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# DICHLORVOS INDUCED NEPHROTOXICITY IN RAT KIDNEY: PROTECTIVE EFFECTS OF *ALSTONIA BOONEI* STEM BARK EXTRACT

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**Keywords:**

Dichlorvos, oxidative stress, nephrotoxicity, *Alstonia boonei*

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**ABSTRACT: Purpose:** Dichlorvos (DDVP) causes toxicity in animals including humans. *Alstonia boonei* stem bark is widely used as a culinary medicine in the Ayurvedic system of medicine, possessing a number of pharmacological properties. This study was designed to evaluate the effectof *Alstonia boonei* (AB) against dichlorvos induced nephrotoxicity in wistar rats. **Methods:** Dichlorvos (50 mg/kg body weight) was orally administered in wistar rats for 14 days followed by the treatment of *Alstonia boonei* (200 and 400 mg/kg body weight) for 14 days. Changes in the kidney biochemical marker namely, glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), aminotransferase (ALT), aspartate aminotransferase (AST) activities and levels of malondialdehyde (MDA), urea, and creatinine in the serum were estimated. Histological examinations of the kidney were monitored. **Results:** Exposure to dichlorvo slowered activities of kidney antioxidants, while it increased LPO levels. Levels of all disrupted parameters were alleviated by co-administration of AB extract. The malondialdehyde concentration of the rats treated with 200 and 400 mg/kg body weights of the extract significantly decreased ($p<0.05$) when compared with the untreated dichlorvos rats. But the creatinine concentration decreased significantly ($p<0.05$) when 200 and 400 mg/kg body weights of the extract of the treated dichlorvos animals were compared with the dichlorvos control. Furthermore, histological alterations in kidney were observedin dichlorvos untreated rats and were ameliorated in dichlorvos-induced treated rats with *Alstonia boonei*. **Conclusion:** In conclusion, the results indicated that dichlorvos showed a protective effect on dichlorvos induced nephrotoxicity.

**INTRODUCTION:** Organophosphate (OP) pesticides are among the most widely used synthetic chemicals for controlling a wide variety of pests.

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The main target of OP pesticides is acetyl cholinesterase (AChE) which hydrolyses acetylcholine in cholinergic synapses and neuromuscular junctions [1]. Pesticides are chemicals used to control the insects/pests in agriculture, household, and public health programmes. Indiscriminate uses of pesticides have led to a serious risk of toxicity in animals including humans. Dichlorvos (DDVP) is an organophosphate pesticide used to protect greenhouse plants, fruits and vegetables against mushroom flies, aphids, spider, mites, caterpillars,

thrips, and white flies. It is also used to treat a variety of parasitic worm infections in livestock, dogs, and humans. Dichlorvos is reported to cause toxicity of reproductive system, [2] pancreas [3], kidney and spleen [4], brain [5, 6], and immune system [7].

Pesticides are believed to damage the lipoidal matrix in cell, generating reactive oxygen species (ROS) and promoting oxidative stress.The lipophilicityoforganophosphate insecticides favors their incorporation in membranes. Therefore, insecticides may result from physicochemical changes at the level of membrane lipid structure and organization [8, 9].Endogenous non-enzymatic (glutathione, GSH) and enzymatic (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) antioxidants detoxify these ROS and protect cells. Due to continuous exposure of pesticides, level of these endogenous antioxidants decreases, leading to accelerated cell death. Use of cytoprotective agents in the form of exogenous antioxidants may help in scavenging excess ROS, helping in cell survival and longevity. A number of medicinal plants are reported to possess ROS scavenging and cytoprotective activity [10-13].

*Alstonia boonei* De Wild is large deciduous evergreen tree, usually up to 45m tall and 1.2m in diameter, belonging to the family Apocynaceae consisting of about 40-60 species. It is a native of tropical and subtropical Africa, Southeast Asia, Central America and Australia. ‘Alstonia’ named after Dr. C. Alston (1685-1760), a Professor of Botany at Edinburgh University. Its reported for treatment of malaria, intestinal helminthes, rheumatism, muscular pain, insomnia, and hypertension. It contains phytochemicals such as saponin, alkaloids, tannins and steroids [14, 15]. Although, medicinal importance of stem bark extract of *A. boonei* in ameliorating some disease conditions reported by several authors, however, to the best of our knowledge, there is lack of information on the effect of this plant on its neuroprotective potentials. *A. boonei* has been shown to possess many pharmacological and physiological activities such as antioxidants [16].

