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Protective Effect of Hydroethanolic extract of *Datura metel* leaves against Nicotine induced toxicity in albino rats

Gbenga Oluyemi Afolayan[1], Ifedolapo Adesola Adejumo[1]. Kehinde Sola Salako[2], Ayomide Mautin Akwe[1]

[1]Department of Pharmacology, Therapeutics & Toxicology, Faculty of Basic Medical Sciences, University of Lagos, Nigeria.

[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Nigeria.

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* *Corresponding Author:*

iadejumo@unilag.edu.ng
+2348059410281
https://orcid.org/0000-0002-5492-0895

ABSTRACT

Background: *Datura metel* (*D.metel*) is a plant with both poisonous and medicinal properties that have been proven to have great pharmacological potential. It has a wide range of traditional applications and uses in folklore medicine to treat various ailments. Conspicuous marketing of new nicotine-containing formulations has caused an upsurge of nicotine toxicity which is increasingly germane to public health . Fatal overdose of nicotine could occur as a result of suicidal intent or by accident. The current review focuses on the protective effect of hydroethanolic extract of *Datura metel* leaves against nicotine-induced toxicity in albino rats.

Methods: For a period of 7-days, nicotine (1mg/kg/day, i.p) was administered to five groups of rats in addition to hydroethanolic extract of *D.metel* leaves at doses of 250, 500, 1000 mg/kg and vitamin E at 50mg/kg/day, p.o . On the 8th day, the animals were sacrificed, and organs (kidney, heart, liver) were taken for histopathological analysis. The phytochemical analysis and acute toxicity studies were also determined.

Results: Phytochemical analysis revealed the presence of alkaloid, saponin, phenol, flavonoid, anthraquinone and steroid. No observable signs of delayed toxicity and mortality for 14 days post-treatment up to a maximum dose of 8g/kg/p.o. The intraperitoneal LD_{50} value was estimated as 3076.1mg/kg/i.p. Post-treatment with *D. metel* ameliorated the nicotine induced pathomorphological changes .

Conclusion:The dose related attenuation of pathomorphological changes is suggestive of D.metel's protective ability. A basis for further exploration of the potential of *D.metel* as a possible therapeutic agent in human nicotine induced toxicity can be deduced.

1. Introduction

Acute poisoning accounts for a small percentage of cases presented in the emergency units[1] Despite its importance, there is a paucity of information on the *adult* population in *Nigeria*[2]. *Annually, the pattern of acute poisoning in adults is indirectly accountable for about a million illnesses*[3].Poison exposure may occur in multiple settings including occupational, environmental, recreational, or medicinal[4]. The various routes of exposure by which poison is introduced into the body system are oral and non-oral routes: inhalation, insufflation, ingestion, cutaneous, mucous membrane, and injection[5]. The toxic effects range in their intensity from mild, moderate to lethal symptoms.A general approach to acute poison management is administering intravenous fluids to induce diuresis to

eliminate the poison, activated charcoal to bind the poison, antidotes for poison reversal, anxiolytics to calm the patient, antiseizure to abort seizures and a ventilator to assist respiration[6].

Nicotine, a member of the Solanaceae family, is a volatile, naturally occurring alkaloid, derived from tobacco leaves[7]. It is the major toxic, pro- carcinogenic and addictive psychoactive constituent of tobacco[8]. Historically, nicotine-based insecticides caused most cases of nicotine intoxication[9]. However, the rampant use of e-liquid containing nicotine from the electronic form of cigarettes has catalysed a market for highly concentrated nicotine that has reported undesirable events such as seen in accidental ingestion in children[10]. Furthermore, a condition known as green tobacco sickness following cutaneous exposure or undesired ingestion during tobacco harvesting in young, inexperienced tobacco harvesters who do not consume tobacco has also been established[11]. Consequent effects of fatal overdoses of nicotine though rare can potentially be life-threatening[12]. Nicotine poisoning tends to produce symptoms that follow a biphasic physiologic response[13]. At low doses, nicotine causes stimulatory effects (i.e., hyperactivity) on nicotinic-type acetylcholine receptors, and inhibitory effects (i.e., hypokinesis and akinesis) in higher doses or on prolonged exposure to the neuromuscular junction causing neuromuscular blockade[14]. Acute nicotine ingestion exhibits early symptoms such as nausea and vomiting, excessive salivation, abdominal pain, pallor, sweating, hypertension, tachycardia, ataxia, tremor, headache, dizziness, muscle fascinations, and seizures. This is followed by hypotension and bradycardia, progressive muscular weakness and/or paralysis, respiratory failure, central nervous system depression, coma, and death[15,16]. Conversely, basic knowledge of pharmacology using plant-derived therapeutic agents in human and animal studies suggests that early antagonism of nicotine toxicity is associated with a better outcome. The rapid and effective intervention and treatment of nicotine-poisoned patients during hospital admission reduces the number of deaths and improve the prognosis for surviving patients. Atropine is the mainstay of treatment in nicotine-induced toxicity mediated by blockade of muscarine-sensitive receptors though not so effective at the nicotine-sensitive synapses[17]. *The important Datura* species include the following : Downy thorn apple *(D.metel synonym D.fastuosa*), angel's trumpet (*D.innoxia*), moon flower (*D.discolor*), jimson weed (*D.stramonium*) all belonging to the Family Solanaceae[18].

