ORIGINAL ARTICLE

Hepatoprotective effects of Common Sage (Salvia officinalis) leaves on Cyanide-Induced Hepatotoxicity in Male Wistar Rats

Azukaego Thomas Hughs Mokogwu[1], Emeka Edward Okocha[2], Kingsley Chukwuka Amaihunwa[1], Enekabokom Nwoke Ekene[3], Benson O Eyenubo[4], Osinachi Mark Nwachukwu[1], Ikemefune Ochonogor[1], Godwin O Avwioro[1]

ABSTRACT

Common sage *(Salvia officinalis)* leaf is used worldwide as a medicinal plant for various ailments. Its hepatoprotective effect was investigated on cyanide-induced hepatotoxicity in rats. In this experimental study, thirty male Wistar rats, divided into five groups having six rats in each group (A, B, C, D and E). Group A which was the control, received 0.4ml/day corn oil, while group B had 0.4ml of potassium cyanide (7mg/kg/day) and group C received 0.4ml of sage plant leaf extract (500mg/kg/day) in saline. Group D received 0.4 ml sage plant leaf extract (500mg/kg/day) plus 0.4 ml potassium cyanide (7mg/kg/day) and group E received 0.4ml of sodium thiosulphate ($Na_2S_2O_3$) (600 mg/kg/day) plus 0.4ml of potassium cyanide (7mg/kg/day). Treatments were by oral route, daily, and weighed at intervals of 2 days for two weeks. Serum aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase) (ALP) activity and total proteins (TP) and total bilirubin (TB) were measured as liver function indicators. While liver malondialdehyde (MDA) and Superoxide Dismutase (SOD), glutathione-S-transferase (GST), reduced glutathione (GSH) and catalase were assessed as indices of oxidative stress. Standard methods were utilized for all the measurement. The increased activities of the liver function enzymes by cyanide-induction were restored by co-administration of either the sage *(Salvia officinalis)* leaf or sodium thiosulphate ($Na_2S_2O_3$). The MDA level which was increased with reduced activities of GSH, GST, SOD and catalase on treatment with cyanide only, were all reversed on co-treatment of cyanide with sage leaf extract or chemical drug (sodium thiosulphate). The results revealed that common sage leaf protects the liver against cyanide-induced oxidative stress.

Keywords: Sage leaf, cyanide, hepatotoxicity, oxidative stress, sodium thiosulphate

International Journal of Human and Health Sciences Vol. 10 No. 04 October'26

DOI:https://doi.org/10.31344/ijhhs.v10i4.991

INTRODUCTION

Cyanide poisoning is a world health burden that affects both humans and animals.[1] Its chemicals or compounds associated with latent reactive cyanide anions (CN^-) commonly exist as potassium, sodium, hydrogen as well as cyanogenic glycosides encountered in certain plants.[2,3] Cyanides are frequently found in the environment, and are known for their rapid and potential hazardous effects on living organisms, most particularly for inhibition of cellular respiration that hinders the production of adenosine triphosphate (ATP) that is essential for cellular energy. This leads to an increase in the generation of oxygen-free radicals that often results to considerable decrease in the activity of superoxide dismutase, catalase as well as antioxidant vitamins, in animal lenses and

1. Department of Medical Laboratory Science, Faculty of Allied Science, Delta State University, Abraka, Nigeria.
2. Prince Abdul Mohsen Hospital, Al-Ula, Saudi Arabia.
3. Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Orowukwo, Port-Harcourt, Nigeria.
4. Department of Science Laboratory Technology, Faculty of Science, Delta State University, Abraka. Nigeria.

Correspondence to: Azukaego Thomas Hughs Mokogwu, Department of Medical Laboratory Science, Faculty of Allied Science, Delta State University, Abraka, Nigeria. Email: tmokogwu@yahoo.com

retinas. Thus, its accumulation affects various body organ dysfunction, with resultant dizziness, seizures, unconsciousness and arrhythmia and often death.[4-6] Various researchers have shown that in humans, cyanide poisoning occurs from accidental/intentional exposure from industry; in mining, electroplating and manufacturing, combustion of plastics and tobacco as well as consumption of contaminated cyanogenic compounds like cassava. with consequent risk of chronic cyanide toxicity that may lead to neurological disorders.[7-9] Animals are also found to be highly vulnerable to cyanide hepatotoxicity particularly via ingestion of cyanogenic plants often during grassing.[10-12]