The increasing use of dichlorvos interested the researchers in reducing the damages caused for this class of pesticides in the environment. This stimulated us to examine the potential protective effect of *Alstonia boonei* extract on dichlorvos-induced nephrotoxicity in albino rats.

## MATERIALS AND METHODS:

### Chemicals

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate – DDVP) was bought from a local chemist in Ibadan, Nigeria. Thiobarbituric acids (TBA) were bought from Aldrich Chemical Co. (Milwaukee, WI, USA). Glutathione, hydrogen peroxide, 5, 5’-dithios-bis-2-nitrobenzoic acid (DNTB) and Epinephrine bought from Sigma Chemical Co., Saint Louis, MO USA. Trichloroacetic acid (TCA) and Thiobarbituric acid (TBA) was bought from British Drug House (BDH) Chemical Ltd., Poole, UK. Other reagents were of analytical grade and the purest quality available.

### Collection and extraction of *Alstonia booneis*tem bark

The stem bark of *Alstonia boonei*was procured from local suppliers in Ado-Ekiti (Ekiti State) and authenticated at the Department of Plant Science, Ekiti State University. The stem bark of *Alstonia boonei*air-dried and crushed into fine powder. The powdered part extracted with ethanol using maceration and the extract concentrated in vacuum at $40^0$C with rotary evaporator and water bath to dryness. The yield of the extraction was 5.01%.

### Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out with ethanolic extracts of *Alstonia boonei* stem bark for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods [17].

### Animals

Male Wistar rats (*Rattusnorvegicus*) weighing between 80-120 g bought from the animal house of the Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, Nigeria. Animals kept in aired cages at room temperature (28-$30^0$C) and preserved on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum.*

### Ethical approval

Rats handling and treatments conform to guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use. The ethical committee of the Afe Babalola University approved this study. All animals in this

study follow the institutional Animal Ethical Committee according to guidelines given by Committee for Control and Supervision of Experiments on animals (CPCSEA).

**Induction of Nephrotoxicity and drug feeding schedule**

Dichlorvos induced in groups II, III and IV. Briefly, dichlorvos dissolved in distilled water and after that managed by intravenous injection (through tail vein) at a dose of 50 mg/kg body weight.

**Study Design**

Twenty male rats divided into four groups of five rats each. Group I – Control (distilled water); Group II–Dichlorvos (50 mg/kgb.w.); Group III – *Alstonia boonei* (200 mg/kg b.w.) (14 days) + DDVP (50 mg/kg b.w.); Group IV – *Alstonia boonei* (400 mg/kg b.w.) (14 days) + DDVP (50 mg/kg b.w.).

**Preparation of tissue homogenate**

Kidney tissues were quickly removed, washed in ice-cold, isotonic saline, and blotted individually on ash-free filter paper. The tissues were then homogenized in 0.1M 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride buffer, pH 7.4 using a Potter-Elvehjem homogenizer at 4 °C, the crude tissue homogenate was then centrifuged at a speed of 9000 rpm for 15 min in cold centrifuge, and the supernatant was kept at -20 °C for estimation of GSH, SOD and CAT activities.

**Preparation of Serum**

Blood collected from the heart of the animals into plain centrifuge tubes and allowed to stand for 1 h. Serum prepared by centrifugation at 3,000 gfor 15 min in a Beckman bench centrifuge. The clear supernatant used for estimating serum lipid profile and enzymes.

**Biochemical tests**

Protein contents of the samples tested by the method of [18] using bovine serum albumin as standard. The alanine and aspartate aminotransferases (ALT and AST) tested by the combined methods of [19] and [20]. Lipid peroxidation level was tested by the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA), a product of lipid peroxides as described by [21]. The tissue superoxide dismutase (SOD) measured by the nitro blue tetrazolium (NBT) decrease method of [22]. Catalase (CAT) tested spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by [23]. Reduced glutathione level by the method of [24] this method is on developing a stable (yellow) colour when 5', 5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) mix to sulfhydryl compounds. The chromophoric product resulting from Ellman's reagent with the reduced glutathione (2-nitro-5-thiobenzoic acid) holds a molar absorption at 412 nm which is part to the reduced glutathione in the test sample. The glutathione peroxidase (GPx) tested by the method of Beutler E *et al*. [25].