*D.metel i*s one of the folklore medicinal herbs used in traditional Chinese and Indian Ayurveda medicine[19]. Just like other variants of *Datura* plants, it contains tropane alkaloids such as scopolamine, hyoscyamine, and atropine predominantly in its seeds and flowers which is responsible for its broad spectrum of pharmacological properties[20]. Admist the safety concerns , the ethnopharmacologic importance of *D.metel* still remains helpful clinically. A diverse unorthodox clinical application has found it successful as narcotic, analgesic, antihelminthic, cytotoxic, wound healing and antiinflammatory[21]. Herbicidal activity of *D.metel* via allelopathic effect on the noxious weed, *Parthenium hysterophorus L* has been established from literature[22]. However, it is noteworthy that the ingestion of *D. metel* in any form is dangerous and banned in some countries due to the tropane alkaloid content, hence should be treated with extreme caution[23]. Further studies to ascertain the possible use of *D. metel* as a potential therapeutic agent in nicotine intoxication in humans is therefore advocated. This study aimed to evaluate the protective effect of the hydroethanolic extract of *Datura metel* leaves against nicotine-induced toxicity in albino rats.

2.0 Materials and Methods

2.1 Materials

Ethanol (Emsure® ACS, ISO, Reag. Ph Eur), Nicotine (Sigma Aldrich, Germany), Vitamin E (Emzor Pharmaceutical Industries Limited), Light Microscope (Leica DM- 500, Leica Microsystems, Germany), Leica Tissue Processor (Microm STP 125, Thermofisher, USA) Haematoxylin & Eosin (Haematoxylin Stain Sigma Aldrich, Germany), Coverslips and glass slides (eltech microscope frosted slides), Rotary Microtome (Microm HM 325, Thermofisher, USA).

2.1.1 Collection and identification of Datura metel plant

Fresh leaves and stems of *D.metel* were collected from, Yaba, Lagos Nigeria with Latitude 6.5037°N and Longitude 3.3684° E . The botanical identification and authentication of the plant were carried out at the herbarium unit of the Department of Botany, University of Lagos Mr O.O Oyebanji and a voucher specimen was deposited with an allotted number (LUH 6590). The experimental procedures adopted in this study was approved by the Health Research Committee of the College of Medicine , University of Lagos, Nigeria (CMUL/HREC/10/19/558).

2.2 Preparation and extraction of *Datura metel*

Fresh leaves of *D. metel* were thoroughly washed under running water to remove dirt and soil, cut into tiny pieces, and air-dried under shade at room temperature and optimal ventilation. A preliminary method using 3.5g of the powder to determine the most homogenous solvent was done using distilled water/ethanol/hydro-ethanol (70:30) at room temperature and at 100^0 C in a water bath. The powder dissolved best in hydro ethanol (70:30) forming the basis of using it for our experiment. 100g of the fine powdered sample was macerated in 2.5 L of 70% hydro ethanol (70% ethanol, 30% distilled water) with continuous stirring for 72h. The homogenate was then filtered through a muslin cloth followed by Whatman no. 1 filter paper . The filtrate was then transferred to an aerated oven preset at 40°C for 48hrs and completely dried until a deep brown, aromatic solid residue was obtained. The residue, thus obtained, was stored in air and moisture tight container and was freshly reconstituted when needed[24].

2.3 Experimental animals

Fifty -five bred animals (rats; weighing 150- 200g and mice weighing 20-25g; 10-12week old), were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. The animals were properly housed in transparent plastic cages padded with wood shavings under standard conditions (28±2 ºC) temperature, 55±5% relative humidity and 12h light: 12h dark cycle. Animals were maintained on standard rodent pellets (Livestock Feeds PLC, Ikeja, Lagos, Nigeria), and provided with water *ad libitum.* The rats were acclimatized for fourteen days prior to commencement of experimental procedures which were per the provisions of the Experimentation Ethics Committee on animal use of the College of Medicine, University of Lagos, Nigeria and the United States National Academy of Sciences guide for the care and use of laboratory animals[25].

