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Evaluation of anti-oxidant, anti-cholinesterase activity and dopamine levels of methanolic leaves extract of *Solanum incanum* in arsenic-induced neurodegeneration in Wistar rats

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Abstract
The study investigated the anti-oxidant, anti-cholinesterase activity and dopamine levels of methanolic leaves extract *Solanum incanum* in arsenic-induced neurodegeneration. Animals were divided into four groups: A Control (maintained on food and water only), B treated with 300 mg/kg *S. incanum*, C treated with 20 mg/kg As_2O_3, and D treated with 300 mg/kg and then 20 mg/kg As_2O_3. *Serum* anti-oxidant activities were determined by measuring the Catalase (CAT) and Super-oxidase (SOD) activities using enzyme-linked immunosorbent assay (ELISA). Serum acetylcholinesterase inhibitory activity and dopamine levels were also quantified using ELISA. Prepared slides from the brain were dehydrated using a dry air oven in xylene for 30 minutes and later mounted on the microscope and viewed using oil immersion ×1000 magnification. The result of CAT and SOD assays showed significant decrease (P<0.0002) when control groups (40 µmol/min/mL and 107.7 U/mL) were compared with the group exposed to arsenic only (20 µmol/min/mL and71.32 U/mL) respectively. The activity of cholinesterase was significantly reduced (P<0.0006) in group exposed to arsenic only (30.30 U/mL) compared to the control group (45.42 U/mL) But, there was no significant difference between the control group and group treated with *S. incanum* methanol extract and then arsenic and group treated with arsenic and then *S. incanum* methanol extract. There was no observed significant difference (P>0.5) in dopamine levels between the groups. Histopathological lesions observed include degenerative vacuolated neurocytes. For histopathological study, there were dystrophic changes in the form of shrunken hyperchromatic neurocytes which were irregular with chromatolysis and abnormal Nissl granule distribution in groups exposed to arsenic only as well as arsenic *S. incanum*. Because of the presence of significant anti-oxidant and anticholinesterase activities as well as neuronal cells protective effect, *S. incanum* is a potential source of remedy against arsenic poisoning.

Keywords: Antioxidant activity, cholinesterase inhibitors, crude extract, dopamine activity, oxidative, toxicity study

Introduction
Nerve cells in the brain and peripheral nervous system lose function over time and eventually die in neurodegenerative disease (Borah *et al*., 2021) [7]. Although therapies may alleviate some of the physical or mental symptoms associated with neurodegenerative disorders, there are presently no known cures or ways to decrease disease development (Lee *et al*., 2019) [16]. Scientists have discovered that a person's genes and environment factors increase the chance of acquiring a neurodegeneration (Scheiblich *et al*., 2020) [33]. Chronic arsenic exposure has been related to a variety of negative health impacts in exposed individuals, including skin disorders, cancer, diabetes, cardiovascular disease, reproductive, developmental, and neurodegeneration (Mostafalou and Abdollahi, 2013) [23].

The biological properties of *Solanum incanum* or garden egg leaf and seed as well as its derivatives have been reported, including antioxidant neuroprotectant properties (Patel *et al*., 2021) [28]. Studies shows that garden egg is used in the treatment of variety of diseases conditions such as diabetic retinopathy, uveitis, light induced retinopathy, and ischemia/reperfusion injury (Yang *et al*., 2019) [45]. The plant is said to contain important minerals and vitamins such as proteins, riboflavin, thiamine iron, calcium, nicotinamide, glucose, Vitamin C water, fiber, fat, and carotenes, all of which are beneficial to one's health (Abdisa, 2019) [1]. Carotene is converted to Vitamin A in the body, which is required for

night blindness prevention and xerophthalmia (Gomes *et al*., 2013) [10]. The high concentration of α-chaconine in this plant have anticholinesterase action in nerve tissues, which contributes to its therapeutic usefulness (Väänänen *et al*., 2005) [41].