Several works have shown that the key detoxifying enzyme rhodanese detoxifies cyanide in the liver with the formation of thiocyanate (SCN) that is excreted into the urine, at a lower intrinsic toxicological risk. Also, sodium thiosulfate is a pharmacologic agent in the treatment of/ management of cyanide poisoning particularly as it provides sulphur for the cyanide detoxification enzyme rhodanese.[13-15] Ola-Mudathir & Jeje opined that the prevalent flavonoid in onions is querectin and demonstrated and showed that querectin, protects the liver against cyanide-induced oxidative stress.[15] Zhang et al. stated that medicinal values of plants lie in the chemical compounds that produce definitive physiological actions in humans, concluding that their role in modern medicine is indispensable.[18] A good example of such is *Salvia officinalis* (Common Sage) plant whose leaves extract have been utilized in various therapeutic uses. Studies have shown that this plant which belongs to the Lamiaceae family with its unique aromatics odor is commonly used as flavor specie and herbal medication.[19] Equally, several studies have shown that Salvia *officinalis* leaf has biologic potential that include anti-proliferative, anti-hypertensive, anti-hyperglycemic, antimicrobial, and anti-inflammatory, can protect the liver in parasitaemic hepatic injury, etc.[20-24] Other works have also shown that Salvia *officinalis* leaf contains high concentration of flavonoid with antioxidant potential,[20,25] and currently has very high flavonoid content in mg querectin equivalent per gram extract (mgQE/g extract) as well as very potent antioxidant and anti-inflammatory activity.[26] Therefore, search for the inexpensive management of hepatotoxicity with little and no side effect, has led to this study- to investigate the hepato-protective and antioxidant activity of common sage *(Salvia officinalis)* leaf on cyanide-induced hepatotoxicity in male Wistar rats.

METHODS

Authentication and preparation of plant extract: Sage plant from Vom-Jos, Plateau State was identified, authenticated and a voucher number (FS/20/21/00121) was assigned to it and stored for future reference. The plant has currently been propagated and thrives well at our garden at Asaba, Delta State (fig. 1). The leaves were cut into small pieces, dried at a temperature of 30 -40^{o}C for 21 days. The dry leaves were pulverized to obtain a fine powder. 50g was weighed and added to 400 ml absolute methanol in a flask. It was shaken at regular intervals for 3 days at room temperature. The mixture was filtered using muslin cloth and concentrated using Soxhlet apparatus. The extract obtained was kept in an airtight sample bottle, labelled and stored in the refrigerator.

Preparation of the extract solution: Weighed quantity of methanol extract of the Sage *officinalis* leaf (500mg/kg) was suspended in distilled water.

Animal experimentation: Thirty apparently healthy male Wister rats weighing between 150-250g were used in the study. The animals were properly housed, fed with standard growers' marsh and water *ad libidum*.

Acute oral toxicity study: This was performed using OECD-423 guideline for animal study.[27] Female albino mice weighing 20-24g, divided into two groups of six animals each were used. One group was given 2,000mg/kg body weight of the Sage leaves extract while the second group received 5,000mg/mg body weight of the sage leaf extract orally. They were observed for symptoms of toxicity and mortality for 72 hours.

Experimental design: Thirty Wistar rats were randomly divided into five groups of six rats each (A, B, C D and E) and had oral administration of the plant extracts. Group A served as control and received 0.4ml/day corn oil. Group B was given 0.4ml of potassium cyanide (7mg/kg body weight). Group C was given orally 0.4ml of sage plant leaf extract (500mg/kg/day), which was dissolved in normal saline. Group D was treated with 0.4 ml sage plant leaf extract (500mg/kg/

day) and 0.4 ml potassium cyanide (7mg/kg body weight/day). Group E was given

0.4ml of $Na_2S_2O_3$ (600 mg/kg b.wt/day) plus 0.4ml of potassium cyanide (7mg/kg body weight/day). Groups D and E were pre-administered with 0.4ml of sage plant leaf extract 500mg/kg body weight/day and 0.4ml of sodium thiosulphate ($Na_2S_2O_3$) (600 mg/kg body weight/day) respectively for 2 weeks. The animals were observed daily and weighed at intervals of 2 days for two weeks. At the end of the protocol period of two weeks, blood samples were taken from the retro-orbital plexus of each animal using plain capillary tube into 5ml blood container. The blood was allowed to cloth and centrifuged at 3,000 rpm for 5 minutes for separation of serum. The separated serum was then labelled and stored at -8ºC pending biochemical analysis.