2.4 Acute toxicity testing (LD_{50} determination) of *D. Metel* in mice

Twenty-five albino mice were employed for the oral acute toxicity testing. Five groups of five mice were fasted for 12 h and administered *D. metel* at doses of 500, 1000, 2000, 4000, 8000mg/kg/p.o. The intraperitoneal acute toxicity testing was carried out using fifteen animals of which five mice were administered *D. metel* hydroethanolic extract at doses of 2000, 2500, and 3000 mg/kg/i.p. Behavioural changes, as well as symptoms of toxicity, were observed for the first 2 h and then, 24 hours post-treatment for mortality. The mice were monitored for 14 days for any signs of delayed toxic reactions or mortality. The median lethal dose (LD_{50}) was estimated using the log-dose probit analysis method according to Miller and Tainter[26].

2.5 Phytochemical analysis of the hydroethanolic extract of *D. Metel*

The hydroethanolic extract of *D.metel* was analysed via series of chemical tests to determine the phytochemical compounds it contained according to methods adopted by Adeneye and Adeyemi with slight modifications[27].

2.6 Determination of *D. metel* leaves activity in nicotine-induced toxicity

The assay was carried out with slight modifications according to the prorocol adopted by Das *et al*[28]. Nicotine (1mg/kg/day, i.p) was administered to five groups, A-E of six rats (n=6) for seven days. Hydroethanolic extract of *D. metel* leaves was administered to groups, B,C and D at doses 250, 500, and 1000 mg/kg while vitamin E (50mg/kg/day, p.o) was administered to the group E as a negative control. The groups were as follows:

Group A (Control): Nicotine (1mg/kg body $mass^{-1}day^{-1}$, i.p.)

Group B: Nicotine/ *Datura metel*: Nicotine (1mg/kg, i.p.) + *Datura metel* (250mg/kg body $mass^{-1}day^{-1}$, p.o)

Group C: Nicotine/ *Datura metel*: Nicotine (1mg/kg) + *Datura metel* (500mg/kg body $mass^{-1}day^{-1}$, p.o)

Group D: Nicotine/ *Datura metel*: Nicotine (1mg/kg) + *Datura metel* (1000mg/kg body $mass^{-1}day^{-1}$, p.o)

Group E: (Control) Nicotine/ Datura metel: Nicotine (1mg/kg) + Vitamin E (50mg/kg body $mass^{-1}day^{-1}$, p.o).

The rats had regular daily food and water intake, and their general well-being was observed. On the 8^{th} day, the animals were humanely sacrificed, and organs (kidney, liver, heart) were perfused with cold normal saline before being harvested and stored in formal saline for histopathological analysis.

2.7 Statistical Analysis

Data are expressed as the mean ± standard error of the mean (SEM). Using Graph-Pad Prism 6 (Graph-Pad Software Inc., California, United States of America), a probit vs log dose graph was plotted and the LD_{50} was calculated using linear regression analysis. Statistical significance was considered at $p < 0.05$.

3. Results

3.1 LD_{50} determination of *D. metel*

Result of acute toxicity testing of the hydroethanolic extract of *D. metel* treated mice at a maximum dose of 8000 mg/kg, p.o. exhibited the following behaviours in the initial 2-hrs post-treatment observation period: calmness, sedation and reduced locomotion. However, this did not result in death or observable delayed toxic symptoms in the experimental group up until 14days post-treatment. However, the intraperitoneal administration as shown in Figure 1 estimated the LD_{50} to be the antilog of 3.488 (3076.1mg/kg/i.p).

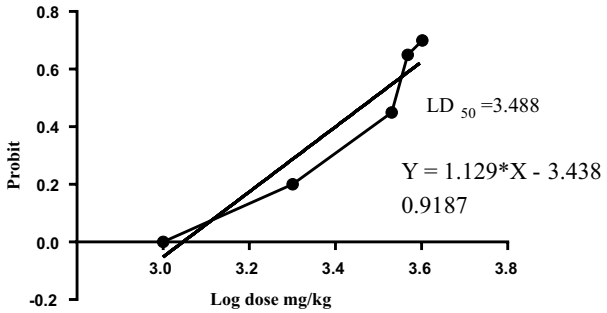

Figure 1: Estimation of LD_{50} of intraperitoneally administered hydroethanolic extract of *Datura metel* leaves

3.2 Phytochemical analysis of hydroethanolic leaf extract of *D. metel*

Qualitative and Quantitative phytochemical analysis of the hydroethanolic leaf extract of *D. metel* revealed the presence of alkaloid (20.445), saponin (8.205), phenol (39.81), flavonoid (9.855).