Cholinesterase inhibitors work by preventing cholinesterase from hydrolyzing acetylcholine into its constituent's acetate and choline. This increases acetylcholine availability and duration of action in neuromuscular junctions (Hassan *et al.*, 2020) [12]. There are two active sites in the cholinesterase enzyme: an anionic site created by tryptophan and an esteractic site formed by serine (Sharma, 2019) [36]. Organophosphates and arsenic, for example, interact with the serine esteractic site of cholinesterase, preventing it from cleaving acetylcholine. As a consequence, acetylcholine will continue to build up and activate receptors in the brain (Colovic *et al.*, 2013) [9]. Cholinesterase inhibitors are divided into three categories: reversible, irreversible, and pseudo-reversible (Strelnik *et al*., 2016) [38]. In general, reversible cholinesterase inhibitors are used for therapeutic purposes. Irreversible and pseudo-reversible inhibitors, on the other hand, are often employed as insecticides and biowarfare (nerve agents) (Colovic *et al.*, 2013) [9].

Despite the growing popularity of *Solanum incanum* leaf and seed as a supplements and or therapeutic agents, little is known about its antioxidant and anti-cholinesterase effects. As a result, the present study evaluated the effect of methanol leaf extract of *Solanum incanum* on antioxidant enzymes, cholinesterase inhibition and histopathologic effect in the brain of Wistar rats.

Materials and Method

Plants Collection and Identification

Solanum incanum leaf was obtained from Gwaski village, Sakwa ward, Hawul LGA, Borno State, Nigeria. S. Sanusi from the University of Maiduguri's Department of Biological Science in Borno State, Nigeria, identified it, and a voucher number (DCPT 014) was assigned. The leaf was cleaned and air-dried for two weeks at ambient temperature (26 ± 1 °C).

Plant Extraction

Solanum incanum leaves were washed and crushed to semi-powdered form (40–60 mesh). The powder was allowed to dry for two weeks at room temperature (26 ± 1 °C). In flat bottom flasks (Sigma-Aldrich, USA), 200 g of leaves sample were soaked for 3 days in 1000 mL of 80 percent methanol. To get a high yield of the extract, the semi-powdered leaves in methanol was shaken daily for three days at 25 ± 1 °C. The extract was then filtered using new white clean muslin cloth and concentrated to semisolid form using a rotary evaporator (IKA® RV 10, USA) at 42 °C. The semi-solid crude extract was then weighed and put into sample vials, which were then kept at 4 °C until needed. Yield (%) = [wt of extract (g)/wt of plant material (g)] × 100 (Hassan *et al*., 2020) [12].

Plants Sample Dilution and Dose Preparation

Stock solution was prepared by dissolving 100 g of *S. incanum* leaves extract in 1 L of 100% DMSO (100 g/L). Preparation of sub-stocks solution was done by diluting the stock solution to 10 mg/mL with distilled water. Working solution was prepared from sub-stock solution using twofold serial dilution with distilled water at concentrations of interest (1 mg/mL). DMSO (vehicle) was maintained at 0.1% in all concentration of extract (Hassan *et al.*, 2020) [12].

Toxicity study of the extract

Oral toxicity study of the extract) was carried out in accordance with the Organization for Economic Cooperation and Development's guidelines (OECD) 423(7) (Alli *et al.*, 2011) [4]. The Usmanu Danfodiyo University Institutional Animal Care and use Committee (IACUC) Faculty of Veterinary Medicine Usmanu Danfodiyo University Sokoto, Nigeria approved the protocol for these experiments under number UDUS/IACUC/AUP-R005/2020). For the acute toxicity study of the crude extract, rats were treated with 1000, 500 and 250 mg/kg of the extract for 2 days (Clemente *et al*., 2019) [8]. Probit analysis was used to determine the LC_{50}.In chronic toxicity study of the crude extract, 500, 250 and 125 mg/kg of the extract were administered for 14 days (Adekola *et al*., 2020) [2].

Induction of Neurodegeneration using Arsenic

Neurodegeneration in in rats was induced by administering arsenic trioxide (As_2O_3) (20 mg/kg bw) orally for 5 days (Patlolla and Tchounwou, 2005) [29].