Biochemical parameters: Serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase) (ALP), gamma-glutamyl transferase (GGT), total proteins (TP) and total bilirubin (TB) were measured using Mindray assay kits (Shenzhen China) using an AS-120 Auto-Analyzer (E. LabBST, China: BA-88A). The animals were sacrificed and then cervical decapitation was conducted before laparotomy section. The livers, kidneys and hearts were weighed. The livers only from the animals, per group, were then homogenized in phosphate buffer (p^H 7.4) and centrifuged in a cold centrifuge (4^0C) at 10,000g for 10 minutes to obtain the post-mitochondrial fraction for evaluation of oxidative stress and antioxidant enzymes per each of the animals. While oxidative stress parameters were assessed using established methods.

Determination of oxidative stress (lipid peroxidation assessment): Thiobarbituric acid reactive compounds (TBARS) which were generated during lipid peroxidation were measured to assess lipid peroxidation.

Determination of catalase activity: Catalase activity was measured. The principle was based on the fact that when dichromate in acetic acid is heated in the presence of H_2O_2, it is reduced to chromic acetate, with per chromic acid as an unstable intermediate. The chromate acetate generated is read calorimetrically at 580 nm. The presence of dichromate in the test mixture does not affect the measurement.[34]

Determination of superoxide dismutase (SOD) activity: The activity was done. It was based on the capacity of superoxide dismutase to limit the auto-oxidation of adrenaline at p^H 10.2. The oxidation of adrenaline to adreno-chrome is known to be caused by superoxide anion (O_2) produced by the xanthine oxidase process. With rising pH and likewise with increasing adrenaline content, the quantity of adreno-chrome, synthesized per superoxide anion increased. Thus, the hypothesis that adrenaline auto-oxidation occurs via at least two mechanisms, one of which is a free radical chain reaction involving superoxide radicals that might be blocked by SOD.[35]

GST: The activity of glutathione-S-transferase in serum was estimated; with 1-chloro-2,4-dinitrobenzene as the second substrate, glutathione-S-transferase activity is increased and it was used as the substrate. This upon conjugation with reduced glutathione, increased the absorption wavelength to a longer wavelength. The increase in absorption at 340 nm wavelength allows for direct enzymatic reaction measurement.[36]

GSH: The reduced form of glutathione contains the majority of cellular non-protein sulfhydryl groups. This principle is based on the production of a rather stable yellow color, when sulfhydryl substances were treated with 5, 5 – dithiobis – (2-nitrobenzoic acid) (Ellman's reagent). Ellman's reagent reacts with reduced glutathione to produce 2-nitro-5-thiobenzoic acid, a chromophoric compound having a molar absorbance of 412 nm. The absorbance at 412 nm is proportional to the reduced GSH concentration.[37]

***Determination of tissue protein level*:** The principle was based on the –CO-NH-bond in polypeptide chain reaction with copper sulphate in an alkaline medium to give a blue colored complex. The tyrosine and trptophan residues of protein cause reduction of the phosphomolybdate and phosphotungstate components of the Folin-Ciocalteau reagent to give bluish products which contribute towards enhancing the sensitivity of the method.[38]

Statistical Analysis: Data was expressed as Mean±SEM. One-way ANOVA was used to compare the findings, with Turkey's posthoc test used to compare the means of the different treatment groups. The level of significance was taken at $p<0.05$. Graph Pad Prism Version 5.0 for Windows was used for the data analysis. (Graph Pad® Software, San Diego, CA, USA).

RESULTS

Acute toxicity: The acute toxicity test revealed that Sage plant leaf powder extract at 2,000 and 5,000 mg/kg body weight was non-toxic to the mice. The weight of rats for the cyanide-induced hepatotoxicity were not significantly different ($p>0.05$) from both the control and other treated groups (Table 1).

Biochemical parameters: Table 2 shows the results of the biochemical parameters analyzed in the various groups of the Wistar rats. From the results, all animals treated with cyanide only raised the activity of aspartate, alanine transaminases as well as gamma glutamyltranferase and alkaline phosphate at a highly significant ($p<0.001$) level when compared with the control as well as treatment with the extract of salvia officinalis leaves only. However, there were significant reductions in both the activities of these enzymes as well as the levels in TB and increase in TP, in the animals treated with either the Salvia officinalis leaf extract or the $Na_2S_2O_3$ co-administration of the cyanide when also compared with treatment with cyanide only at a significant level of $p < 0.05$. There was reduction in the level of total proteins in rats treated with cyanide only when compared with both the control as well as in other groups at a significant level of $p<0.05$.

