2-oxoglutarate. The transfer of an amino group between L-aspartate and 2-oxoglutarate with the formation of oxaloacetate and L-glutamate is catalysed by ALT (Zamin et al., 2002).The aminotransferases are most frequently used as they are indicators of liver injury and considered markers of hepatocellular necrosis. The total bilirubin concentration as well as, the albumin and total protein (TP) concentrations in this study were not altered. These observations showed that extract of *Laurus nobilis* did not affect hepatocytes function nor interfered with bilirubin metabolism, and so, indicated that there were no disturbance of hepatocytes themselves. The non affectation of protein concentration means that the extract did not trigger proteolysis nor cause a reduction in protein synthesis because there were no damages to hepatocytes upon the administration of extract. The non significant effect in the level of albumin which is the main protein of plasma and synthesized only in the liver indicated that its synthetic function was unaffected by the extract. Although, the half-life of albumin is between 7-26 days, therefore it is difficult to conclude from this study that albumin was unaffected since extract administration took place for two weeks.

CONCLUSION

The extracts of *Laurus nobilis* neither promoted lipid peroxidation nor inhibited *invivo* enzymatic antioxidative processes in Wistar rats. The extracts did not impact negatively in hepatic functions of normal rats without induced hepatotoxicity.

REFERENCES

- Aqili Khorasani, M. S. (1992). Collection of drugs (Materia media)–Engelab–e–Eslami Publishing and Educational organization, 624–630,
- American Physiological society. (2002). Guiding principles for research involving animals and human beings. *Am J physiol Regul Integr Comp Physiol*, 283, 281-283.
- Barla, A., Topçu, G., Öksüz, S., Tümen, G., & Kingston, D. G. (2007). Identification of cytotoxic sesquiterpenes from Laurus nobilis L. *Food chemistry*, *104*(4), 1478-1484.
- Baytop, T. (1985). Therapy with Medicinal Plants in Turkey (Past and Present). İstanbul: Nobel Tıp Kitabevleri, 194.
- Chatterjee, M., Sarkar, K., & Sil, P. C. (2006). Herbal (Phyllanthus niruri) protein isolate protects liver from nimesulide induced oxidative stress. *Pathophysiology*, *13*(2), 95-102.
- Condezo-Hoyos, L., Rubio, M., Arribas, S. M., España-Caparrós, G., Rodríguez-Rodríguez, P., Mujica-Pacheco, E., & González, M. C. (2013). A plasma oxidative stress global index in early stages of chronic venous insufficiency. *Journal of vascular surgery*, *57*(1), 205-213.
- Del Rio, D., Stewart, A. J., & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, metabolism and cardiovascular diseases*, *15*(4), 316-328.
- Kupeli, E., Orhan, I., & Yesilada, E. (2007). Evaluation of some plants used in Turkish folk medicine for their anti-inflammatory and antinociceptive activities. *Pharmaceutical biology*, *45*(7), 547-555.
- Gasparyan, G., Tiratsuyan, S., Kazaryan, S., & Vardapetyan, H. (2015). Effect of Laurus nobilis extract on the functioning of liver against CCl4 induced toxicity. *Journal of Experimental Biology and Agricultural Sciences*, *3*(2), 174-183.
- Ghadiri, E., Ahmadi, R., Moridikyia, A., Mahdavi, E., & Tavakoli, P. (2014). Laurus nobilis has Antibacterial Activity against Staphylococcus aureus. In International Conference on Food, *Biological and Medical Sciences*, Jan (pp. 28-29).
- Goldberg, D. M. (1984). Glutathione reductase. *Methods of enzymatic analysis*, 3, 258-265.
- Gülçin, İ. (2006). Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology*, 217(2-3), 213-220.
- Khan, A., Zaman, G., & Anderson, R. A. (2009). Bay leaves improve glucose and lipid profile of people with type 2 diabetes. *Journal of clinical biochemistry and nutrition*, *44*(1), 52-56.
- Kilic, A., Hafizoglu, H., Kollmannsberger, H., & Nitz, S. (2004). Volatile constituents and key odorants in leaves, buds, flowers, and fruits of Laurus nobilis L. *J Agric Food Chem*, 52, 1601-1606.
- Liu, M. H., Otsuka, N., Noyori, K., Shiota, S., Ogawa, W., Kuroda, T., ... & Tsuchiya, T. (2009). Synergistic effect of kaempferol glycosides purified from Laurus nobilis and fluoroquinolones on methicillin-resistant Staphylococcus aureus. *Biological and Pharmaceutical Bulletin*, *32*(3), 489-492.
- Lu, M., Yuan, B., Zeng, M., & Chen, J. (2011). Antioxidant capacity and major phenolic compounds of spices commonly consumed in China. *Food Research International*, *44*(2), 530-536.