Protective and therapeutic effects of the extract on arsenic-induced Neurodegeneration

Neuroprotective and therapeutic effects of the extract on arsenic-induced neurodegeneration were carried out in accordance with the OECD guidelines. Animals were divided into four groups of 5 rats each. The groups were treated as follows:

A. Normal control, received only food and water.

B. In addition to food and water, the rats were administered 300 mg/kg bw of *S. incanum* methanol extract.

C. Rats received 20 mg/kg bw of arsenic trioxide (As_2O_3) in addition to food and water.

D. Rats were given 300 mg/kg bw of *S. incanum* methanol extract for 10 days followed by administration of 20 mg/kg bw of arsenic trioxide (As_2O_3) for 5 days in addition to food and water.

degenerative vacuolated neurocytes degenerative vacuolated neurocytes *S. incanum* methanol extract E. Rats were given 20 mg/kg bw of arsenic trioxide (As_2O_3) for 5 days followed by administration 300 mg/kg bw of *S. incanum* methanol extract for 10 days in addition to food and water.

Animals were anaesthetized with chloroform vapor, forty-eight (48) hours after the last treatment, blood was collected via cardiac puncture with 5 ml syringe and needle and transferred into EDTA free bottles. The brain of each rat was removed after the head was dissected with the use of a dissecting kit. Before putting into a clean sample container containing 10% neutral buffered formalin, the dissected brains were dipped into a beaker containing normal saline to wipe away excess blood (Parasuraman *et al*., 2010) [27].

Biochemical Tests

Total proteins (Kashyap *et al*., 2020) [14], Catalase (CAT) activity (Zhang *et al*., 2017) [46], Super-oxidase (SOD) activity (Warsinggih *et al*., 2020) [44], Cholinesterase (Ache) inhibitory activity (Hadda *et al*., 2017) [11] and Dopamine (DA) levels (van Nie *et al*., 2020) were determined from serum using enzyme-linked immunosorbent assay (ELISA)

assay kits from Thermo Fisher Scientific, USA as described in the manufacturer's guide.

Histological Study

The brain was immersed in fixative (10% neutral buffered formalin) for 3 days and then transferred directly to 70% alcohol where it was graded to 90%,100% alcohol for 8, 12 and 15 hours respectively. Alcohol was then replaced with Xylene and incubated 4 hours, followed by embedding the tissues and insertion into paraffin wax to harden tissue for easy cutting into thin sections with the microtome. Casting of tissues into block of paraffin in 'L' block was carried out to remove air bubbles followed by it solidification. Prepared slides were dehydrated using a dry air oven in xylene for 30 minutes and latter mounted on the microscope and viewed using oil immersion ×1000 magnification (Sabdyusheva *et al*., 2020) [31].

Results

Toxicity study

The chronic toxicity studies revealed the LC_{50} of 676.10 mg/Kg body weight for the extract and 45.70 mg/Kg body weight for the arsenic trioxide (As_2O_3).

Total Protein

Result of total protein shows protein level of 7.47g/dL in the control group compared to the group exposed to arsenic only. There is significant decrease in protein level at $P<0.01$ in the group that was exposed to arsenic (As_2O_3) only. (Figure 1).