3.3 Effect of *D. metel* leaves in nicotine-induced toxicity on the kidney, liver and heart

The histopathological analysis of the kidney showed the protective role of *D. metel* extract in a dose-dependent manner. The effect shown by the doses of 500 and 1000 mg/kg are comparable to the effect shown by the Vitamin E which served as the positive control (Figure 3). The histopathological analysis of the liver showed the protective role of *D. metel* extract. The protective effect was only conspicuous at the dose of 500 mg/kg (Figure 4). The histopathological analysis of the heart showed the protective role of *D. metel* extract. The protective effect was only conspicuous at the doses of 500 and 1000 mg/kg (Figure 5).

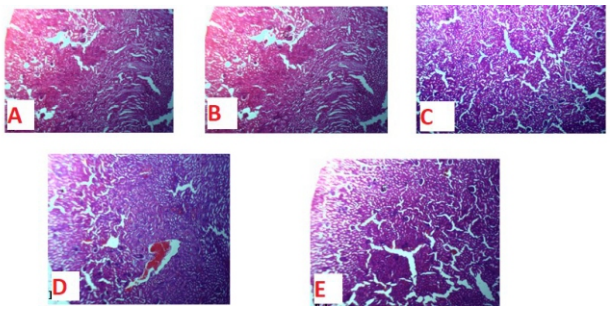

Figure 2: The histolopathological effect of the hydroethanolic extract of *D-metel* on the kidney of rats

(A) Nicotine Kidney with an increased width of space around the glomeruli (B) Nicotine Kidney + Ext.250 mg/kg with a reduced width perivascular space around the glomerulus and the taft of the glomerulus not as thick as A (C)) Nicotine Kidney+ Ext. 500 mg/kg with normal appearing glomeruli G and tubules T (D) Nicotine Kidney+ Ext. 1000 mg/kg(showing normal appearing glomeruli, G and tubules, T (E) Nicotine Kidney+ Vit.E at 50 mg/Kg Showing normal appearing glomeruli G

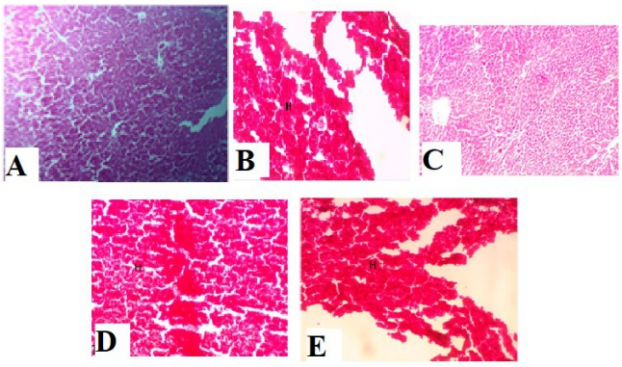

Figure 3: The histolopathological effect of the hydroethanolic extract of *D. metel* on the liver of rats

(A) Nicotine Liver with distension of central vein (B) Nicotine Liver + Ext. 250 mg/kg with necrotic hepatocytes (C) Nicotine Liver+ Ext. 500 mg/kg with well preserved architecture and hepatocyte (D) Nicotine Liver + Ext. 1000 mg/kg with necrotic hepatocyte (E) Nicotine Liver+ Vit.E. 50 mg/kg showing a normal liver

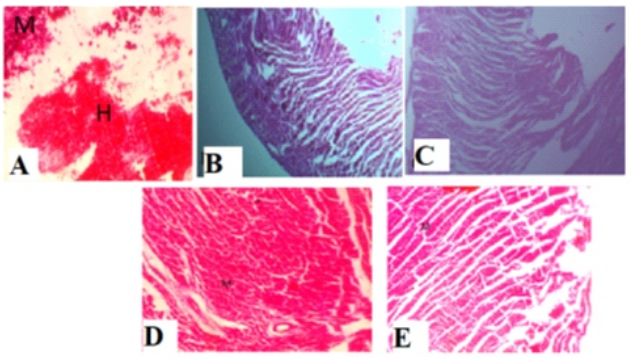

Fig 4 showing the histolopathological effect of the hydroethanolic extract of *D-metel* on the heart of rats (A) Nicotine heart with a necrotic myocardial muscle, M and extensive hemorrhage, H (B) Nicotine Heart+ Ext. 250 mg/kg with distortion of cyto- architecture of the cardiac muscle (C) Nicotine Heart+ Ext. 500 mg/kg with a well preserved myocardial muscle (D) Nicotine Heart+ Ext. 1000 mg/kg showing normal myocardial muscle, M (E) Nicotine Heart + Vit.E 50 mg/kg with well preserved myocardial muscle