It was observed that the level of increase activity in ALT is far higher than the corresponding increase activity in AST enzymes. Also, there was a noticeable increase in the level of the total proteins when cyanide is co-administered with either Salvia officinalis leaf extract or the sodium thiosulphate. There was equally no significant ($p>0.05$) difference in the levels of the GGT in the control group A compared to co-administration of cyanide with either the Salvia officinalis leaf or sodium thiosulphate (Table 2).

Oxidative stress parameters: The MDA level was raised on treatment with cyanide only, at a significant difference of $p<0.01$ and $p<0.05$ in comparison with control and Salvia officinalis leaf extract only. However, the activities of GSH, GST, SOD and Catalase were all reduced significantly ($p<0.05$) when compared with ether control (group A) or sage leaf extract (group C) only. Also co-administration of cyanide either with Sage leaf extract or the commonly used chemical drug (sodium thiosulphate) significantly reduced the malondialdehyde (MDA) at a significant level of $p<0.05$ compared with the control. Equally, the activities of GST, GSH, SOD and Catalase were significantly ($p<0.05$) increased on co-administration of cyanide with either the treatment drug- sodium thiosulphate or Salvia officinalis leaves extract (groups D & E) respectively also in comparison with treatment with cyanide only (group B) (Table 3).

Organ weight: The weights of the organs on treatment with cyanide only, particularly the liver, were significantly increased in comparison with the control group A ($p<0.01$ and $p<0.05$) (Table 4). Also, the co-administration of cyanide with either the treatment drug for cyanide poisoning in this case sodium thiosulphate led to the reduction of the organs weight when compared with the cyanide treatment only. Moreover, co-administration of cyanide with the sage leaf equally led to reduction of the organ weights in comparison with the cyanide treatment only. There was a noticeable increase in the liver proteins level on treatment with cyanide only in comparison with either the control group A or sage (*Salvia officinalis)* leaf extract. However, a corresponding reduction were noticed in the liver proteins level on co-administration of cyanide either with sage leaf extract or with common drug treatment (i.e., sodium thiosulphate) (Table 4).

Table 1: Rats' weight in different groups - initial and after two weeks administration of cyanide

Rats' Weight	Group A Control (corn oil)	Group B Cyanide only	Group C Sage leaf extract only	Group D Sage leaf extract plus cyanide	Group E $Na_2S_2O_3$ plus cyanide
Initial	194.00±5.45^a	196.33±6.11^a	195.67±20.50^a	193.00±16.64^a	193.33±4.16^a
After	203.63±16.25^a	201.93±4.65^a	215.43±12.47^c	217.43±13.28^c	204.83±17.18^a

Values are expressed in Mean±S.E.M; values sharing the same superscript in the row did not differ significantly ($p>0.05$); however, values sharing different superscript in the same column are statistically significant ($p<0.05$).

Table 2: Effect of sage plant leaf on rats' liver function parameters in cyanide-induced hepatotoxicity

Groups	TP (g/l)	GGT (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (µmol/l)
A	88.00±1.20	27.27±2.19	30.00±6.85	30.10±1.20	40.27±6.17	14.18 ± 2.75
B	46.10±3.60**	60.17±3.18**	110.10±10.18***	168.40±3.60***	156.90±8.66***	68.43±4.96***
C	92.50±2.48*	29.00±3.12*	44.60±3.82*	43.48±2.47*	53.21±10.31*	12.10±3.37*
D	82.40±2.30*	36.10±10.80*	60.10±8.51*	58.60 ± 2.63*	98.67±12.49**	48.64±3.19**
E	87.04±3.2	30.00±08.16*	70.16±6.18**	60.12±3.18**	90.35±12.12**	50.06±3.18**

Values are expressed as Mean±SEM; ***=p<0.001 (values compared to control groups), **=p<0.01 and *=p<0.05 (values compared to cyanide groups).

Table 3: Effects of sage leaf extract on rats' liver MDA, GSH, GST, SOD and CATALASE levels on cyanide-induced hepatotoxicity

Groups	MDA (µg/g tissue)	GSH (µg/g tissue)	GST (nmol/mg protein)	SOD (U/mg protein)	Catalase (Kat)
A	0.598±0.102	6.60±0.346	0.465±.056	3.464±0.326	0.2008±0.006
B	2.801±0.38**	3.94±0.100*	0.165±0.050*	2.000±0.098*	0.0680±0.006*
C	0.630±0.038	7.60±0.520	0.484±0.044	3.43±0.304	0.198±0.006
D	1.142±0.116*	6.10±0.460	0.406±0.025	3.501±0.156*	0.146±0.006#*
E	1.110±0.113 *	7.9±0.40 *	0.600±0.026*	2.60±0.345	0.156±0.008*

*=p<0.05 (as compared to control).