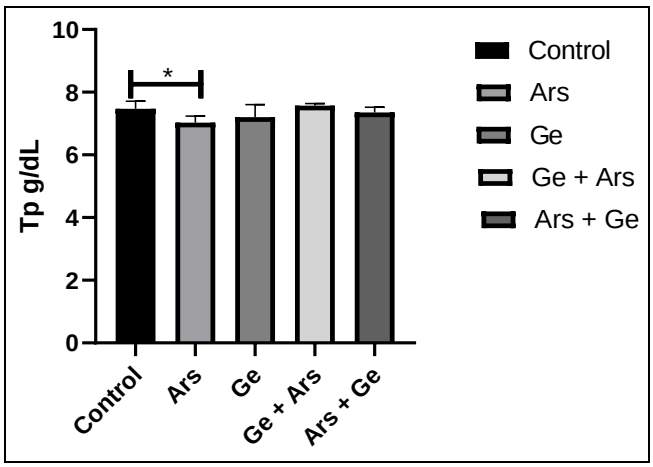

Fig 1: Serum Total protein (TP) of Wistar rats treated with arsenic (Ars), *S. incanum* methanol extract (Ge), *S. incanum* methanol extract (Ge) and then arsenic (Ars), arsenic (Ars) and then *S. incanum* (Ge). *$P<0.01$ represents significant different between the control group and group exposed to arsenic (Ars) only. The values represent mean ± SEM from independent experiments.

Catalase (CAT) Activity

Result of catalase (CAT) activity showed and with significant different ($P<0.0002$) between the control group (40 µmol/min/mL) and group exposed to arsenic (20 µmol/min/mL) only. There was no significant difference between the control group and groups treated with crude extract, crude extract and arsenic (As_2O_3), arsenic (As_2O_3) and then crude extract (Figure 2).

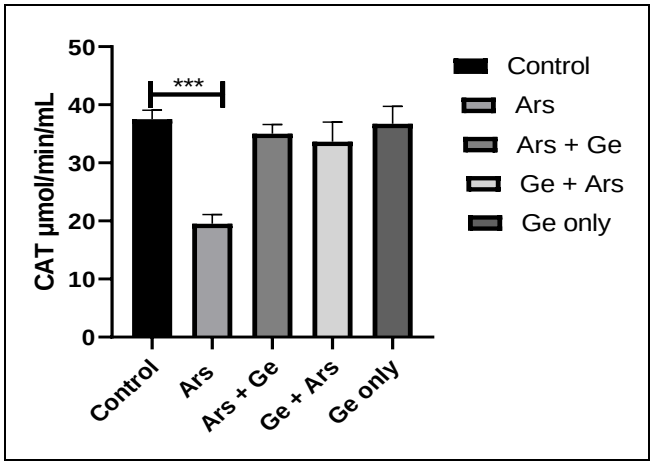

Fig 2: Catalase (CAT) activity of Wistar rats treated with arsenic (Ars), *S. incanum* methanol *extract* (Ge), *S. incanum* methanol extract (Ge) and then arsenic (Ars) , arsenic (Ars) and then *S. incanum* methanol extract (Ge). ***$P<0.0002$ represents significant different values of the control group and group exposed to arsenic (Ars) only. The values represent mean ± SEM from independent experiments.

Superoxidase (SOD) Activity

Result of superoxidase (SOD) activity showed and with significant different ($P<0.0048$) between the control group (107.7 U/mL) and group exposed to arsenic (71.32 U/mL) only. There was no significant difference between the control group and groups treated with crude extract, crude extract and then arsenic (As_2O_3), arsenic (As_2O_3) and then crude extract (Figure 3).

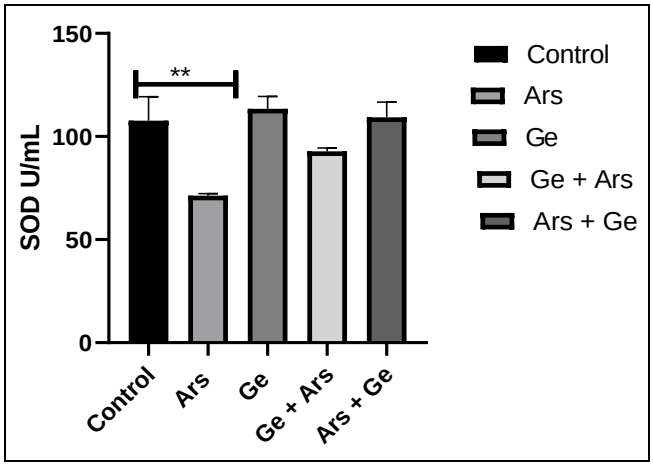

Fig 3: Superoxidase (SOD) activity of Wistar treated with arsenic (Ars), *S. incanum* methanol extract (Ge) and arsenic (Ars), arsenic (Ars) and *S. incanum* methanol extract (Ge). **$P<0.0048$ represents significant different values of the control group and group exposed to arsenic (Ars) only. The values represent mean ± SEM from independent experiments.