4. Discussion

Nicotine, a psychoactive component of tobacco, maintains smoker's addiction and is responsible for the multiple toxidromes that underlie nicotine toxicity[29]**.** It activates and desensitizes nicotinic cholinergic receptors which demonstrate a bewildering heterogeneity in structure, function and distribution within the nervous system mediating the complex symptoms observed in nicotine poisoning[30]. About four-fifth of the world population (4bllion people) use traditional medicine at the level of primary health care according to The World Health Organization (WHO)[31] . Ethnopharmacology has revealed various sources of many pharmaceutical drugs to evolve from secondary metabolites derived from plants.

Members of the *Datura* species including *Datura metel*, contain a copious quantity of belladonna alkaloids such as scopolamine and atropine; that can produce both local and systemic anticholinergic toxicity[32]. The observed discrepancy in the toxicological dose-response effect of the different species may be due to the varying levels of toxic metabolites contained in them.This study focuses on the protective effectiveness with post-treatment of hydroethanolic extract of *Datura metel* leaves to increase survival following nicotine toxicity in rats. In this study, the acute toxicity of hydroethanolic extract of *Datura metel* leaves was evaluated by intraperitoneal administration with an LD_{50} estimated to be 3076.1 mg/kg/i.p. Additionally, the extract showed no mortality when administered orally up to a maximum dose of 8000 mg/kg/po. This shows that *D. metel* is relatively safe upon oral ingestion and this is in tandem with the results obtained from Ganesh *et al* where *Datura fastuosa* was found to be safe up to dosage of 2000 mg/kg body weight with no symptoms of toxicity or mortality[33].

The phytochemical constituents in our study revealed the presence of alkaloid, saponin, phenol, flavonoid, anthraquinone and steroid. These findings are similar to a study by Akharaiyi *et al*[34] that demonstrated the presence of the aforementioned in the phytochemical analysis of *Datura metel* leaf aqueous and ethanol extract.Conversely, this was similar to the preliminary phytochemical investigation was performed on methanolic and hydroalcoholic extract of *Datura fastuosa* (syn: *Datura metel*) revealed the presence of alkaloids, tannins, cardiac glycosides, flavonoids, carbohydrates, amino acids and phenolic compounds[35]. The study showed the histopathological findings of the nicotine treated rats revealed significant pathomorphological alterations in the kidney such as widening of space around the glomeruli and thickening of the glomeruli tuft and adhesion to bowman's capsule. Administration of the *D. metel* extract at a dose of 500mg/kg effectively reduced pathological changes induced by nicotine damage showing normal appearing glomeruli and tubule.

This contrasts with a study by Imo *et al*[36] where the results showed that some parts of *Datura metel* (leaf, seed and fruits) posed mild toxic effect, while other parts exhibited nephroprotection by regulating the kidney function of male albino rats. Studies have demonstrated that blockage of central and peripheral cholinergic neurotransmission by the parts of the *Datura* species have severe anticholinergic fatal adverse effects[37]. From our study, the liver of the nicotine - treated rats showed a distention of the central vein and distortion of the general cytoarchitecture of the liver. The *D. metel* treated rats at 500mg/kg showed a well-preserved architecture and hepatocyte. This differs from a study by Sanni *et al*[38] which demonstrated the histological analysis revealed the presence of intracellular accumulations (glycogen or lipids) in the liver suggesting that the palm wine extract of *Datura stramonium* had the potential to cause liver damage.

Similarly, toxicological assessment of the pretreatment of rats with extract of *Datura fastuosa* seed through histological evaluation demonstrated a decrease in the necrotic changes in the liver architecture[39]. The histological analysis of the heart of nicotine-treated rats exhibited a