Table 4: Effect of sage plant leaf extract on rats' organs and liver proteins in cyanide-induced hepatotoxicity

Groups	Liver Proteins (mg/g tissue)	Heart	Kidney	Liver
A	268.312±13.10	0.32±0.07	0.31±0.06	3.40±0.16
B	235.132±22.2*	0.84±0.18**	0.64±0.16**	6.10±0.28**
C	278.234 ± 18.60	0.48±0.28*	0.43±0.16*	4.80±0.38*
D	196.213 ± 17.67	0.38±0.15*	0.34±0.21*	3.80±0.16*
E	193.703 ± 19.72	0.42±0.08*	0.48±0.38*	4.00±0.42*

Values are expressed as mean±SEM; **=p<0.01 (values compared to control groups), *=p<0.05 (values compared to cyanide groups).

DISCUSSION

Cyanide poisoning exhibits its hazardous effects in humans and animals by blocking cytochrome oxidase in the mitochondria thus preventing cellular respiration [5,39]. The mechanism of this inhibition involves the cyanide blocking a very important enzyme (cytochrome c oxidase) crucial for the production of energy in form of adenosine triphosphate (ATP) in the system and prevents the cells from utilizing oxygen thus creating cytotoxic hypoxia irrespective of adequate oxygen levels in the systemic circulation and causes the cells to switch to anaerobic metabolism [4,15,40]. This results to lactic acid accumulation with metabolic acidosis and to the production of free radicals such as superoxide anions with resultant lipid peroxidation[1,4,7,41,42]. Thus our observation of high increase in malondialdehyde level as a result of lipid peroxidation and decrease activities of GSH, GST, SOD and Catalase are in agreement with the above accretion. Also, our findings of decreased in SOD and Catalase is in line with the work [43] that noted a decrease in SOD, Catalase and antioxidant vitamins in the lens and retina of rabbits exposed to cyanide. Some researchers have documented the dosage of cyanide poisoning in various different species, noting that most illness disorders, have their underlying cause of oxidative stress particularly, as increase in reactive oxygen stress (ROS) or reduction in the antioxidant defense mechanism as the most common symptom [1,10,44]. They alluded that tissue damage and death often results from an autocatalytic chain of metabolic stress amplified and spread the oxidative stress that leads to increased free radicals such as OH^-, O_2 and H_2O_2 generation and antioxidant depletion. Also, other works have adduced that the reduced synthesis of sulphur-containing amino acids might be one reason for the lower glutathione content in the liver. The utilization of such amino acids by rhodanese and mercaptopyruvate is a known example [1,4,15,45].

The co-administration of *Salvia officinalis* leaf and sodium thiosulphate to the cyanide, which led to the decrease of malondialdehyde as well as an increase in the activities of antioxidant enzymes GST, SOD and Catalase and the non-enzymatic GSH showed that these additions reduced oxidative stress. These findings are similar to other works[15, 46.] Our studies revealed that Salvia *officinalis* leaf and sodium thiosulphate protect the liver of rats from cyanide-induced hepatoxicity thus highlighting the hepatoprotective effects of Salvia *officinalis* leaves through scavenging reactive oxygen species generated by the cyanide. Also, the sulphur content of the sodium thiosulphate may perhaps explain the noticeable significant increase in the activity of GSH and GST observed in the rats co-administered with it and cyanide.

Sodium thiosulphate is commonly used to convert cyanide to thiocyanate (CN) through the action of rhodanese which is an enzyme made up of various amino acids in the liver[15,16,17,47]. Thiocyanate is a less toxic chemical compound that is excreted easily by the kidneys and is often administered alongside with hydroxocobalamin and sodium nitrite[47,48]. The growth of advanced atherosclerotic plaques within the coronary arteries is linked to the SCN in the plasma which is associated with lipid peroxidation [1,10,15,49]. The increase in weight of the rats' liver and other organs may have been due to the alterations in the levels of MAD, GSH, GST, SOD and Catalase as a result of lipid peroxidation with perhaps inflammation of the organs.