Cholinesterase (AChE) Inhibitory Activity

Result of cholinesterase (AChE) inhibitory activity showed and with significant difference ($P<0.0006$) between the control group (30.30 U/mL) and group exposed to arsenic (45.42 U/mL) only. There was no significant difference between the control group and groups treated with *S. incanum* methanol extract, *S. incanum* methanol extract and then arsenic (Ars), arsenic (Ars) and then *S. incanum* methanol extract (Figure 4).

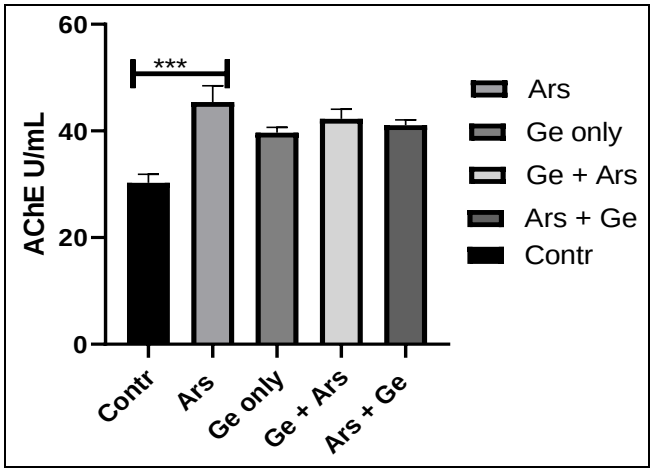

Fig 4: Acetyl cholinesterase (AChE) assay of Wistar treated with arsenic (Ars), *S. incanum* methanol extract (Ge), *S. incanum* methanol extract (Ge) and arsenic (Ars) , arsenic (Ars) and *S. incanum* methanol extract (Ge).$^{***}P$<0.0006 represents significant different values of the control group and group exposed to arsenic (As_2O_3) only. The values represent mean ± SEM from independent experiments.

Dopamine (DA) Levels

There was no observed significant different (P > 0.5) in dopamine levels between the groups. The values obtained were 128.3 U/mL (control), 121.7 U/mL (arsenic), 113.2 U/mL (*S. incanum* methanol extract), 131.2 U/mL (*S. incanum* methanol extract + arsenic) 123.4 U/mL (arsenic + *S. incanum* methanol extract). (Figure 5).

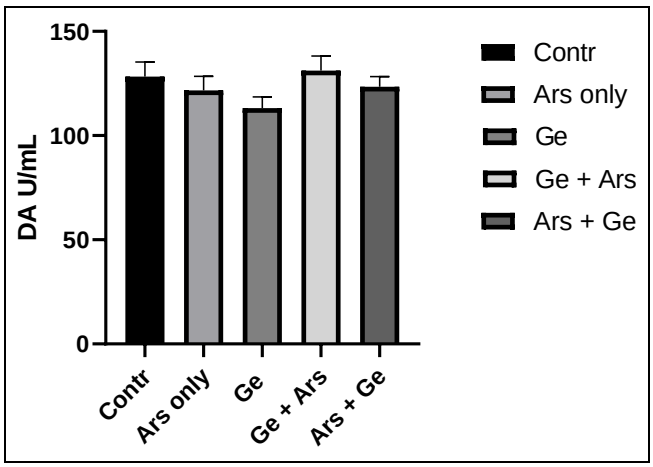

Fig 5: Dopamine (DA) levels from Wistar treated with arsenic (Ars), *S. incanum* methanol extract, *S. incanum* methanol extract and arsenic (Ars), arsenic (Ars) and *S. incanum* methanol extract). There was no significant different (P > 0.5) between the control groups and groups exposed to arsenic (Ars), *S. incanum* methanol extract, *S. incanum* methanol extract and arsenic (Ars), arsenic (Ars) and *S. incanum* methanol extract. The values represent mean ± SEM from independent experiments.