necrotic myocardial muscle with extensive haemorrhage was seen. The *D. metel* treated rats at 500mg/kg showed a well-preserved myocardial muscle. This was not in tandem with the vacuolization and degenerative changes of cardiac myofibers observed in a study by Verma *et al*[40] on the pathomorphological effect of *Datura stramonium* seeds extract in rats. The pathomorphological changes were either reversed or blocked in the kidney, liver, and heart by the *D. metel* extract dose at 500 mg/kg and at a milder effect at 1000 mg/kg (except in liver). The protective effect of *D. metel* seen on the studied organs could be attributed to the phytochemicals present in the plant extract. A study by Wink, demonstrated that alkaloids act as antagonists (and agonist) to a variety of neurotransmitter systems via, direct binding to neuroreceptors and impinging on the neurotransmitter metabolism (e.g., cholinesterase inhibition)[41]. Nicotine toxicity whose effect is elicited by binding to acetylcholine brain receptors, resulting in smoking's rewarding and reinforcement effects is antagonised by alkaloid-containing plant extracts such as *D. metel*[42]. This suggests that plants with alkaloid secondary metabolites could be used in further research as psychotropic medicines, or social drugs studies either as simple tools for the modification of specific neurotransmitter targets or in models of drug abuse and addiction[43]. Varying investigations employing animal models have established that berry extracts with high levels of anthocyanins or other polyphenols can reverse brain insult[44]. Phenols and flavonoids are potent antioxidant and anti-inflammatory compounds[45] which help to scavenge free radicals and could also serve as cytoprotectants in nicotine-induced production of free radicals and reactive oxygen species that caused damaged cell and tissue structure[46]. The cytoprotective effect demonstrated by our study may be due to the presence of these bioactive agents.

Limitations of the study was that quantitative analysis of the effect of nicotine on the organs studied was not done due to absence of appropriate equipment. Also, the extrapolation to humans was not done in this experiment. Policy limitations were due to the fact that the plant is only used as a folklore medicinal herb in traditional Chinese and Indian Ayurveda medicine for now, probably due to the extreme care the practitioners must possess as a result of the fatal adverse effect in improper use.

5. Conclusion

The phytochemistry, acute toxicity and histopathology analysis of *Datura metel* documented in this current study suggests a protective role against nicotine induced toxicity in rats. In view of its multiple uses, more activity screening and structural relationship studies are yet to be explored further. However, information presented in this review could be helpful in promoting research aimed at the search and development of new agents for medical application using plant derived products .

References

1. Jepsen, F., & Ryan, M. (2005). Poisoning in children. Current Paediatrics, *15*(7), 563-568. https://doi.org/10.1016/j.cupe.2005.08.006
2. Malangu, N. (2011). Doctoral dissertation, University of Limpopo (Medunsa Campus) Acute Poisoning in Three African Countries: Botswana, South Africa and Uganda http://hdl.handle.net/10386/674.
3. Lentini, P., Zanoli, L., Granata, A., Signorelli, S. S., Castellino, P., & Dell'Aquila, R. (2017). Kidney and heavy metals-The role of environmental exposure. Molecular medicine reports, 15(5), 3413-3419. https://doi.org/10.3892/mmr.2017.6389
4. El-Maddah, E. I. (2012). Pattern of Acute Poisoning in Adult Patients Admitted to Tanta Poison Centre-Egypt. The Egyptian Journal of Forensic Sciences and Applied Toxicology, 220(1245), 1-15.https://doi.org/10.12816/0005060
5. Aki, E. S., & Alessai, G. S. (2019). General approach to poisoned patient. Poisoning in the Modern World-New Tricks for an Old Dog. http:dx.doi.org/10.5772/intechopen.84681
6. Gooch, M. D. (2017). Toxicologic Emergencies. Patient Transport-E-Book: Principles and Practice, 415. https://nursing.vanderbilt.edu/people/bio/michael-gooch
7. Idrees, M. I. T. (2016). Doctoral dissertation, University of Gezira. Determination of Nicotine in Different Brands of Cigarettes Using High–Performance Liquid Chromatography. http://repo.uofg.edu.sd/handle/123456789/2522
8. Hoffmann, D., Hoffmann, I., & El-Bayoumy, K. (2001). The less harmful cigarette: a controversial issue. A tribute to Ernst L. Wynder. Chemical research in toxicology, 14(7), 767-790. https://doi.org/10.1021/tx000260u
9. Alkam, T., & Nabeshima, T. (2019). Molecular mechanisms for nicotine iintoxication. Neurochemistry International, 125, 117-126. https://doi.org/10.1016/j.neuint.2019.02.006