Post cyanide exposure led to the increased activities of AST, ALT, GGT, ALP as well as TB in our work. This is not surprising as generally, drug-induced hepatotoxicity is determined by the measurement of GGT and aminotransferases activities in the serum or plasma of humans and animals. GGT is found both in the kidneys and the liver, however, the hepatic GGT has a direct access to the systemic blood stream while the renal GGT is commonly eliminated almost immediately in the urine. Hence, the liver is responsible for the serum or plasma activity of GGT in the systemic circulation where it is very often released into the bloodstream during cellular damage, cholestasis or excessive production[1,4,15,50]. Such increases in the activities of these enzymes as seem in our study is consistent with the observations in serum of workers exposed to cyanide inhalation in an industry in India, in post death autopsy finding in an electroplating chamber [7], in rats' liver fed with cassava[51], in serum of women exposed to potassium cyanide[51] and in suspected food poisoning through contaminated beverages [52].

CONCLUSION

From our findings, common sage (*Salvia officinalis)* leaf extract, whose antioxidant effect is similar to that of a known cyanide treatment drug like sodium thiosulphate, may be an alternate therapeutic agent for the management of cyanide-induced hepatotoxicity. It is also inexpensive and has little or no side effects.

Conflict of interest: The authors declare no conflict of interest.

Funding statement: No external funding received.

Ethical approval: The study was approved by the Research and Ethics Committee, Faculty of Allied and Health Sciences, Delta State University, Nigeria (Ref. No. REC/FAHS/23/019).

Authors' contribution: All authors were equally involved in study design, data collection, compilation and analysis as well as manuscript writing, editing and final submission.

REFERENCES

1. Kulnides N, Lorsirigool A, Saenkaew K, Santichat N, Tawinwang K, Thumrin P, et al. An Overview of Cyanide Poisoning in Humans and Animals. World Vet J. 2024;14(4):645-51.
2. Giantin S, Franzin A, Brusa F, Montemurro V, Bozzetta E, Caprai E, et al. Overview of cyanide poisoning in cattle from Sorghum halepense and S. bicolor Cultivars in Northwest Italy. Animals. 2024;14(5):743.
3. Parker-Cote JL, Rizer J, Vakkalanka JP, Rege SV, Holstege CP. Challenges in the diagnosis of acute cyanide poisoning. Clin Toxicol. 2018;56(7):609-17.
4. Woolf AD. Tylenol cyanide poisoning in the United States, 1982. History of modern clinical toxicology. Chapter 2.3, 2022 pp. 155-163. DOI: https://www.doi.org/10.1016/B978-0-12-822218-8.00027-2
5. Dorooshi G, Dorostkar A, Rahimi A, Zoofaghari S. An unusual acute cyanide intoxication. Advanced Biomedical Research, 2020; 9: 1-3 DOI:https://www.doi.org/10.4103/abr.abr_128_20
6. Abeyasinghe NL, Perera HJM, Weerasinghe DSK. Case report–Death by subcutaneous injection of cyanide in Sri Lanka. Journal of Forensic and Legal Medicine, 2011; 18(4): 182-183. DOI: https://www.doi.org/10.1016/j.jflm.2011.02.010
7. Jadav D, Saraf A, Shekhawat RS, Kanchan T, Nalwa A. Accidental deaths due to toxic industrial cyanide inhalation: An autopsy case report. Cureus, 2022; 14(5): e25376. DOI: https://www.doi.org/10.7759%2Fcureus.25376
8. Gigengack R.K, Cleffken B.I and Loer S.A. Advances in airway management and mechanical ventilation in inhalation injury. Current Opinion in Anaesthesiology 2020; 33:6, 774-780.
9. Akyildiz BN, Kurtoğlu S, Kondolot M, Tunç A. Cyanide poisoning caused by ingestion of apricot seeds. Annals of Tropical Paediatrics, 2010; 30(1): 39-43. DOI: https://www.doi.org/10.1179/146532810X12637745451951
10. Houlton E, Caldwell DJ, Granfone M. Cyanide toxicity secondary to apricot (Prunus armeniaca) kernel meal ingestion in a canine. Toxicon, 2024; 245: 107764. DOI: https://www.doi.org/10.1016/j.toxicon.2024.107764
11. Kennedy A, Brennan A, Mannion C, Sheehan M. Suspected cyanide toxicity in cattle associated with ingestion of laurel-a case report. Irish Veterinary Journal, 2021; 74: 1-6. DOI: https://www.doi.org/10.1186/s13620-021-00188-0
12. Sabourin PJ, Kobs CL, Gibbs ST, Hong P, Matthews CM, Patton KM, and Wakayama EJ. Characterization of a mouse model of oral potassium cyanide intoxication. International Journal of Toxicology, 2016; 35(5): 584-603. DOI: https://www.doi.org/10.1177/1091581816646973
13. Agency for Toxic Substances and Disease Registry. Facts about Cyanide. Atlanta, GA: US Department of Health and Human Services, Public Health Service 2014
14. Brasel JM, Cooper RC, Pritsos CA. Effects of environmentally relevant doses of cyanide on flight times in Pigeons, Columba livia. Bulletin of Environmental Contamination and Toxicology, 2006; 76(2): 202-209.
15. Ola-Mudathir KF, Jeje SO. Protective Effects of Quercetin on Hepatotoxicity Induced by Cyanide in Male Wistar Rats Afr. J. Biomed. Res. 2023; Vol. 26; 273- 279
16. Petrokovics I, Budai M, Kovacs K, Thompson DE. Past, present and future of cyanide antagonism research: From the early remedies to the current therapies. World J Methodol 2015; 5(2):88-100.
17. Ola-Mudathir FK, Maduagwu EN. Effects of Alium cepa Linn on rhodanese activities and half-life of cyanide in the blood. International Journal of Biological