Histopathologic effect on brain

Results of histopathological finding from Wistar rats showed lesions such as degenerative vacuolated neurocytes, dystrophic changes in the form of shrunken hyperchromatic, irregular with chromatolysis in neurons and abnormal Nissl granule distribution in group exposed to arsenic (Ars) only (plate D).

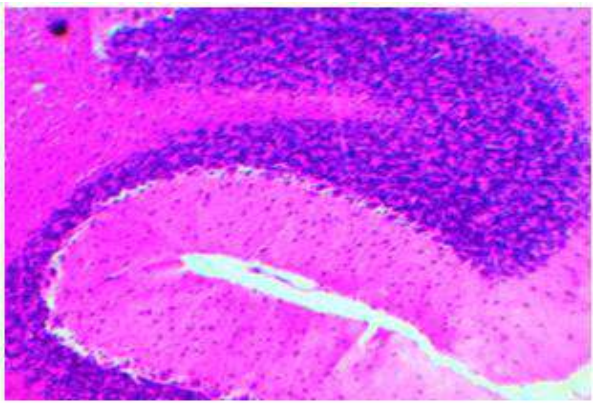

Plate A: Brain section of the Wistar rat fed with feed and water only (control group)

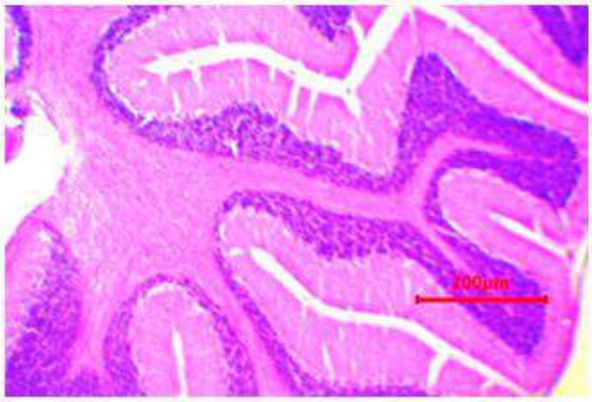

Plate B: Brain section of the Wistar rat exposed to *S. incanum* methanol extract only

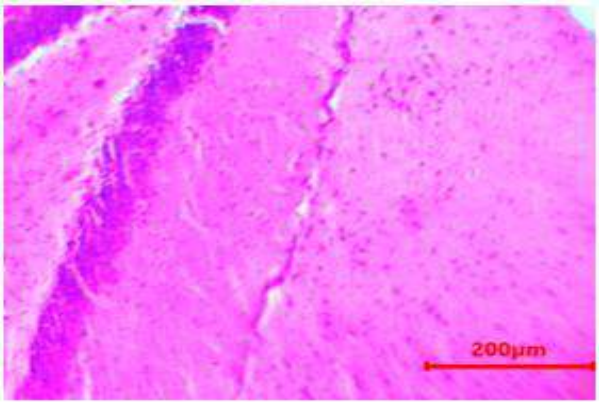

Plate C: Brain section of the Wistar rat exposed to *S. incanum* methanol extract and then arsenic (Ars)