10. Kyriakos, C. N., Filippidis, F. T., Hitchman, S., Girvalaki, C., Tzavara, C., Demjén, T., ... & Vardavas, C. I. (2018). Characteristics and correlates of electronic cigarette product attributes and undesirable events during e-cigarette use in six countries of the EUREST-PLUS ITC Europe Surveys. Tobacco Induced Diseases, 16. https://doi: 10.18332/tid/93545
11. Schep, L. J., Slaughter, R. J., & Beasley, D. M. G. (2009). Nicotinic plant poisoning. Clinical Toxicology, 47(8), 771-781. https://doi.org/10.1080/15563650903252186
12. Pesta, D. H., Angadi, S. S., Burtscher, M., & Roberts, C. K. (2013). The effects of caffeine, nicotine, ethanol, and tetrahydrocannabinol on exercise performance. Nutrition & metabolism, 10(1), 1-15. https://doi.org/10.1186/1743-7075-10-71
13. Abreu-Villaça, Y., Seidler, F. J., Qiao, D., Tate, C. A., Cousins, M. M., Thillai, I., & Slotkin, T. A. (2003). Short-term adolescent nicotine exposure has immediate and persistent effects on cholinergic systems: critical periods, patterns of exposure, dose thresholds. Neuropsychopharmacology, 28(11), 1935-1949. https://doi.org/10.1038/sj.npp.1300221
14. Appiah-Ankam, J., & Hunter, J. M. (2004). Pharmacology of neuromuscular blocking drugs. Continuing Education in Anaesthesia Critical Care & Pain, 4(1), 2-7. https://doi.org/10.1093/bjaceaccp/mkh002
15. Smolinske, S. C., Spoerke, D. G., Spiller, S. K., Wruk, K. M., Kulig, K., & Rumackt, B. H. (1988). Cigarette and nicotine chewing gum toxicity in children. Human toxicology, 7(1), 27-31. https://doi/abs/10.1177/096032718800700105
16. Tafuri, J., & Roberts, J. (1987). Organophosphate poisoning. Annals of emergency medicine, 16(2), 193-202. https://doi.org/10.1016/S0196-0644(87)80015-X
17. Eyer P. (2003). The role of oximes in the management of organophosphorus pesticide poisoning. Toxicological reviews, 22(3), 165–190. https://doi.org/10.2165/00139709-200322030-00004
18. Passos ID, Mironidou-Tzouveleki M. (2016) Hallucinogenic plants in the Mediterranean countries. Neuropathology of drug addictions and substance in misuse. Academic Press. (pp. 761-772) https://doi.org/10.1016/B978-0-12-800212-4.00071-6
19. Bhat, S. D., Ashok, B. K., & Acharya, R. (2012). Exploring the concept of vacha (acorus calamus linn.) Shodhana in Ayurveda. International Journal of Research in Ayurveda & Pharmacy, 3(3). https://www.researchgate.net/publication/289785544
20. Freye, E. (2009). Toxicity of Datura stramonium. In Pharmacology and Abuse of Cocaine, Amphetamines, Ecstasy and Related Designer Drugs). Springer, Dordrecht. (pp. 217-218). https://doi:10.1007/978-90-481-2448-0_34
21. Al-Snafi AE. Medical importance of Datura fastuosa (syn: Datura metel) and Datura stramonium-A review. IOSR Journal of Pharmacy. 2017;7(2):43-58. http://www.iosrphr.org/
22. Ramachandran A, Venkataraman NS. (2016). Allelopathic effects of aqueous leaf extracts of Datura metel L. on Parthenium hysterophorus L. Lifesciences Leaflets.; 72:14-22. http.//api.ning.com/files/UjLFZWxAkgo
23. Michelson (2008). "Datura ingestion: a review of literature and case reports". Journal of Pharmacology and Toxicological Studies. **25** (7): 198–209. http://www.efloras.org/
24. Satyajit Sarker DD, Gray AI. (2010). Humana Press Inc; 2006 National Research Council. Guide for the care and use of laboratory animals. Natural Products Isolation 2nd ed. UK. https://doi.org/10.1385/1-59259-955-9:1
25. National Research Council. (2010). Guide for the care and use of laboratory animals.Guide for the care and use of laboratory animals.8th edition.National Academic Press. https://www.nap.edu
26. Miller LC, Tainter ML (1944). Estimation of LD50 and its error by means of log-probit graph paper. Proc Soc Exp Biol Med. 57:261
27. Adeneye, A. A., & Adeyemi, O. O. (2008). Hypoglycaemic effects of the aqueous seed extract of Hunteria umbellata in normoglycemic and glucose and nicotine induced hyperglycaemic rats. Planta Medica, 74(09), PA245. http://www.healthy-synergies.com
28. Das, S., Gautam, N., Dey, S. K., Maiti, T., & Roy, S. (2009). Oxidative stress in the brain of nicotine-induced toxicity: protective role of Andrographis paniculata Nees and vitamin E. Applied Physiology, Nutrition, and Metabolism, 34(2), 124-135. https://doi.org/10.1139/H08-147
29. Siqueira, L. M., Ryan, S. A., Gonzalez, P. K., Patrick, S. W., Quigley, J., & Walker, L. R. (2017). Nicotine and tobacco as substances of abuse in children and adolescents. Pediatrics, 139(1).