and Chemical Sciences 2015; 9(2):1004. DOI:10.4314/ijbcs.v9i2.36

18. Zhang M, Zhao J, Li XDaX. Extraction and Analysis of Chemical Compositions of Natural Products and Plants. Separations J. 2023; 10(598):1-29.

19. Stuper-Szablewska K, Szablewski T, Przybylska-Balcerek A, Szwajkowska-Michałek L, Krzyżaniak M, Świerk D, Cegielska-Radziejewska R, Krejpcio Z. Antimicrobial Activities Evaluation and Phytochemical Screening of Some Selected Plant Materials Used in Traditional Medicine. Molecules. 2023; 28(1): 244. DOI: 10.3390/molecules28010244

20. Mokogwu TH, Amaihunwa KC, Adjekuko CO, Ekene EN, Okoro EO, Adeosun OG Avwioro GO. In vivo Antimalarial and Liver Function Profiles of Methanol Extract of *Salvia officinalis* (Common Sage) Leaf in *Plasmodium berghei*Infected Mice. *Ethiop J Health Sci.* 2024; 34(4):290. doi: http://dx.doi.org/ 10.4314/ejhs.v34i4.5

21. Lahlou Y, Moujabbir S, Aboukhalaf A, El AB, Bamhaoud T. Antibacterial Activity Of Essential Oils Of Salvia Officinalis Growing In Morocco (National Institute of Public Health) Rocz Panstw Zakl Hig 2023; 74(4):459-468 https://doi.org/1032394/rpzh.2023.0275

22. Mokogwu ATH, Adjekuko CO, Oshilonyah UH, Ikpefan JO, Avwioro GO.

23. Antihypertensive and Cardioprotective Effects of *Salvia officinalis* (*Sage*) Leaf in N^G-Nitro-LArginine Methyl Ester (L-NAME) InducedHypertensive Wistar Rats. *Trop J Nat Prod Res.* 2022; 6(12):2047-2050 Available online at https://www.tjnpr.org

24. Mokogwu ATH, Adjekuko CO, Oshilonyah HU, Avwioro OG. Studies of *Salvia officinalis* leaf extract on some biochemical parameters in rats induced with overdosed-tramadol. Afr J Biotech. 2022b; 21(8):353-360

25. Harizia A, Benguerai A, Elouissi A, Mahi T, Bonal R. Chemical composition and biological activity of *Salvia officinalis* L. essential oil against Aphis fabae Scopoli (Hemiptera: Aphididae). J Plant Diseases Protection. 2021; 128(6):1547-1556. https://doi.org/10.1007/s41348-

26. Privitera G., Luca T., Castorina S., Passanisi R., Ruberto G., Napoli E. Anticancer activity of Salvia officinalis essential oil and its principal constituents against hormone-dependent tumor cells Asian Pacific Journal of Tropical Biomedicine. 2019; 9(1): 24-28Mokogwu,ATH, Okocha EE, Amaihunwa KC, Ochonogor I, Ekene EN, Eyenubo BO, Avwioro GO. Chemical constituents, antioxidant and anti-inflammatory properties of de-chlorophyllized extracts of *Salvia officinalis* leaves 2025; in pre ss

27. OECD. Cooperation and Development. Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation, 2002. Paris OECD Publishing.

28. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases American Journal of Clinical Pathology 1957; 28(1):56-63.

29. Binkley F, Shank EE, Hoagland CE. Modification of the King-Armstrong method for the determination of phosphatase. J. Biol. Chem. 1994; 156:253-256.

30. Gjerde H, Marland J. Determination of gamma-glutamyltransferase in completely haemolysed blood samples. Scandinavian Journal of Clinical and Laboratory Investigation 1985; 45(7):661-664.