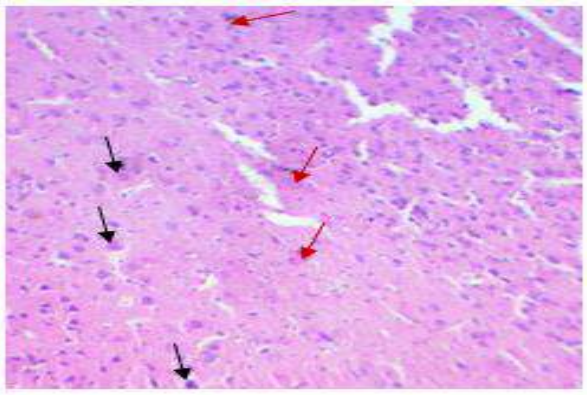

Plate D: Brain section of the Wistar rat exposed to arsenic (Ars) only and Plate

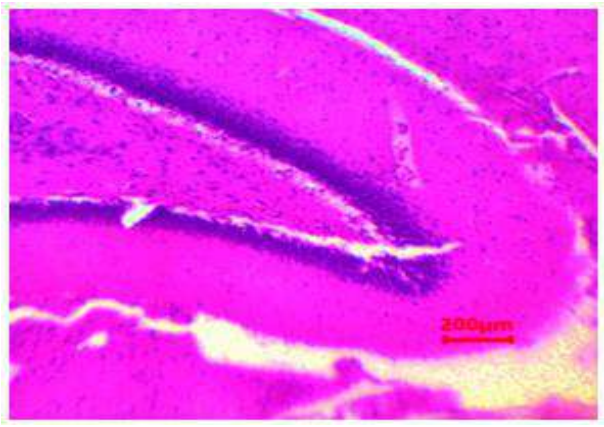

Plate E: Brain section of the Wistar rat exposed to arsenic (Ars) and then *S. incanum* methanol extract.

Fig 6: Histopathological assessment of the brain of Wistar rats: The brain showed degenerative vacuolated neurocytes with red dot (red arrows), dystrophic changes in the form of shrunken hyperchromatic, irregular with chromatolysis in neurons and abnormal Nissl granule distribution with blue dot (black arrows)

Discussion

The main purpose of this study was to determine the efficacy of *Solanum incanum* in protecting and ameliorating the neurodegenerative effect caused by arsenic trioxde (As_2O_3) in Wistar albino rats.

Effect of arsenic trioxide on animals' serum total protein has been studied by many researchers (Sun *et al*., 2021, Mingxing *et al*., 2019). Groups that were exposed to arsenic trioxide (As_2O_3) only showed significant decrease in total protein (p<0.01) compared to the control and other treated groups. On the effect of Solanum incanum on serum protein, there was little research. Some studies have found that Solanum incanum methanol leaf extract can protect serum protein from inflammatory agents by inhibiting cells from activating and releasing inflammatory mediators that promote vasodilation and increased blood vessel permeability. This also prevents plasma proteins and fluids from leaking into the tissues (Anosike *et al*., 2012) [5]. This may be as a result of protein denaturation by the arsenic trioxide (As_2O_3). Sulfhydryl groups have a strong affinity for trivalent arsenicals, and they can bind to destroy cysteines in peptides and proteins (Shen *et al*., 2013) [37]. This is in contrary to the finding by Herrera *et al*., (2021) [13], who reported increased in total protein (TP,) after arsenic administration in albino rats, leading to the damage of cell and followed by acute inflammation marked by significant neutrophil infiltration.

The serum antioxidant enzymes CAT and SOD were examined in this study and the findings demonstrated a significant decrease in both CAT and SOD (P<0.0002, P<0.0048) in Arsenic-treated rats compared to the control group. Antioxidant effect of *Solanum incanum* leave extract may be due to the present of several bioactive compounds. Present of bioactive such as phenol, flavonoid, beta carotene and ascorbic acid proved the antioxidant properties of plant extract (Adelakun *et al*., 2020) [3]. Phenolic compounds work by removing free radicals, binding metal ions, inhibiting enzymatic systems that produce free radicals, increasing the concentration of biologically important endogenous antioxidants, and inducing the expression of a variety of genes involved in the synthesis of enzymes that protect against oxidative stress (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006) [42]. Many flavonoids have been shown to have considerable antioxidant activity in diverse in vitro systems (Kaurinovic & Vastag, 2019) [15]. This is in line with the finding reported by Seif *et al*., (2021) [34] on the anti-oxidative stress effect of *Zingiber officinale* ethanolic extract in in male rats exposed to arsenic. Antioxidant enzymes aid in the fight against free radicals/oxygen-derived species produced during normal physiological processes (Liu *et al*., 2020) [17].