- Luigia, L., & Giuseppe V. (2005). Anthocyanins from Bay (Laurus nobilisL.) Berries. *J Agric Food Chem*, 53(20), 8063–8067.
- Marzouki, H., Piras, A., Salah, K. B. H., Medini, H., Pivetta, T., Bouzid, S., ... & Falconieri, D. (2009). Essential oil composition and variability of Laurus nobilis L. growing in Tunisia, comparison and chemometric investigation of different plant organs. *Natural product research*, *23*(4), 343-354.
- Kingsley, O., Georgina, E. O., Esosa, U. S., Sunday, J. J., & Kazeem, A. O. (2011). Evaluation of Hypoglycemic and Antioxidative Properties of Aqueous Extract of Garcinia kola Seeds in Wistar Rats. *Current Research Journal of Biological Sciences*, *3*(4), 326-329.
- Otsuka, N., Liu, M. H., Shiota, S., Ogawa, W., Kuroda, T., Hatano, T., & Tsuchiya, T. (2008). Anti-methicillin resistant Staphylococcus aureus (MRSA) compounds isolated from Laurus nobilis. *Biological and Pharmaceutical Bulletin*, *31*(9), 1794-1797.
- Peskin, A. V., & Winterbourn, C. C. (2017). Assay of superoxide dismutase activity in a plate assay using WST-1. *Free Radical Biology and Medicine*, 103, 188-191.
- Ramling, P., Meera, M., & Priyanka, P. (2012). Phytochemical and Pharmacological Review on Laurus Nobilis. *Int J of Pharm and Chem Sci*, 1(2), 595-602.
- Singh, A., Bhat, T. K., & Sharma, O. P. (2011). Biochemistry of Hepatotoxicity. Clinical Toxicology. *Journal of Clinic Toxicology*, S4, 001. doi:10.4172/2161-0495.S4-001.
- Slaughter, M. R., & O'Brien, P. J. (2000). Fully-automated spectrophotometric method for measurement of antioxidant activity of catalase. *Clinical Biochemistry*, 33(7), 525-534.
- Vardapetyan, H., Tiratsuyan, S., Hovhannisyan, A., Rukhkyan, M., & Hovhannisyan, D. (2013). Phytochemical composition and biological activityof Laurus nobilis L. leaves collected from two regions of South Caucasus, *Journal of Experimental Biology and Agricultural Sciences*, 1, 45-51.
- Verdian-Rizi, M. (2009). Chemical composition and larvicidal activity of the essential oil of Laurus nobilis L. from Iran. *Iranian Journal of Pharmaceutical Sciences*, 5(1), 47-50.
- Vives-Bauza, C., Starkov, A., & Garcia-Arumi, E. (2007). Measurements of the antioxidant enzyme activities of superoxide dismutase, catalase and glutathione peroxidase. *Methods in Cell Biology*, 80, 379-393.
- Wasowick, W., Neve, J., & Peretz, A. (1993). Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. *Clinical Chemistry*, 39(12), 2522-2526.
- Zamin, J. I., de Mattos, A. A., Perin, C., & Ramos, G. Z. (2002). The importance of AST / ALT rate in nonalcoholic steatohepatitis diagnosis. *Arquivos de Gastroenterologia journal*, 39, 22-26. (Article in Portuguese).