31. Reinhold JG. Total proteins, albumin and globulin. In: Reiner, M. (ed). Standard Methods of Clinical Chemistry, New York Academic Press 1953; 71:88.

32. Malloy HT, Evelyn KA. The determination of bilirubin with photoelectric photometer. Journal of Biological Chemistry 1937; 119(2):481 483

33. Buege JA, Aust, SD. Microsomal lipid peroxidation methods. Enzymol., 1978; 52: 302 -310.

34. Sinha, A. Colorimetric assay of catalase. Analytical Biochemistry 1972; 47.2:389-394

35. Misra, H.P. and Fridovich, I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. Journal of biological chemistry 1972; 247: 3170 -3175

36. Habig WH, Pabst MJ, Jakoby, W.B. Glutathione S-transferases. The first enzymatic method in mercapturic acid formation. Journal of biological chemistry, 1974; 249:7130-7139.

37. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 1963); 61: 882-888.

38. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol Reagent. Journal of Biological Chemistry 1951; 193: 269-275.

39. Kruse F, Nguyen AD, Dragelj J, Schlesinger R, Heberle J, Mroginski MA, Weidinger IM. Characterisation of the Cyanate Inhibited State ofCytochrome c Oxidase. Sci Rep 1951; 10, 3863

40. Jaszczak E, Polkowska Z, Narkowicz Z, Namieśnik J. Cyanides in the environment—analysis—problems

andchallenges. Environmental Science and pollution Research, 2017; 24: 15929–15948.

41. Manoj KM, Ramasamy S, Parashar A, Gideon DA, Soman V, Jacob VD, Pakshirajan K,. Acutetoxicity of cyanide in aerobic respiration: Theoretical andexperimental support for murburn explanation. BiomolecularConcepts. 2020; 11 (1).

42. Yadukul S, Venkataraghava S, Fathima T, Gaonkar VB. Fatal suicidal case of cyanide poisoning-A case report. Journal ofForensic Toxicology and Pharmacology, 2014; 3: 4-6. DOI: http://www.doi.org/10.4172/2325-9841.1000123

43. Kadiri HE, Asagba SO. The chronic effects ofcyanide on oxidative stress indices in the domestic chicken (Gallus domesticus L.). JoBAZ 2019; 80, 30

44. Nagy AL, Ardelean S, Chapuis RJ, Bouillon J, Pivariu D, Dreanca AI, Caloni F. Emerging plant intoxications in domesticanimals: A European perspective. Toxins, 2023; 15(7): 442. DOI: https://www.doi.org/10.3390/toxins15070442

45. Chaudhary M, Gupta R. Cyanide DetoxifyingEnzyme: Rhodanese. Current Biotechnology, 2012; 1: 327-335

46. Bayliak MM, Burdylyuk NI, Lushchak VI. Quercetin increases stress resistance in the yeast Saccharomycescerevisiae not only as an antioxidant. *Annals of Microbiology* 2016; 66, 569–576

47. Avais M, Khan MS, Khan MA, Ashraf K, Khan JA, Ahmad N. Assessing the efficacy of aqueous garlic extract, sodiumnitrite and sodium thiosulfate against prolonged oral cyanide exposure in rabbits. Pakistan Journal of Pharmaceutical Sciences, 2018; 31(2): 412-419. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20183123102

48. Tobarran N, Kershner EK, Cumpston KL, Rose SR, Wills BK. Homicide with intramuscular cyanide injection: A casereport. Toxicology Communications, 2022; 6(1): 20-22. DOI: https://www.doi.org/10.1080/24734306.2021.2015550

49. Kim SC. Management for elevated serum GammaGlutamyl Transpeptidase in primary care settings. AustralasianMedical Journal 2018; 11(2)

50. Oyeyemi FB, Babajide SA, Elelu TK, Oyeyemi WA, Iyiola OA. Biochemical and histological responseson the liver of adult Wistar rats fed with varied levels of cassava. Journal of Chemical, Biological and Physical Sciences. 2012; 2(3) 1350-1357.

51. Dhas PK, Chitra P, Jayakumar S, Rita Mary A. Study of the effects of hydrogen cyanide exposure inCassava workersIndian J Occup Environ Med. 2011; 15(3): 133–136.

52. Nnoli MA, Legbosi NL, Nwafor PA, Chukwuonye II. Toxicological investigation of acute cyanide poisoning of a 29-yearold man: A case report. Iranian Journal of Toxicology, 2013; 7(20): 831-5.