S. incanum methanol extract was tested for it cholinesterase inhibitory activity and effect on dopamine levels. The results obtained showed that there was a significant reduction in cholinesterase enzyme activity (P<0.0006) in the groups treated with arsenic trioxide (Ars) only. There was no observed statistical different in dopamine levels between the groups that were exposed to arsenic trioxide (Ars) only and the other groups. There was no available material on anticholinesterase effects *Solanum incanum's*. Lucky *et al*., (2018) demonstrated the ability of a secondary metabolite from *Moringa oleifera* extract to neutralize free radicals, preventing the formation of senile plaques (A) and neurofibrillary tangles (NFTs) (tau protein) in the hippocampus and cerebral cortex of neurodegenerative induced rats. Neuronal injury and synaptic dysfunction are caused by protein aggregates (A and tau proteins). Several medicinal plants have been shown to have anti-cholinesterase inhibitory activities in the literature (Mathew & Subramanian, 2014 [19], Owokotomo, Ekundayo *et al*., 2015 [26], Samaradivakara *et al*., 2016 [32], Malar *et al*., 2017 [18], Ovais *et al*., 2018) [25]. Furthermore, antioxidants such as vitamin E and vitamin C have been linked to a decreased incidence and prevalence of Alzheimer's disease(AD) and AD patients taking large doses of antioxidants have been shown to have a slower pace of cognitive decline (Polidori & Nelles, 2014) [30]. There is high tendency of correlating the anticholinesterase and antioxidant effect of this plant with it phytochemical content. AChE inhibitory activity has been discovered in a wide range of plants. Alkaloids, ursolic acid, lignans, flavonoids, terpenoids, and coumarins are phytochemicals that may be responsible for this activity (Mottay & Neergheen-Bhujun, 2016) [24]. Result of dopamine in this study contradicts that of Moreno *et al*., (2016) who discovered arsenic exposure resulted in hypoactivity at six months due to increase in dopamine levels. The non-statistical difference observed in this study may be as a result of short exposure period (10 days). Several literatures documented arsenic to have neurotoxic effects induces increase secretion of dopamine (DA) and serotonin (5-HT) due to regulation of norepinephrine (NE) levels (Thakur *et al*., 2021) [40].

Histopathological study also showed degenerative vacuolated neurocytes, dystrophic changes in the form of shrunken hyperchromatic, irregular with chromatolysis in neurons and abnormal Nissl granule distribution in group exposed to arsenic (Ars) only plate D. The histological lesions may be due to the toxic effect of arsenic on the neuronal cells leading to degenerative changes and death of neurons. There was no observed lesion in the control group, group treated with *S. incanum* methanol leave extract only, group treated with *S. incanum* methanol leave extract for 5 days and then exposed to arsenic and group that were exposed to arsenic 5 days before treatment with *S. incanum* methanol leave extract for 10 days. This result is similar with the finding reported by Shaibah *et al*., 2016) [35] and

differed with the finding reported by Mohammod, (2015) [21] who showed edema, intracellular space, edematous changes in arsenic exposed brain tissue of mice. The result also differed with that of Bashir *et al*., (2006) [6], who reported cellular necrosis due to nuclear pyknosis following 24 hours mice exposure to arsenic. Dissimilarity may be due to specie difference. Mohammod and Bashir *et al*., carried out their research on mice while our on study was conducted on Wistar rat.

Conclusions

This study showed that the *S. incanum* methanol extract have significant anti-oxidant and anti-cholinesterase activity. Histopathologic result revealed the protective and curative effect of this extract on arsenic induces neurodegeneration. Because of the presence of significant anti-oxidant, anticholinesterases activity as well as neuronal cells protective and curative effect of this plant, it could be a potential source of lead compounds for the development of drugs that can be used in the management of neurodegenerative diseases.

Data Availability

Data are available from Dr Ibrahim Maina Hassan but with the permission of Usmanu Danfodiyo University Sokoto and TETFund.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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