https://doi.org/10.1542/peds.2016-3436

30. Mazurov, A., Hauser, T., & Miller, C. H. (2006). Selective α7 nicotinic acetylcholine receptor ligands. Current medicinal chemistry, 13(13), 1567-1584. https://doi.org/10.2174/092986706777442011

31. Davidson-Hunt, I. (2000). Ecological ethnobotany: stumbling toward new practices and paradigms. MASA J, 16(1), 1-3. http://www.bionica.info/biblioteca/Davidson-Hunt2000Etnobotanica.pdf

32. Krenzelok, E. P. (2010). Aspects of Datura poisoning and treatment. Clinical Toxicology, 48(2), 104-110. https://doi.org/10.3109/15563651003630672

33. Ganesh S, Radha R and Jayshree N.(2015). A review on phytochemical and pharmacological status of Datura fastuosa linn. International Journal of Multidisciplinary Research and Development; 2(4): 602-605. http://www.allsubjectjournal.com/archives/2015/vol2/issue4/45

34. Akharaiyi FC (2011). Antibacterial, Phytochemical and Antioxidant activities of Datura metel. Int. J. PharmTech. Res. 3(1):478-483. https://doi.org/10.5897/AJMR2014.6805

35. Ratan L , Meenu B, Anju D and Arun N. (2011). Morpo- anatomical and physicochemical studies of dried seeds of Datura fastuosa Linn. . IRJP ; 2 (3): 208-212. https://doi: 10.9790/3013-0702014358

36. Imo, C., Arowora, K. A., Ezeonu, C. S., Yakubu, O. E., Nwokwu, C. D., Azubuike, N. C., & Sallah, Y. G. (2019). Effects of ethanolic extracts of leaf, seed and fruit of Datura metel L. on kidney function of male albino rats. Journal of Traditional and Complementary Medicine, 9(4), 271-27. https://doi.org/10.1016/j.jtcme.2017.09.001

37. Barile, F. A. (2019). Anticholinergic and Neuroleptic Drugs. In Barile's Clinical Toxicology (pp. 251-269). CRC Press. https://www.taylorfrancis.com/chapters/edit/10.1201/9780429154829-17/anticholinergic-neuroleptic-dr

38. Sanni, M., Ejembi, D. O., Abbah, O. C., Olajide, J. E., Ayeni, G., Usman, S. S., et al. (2014). Phytochemical screening and studies on the effects of oral administration of palm wine extract of datura stramonium on the activity of tissue enzymes and histopathology of albino rats.World Journal of Pharmaceutical research. Volume 3, Issue 6, 137-172 https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1409891857.pdf

39. Bauzidi A, Mahdeb N and Kara N.(2011). Toxicity studies of alkaloids of seeds of Datura stramonium and synthesis alkaloids in male rats. J Med Plants Res; 5(15): 342-343. http://www.academicjournals.org/JMPR

40. Verma, S. K., Srivastava, A. K., & Gangwar, N. K. (2012). Pathomorphological changes induced by Datura stramonium seed toxicity in rats. Indian Journal of Veterinary pathology.Volume 36;Issue 2. https://agris.fao.org/agris-search/search.do?recordID=IN2015000104

41. Wink, M. (2012). Medicinal plants: a source of anti-parasitic secondary metabolites. Molecules, 17(11), 12771-12791. https://doi.org/10.3390/molecules171112771

42. Shepard, G. (2005). Psychoactive botanicals in ritual, religion and shamanism. Ethnopharmacology: Encyclopedia of life support systems (EOLSS), 2, 128-182. http://www.eolss.net/

43. Kennedy, D. O., & Wightman, E. L. (2011). Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Adv Nutr .2 (1): 32–50. https://doi.org/10.3945/an.110.000117

44. Willis, L. M., Shukitt-Hale, B., & Joseph, J. A. (2009). Recent advances in berry supplementation and age-related cognitive decline. Current Opinion in Clinical Nutrition & Metabolic Care, 12(1), 91-94. https://doi: 10.1097/MCO.0b013e32831b9c6e

45. Zhang, L., Ravipati, A. S., Koyyalamudi, S. R., Jeong, S. C., Reddy, N., Smith, P. T., .et al (2011). Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. Journal of agricultural and food chemistry, 59(23), 12361-12367. https://doi.org/10.1021/jf203146e

46. Rao, P. S., Kalva, S., Yerramilli, A., & Mamidi, S. (2011). Free radicals and tissue damage: Role of antioxidants. Free radicals and antioxidants, 1(4), 2-7. https://doi.org/10.5530/ax.2011.4.2