When this substance is mixed with reduced glutathione, its absorption shifts to a longer wavelength 340 nm and increase at this wavelength provides a direct measurement of the enzymatic reaction.

**Determination of serum urea concentration**

Urea concentration in the blood was estimated by enzymatic method using Urease enzyme kit by modified Berthelot method [26]. Absorbance was measured using UV-240 Vis spectrophotometer.

**Determination of serum creatinine concentration**

Creatinine level in serum was estimated by the alkaline picrate method using creatinine kit method [26]. Absorbance was measured from UV-240 Vis spectrophotometer.

**Histopathology of tissues**

Kidney was removed and fixed in Bouin's fluid. Fixed materials were embedded in paraffin wax and sections of 5 micrometres thickness were cut. Slides were stained with haematoxylin and eosin for histological examination.

**Statistical analysis**

All the data are expressed in mean ± SEM. The significance of difference in means between control and treated animals was determined by One-way analysis of variance (ANOVA) followed by the Duncan multiple range test for analysis of biochemical data using SPSS (16.0). Values considered statistically significant at $p < 0.05$.

**RESULT:**

**Phytochemicals investigation**

It was found that ethanolic extract contained compounds known to have antioxidant activity like tannins, phlobatannins, flavonoids, anthocyanin, cardiac glycosides and alkaloids **(Table 1)**.

**TABLE 1: PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF ALSTONIA BOONEI STEM BARK**

| Phytochemical | Extract Content |
|---|---|
| Alkaloids | +++ |
| Tannin | ++ |
| Phlobatannins | ++ |
| Saponin | + |
| Flavonoids | +++ |
| Anthraquinones | ++ |
| Phenol | +++ |
| Cardiac glycosides | ++ |

+ = Trace amount Present ++ = Moderate amount present, +++ = Noticeable amount present

**Effects of *Alstonia boonei*stem bark on body weight and relative weight of organs of Dichlorvos-induced nephrotoxicity in rats**

In **Table 2**, there was significant increases ($p<0.05$) in the relative weight of kidney of dichlorvos untreated rats when compared with the control, while treatment with *Alstonia boonei* stem bark (100 and 200 mg/kg) significantly decrease the relative weight of kidney of dichlorvos-induced rats to values that statistically similar ($p>0.05$) to the control. All these changes induced by dichlorvos intoxication significantly ($p < 0.05$) restored to near normal levels on administration of *Alstonia boonei* stem bark.

**TABLE 2: CHANGES IN THE BODY WEIGHT AND RELATIVE WEIGHT OF ORGANS OF DICHLORVOS-INDUCED NEPHROTOXICITY IN RATS TREATED WITH ETHANOLIC EXTRACT OF *ALSTONIA BOONEI***

| Treatment | Body weight (g) | | Weight of organs (g) | Relative weight of organs |
|---|---|---|---|---|
| | Initial | Final | Kidney | kidney |
| Control | 100.25 ± 0.21 | 117.46 ± 5.32 | 0.89 ± 0.27 | 0.68 ± 0.05 |
| dichlorvos untreated | 112.08 ± 1.12 | 128.10 ± 4.96 | 0.54 ± 0.60 | 1.02 ± 0.08* |
| dichlorvos + 200mg/kg | 86.45 ± 2.23 | 125.55 ± 3.11 | 0.67 ± 0.22 | 0.53 ± 0.02** |
| dichlorvos + 400mg/kg | 98.02 ± 3.35 | 131.20 ± 2.09 | 0.78 ± 0.40 | 0.60 ± 0.03** |

Values are means ± S.D. of 5 animals per group, dichlorvos = at 50 mg/kg, dichlorvos Treated = *Alstonia boonei* at 200 mg/kg, dichlorvos treated = *Alstonia boonei* at 400 mg/kg, *significantly different from Control ($p < 0.05$), ** significantly different from dichlorvos untreated ($p < 0.05$).

**Effects of *Alstonia boonei*stem barkon antioxidant parameters and marker enzymes in Dichlorvos-induced nephrotoxicity in rats**

Administration of dichlorvos significantly increased ($p<0.05$) serum, and kidney lipid peroxidation (LPO) products measured as thiobarbituric acid reactive substances respectively **(Table 3)**. However, treatment with *Alstonia boonei*extract completely ameliorated dichlorvos-induced increase in LPO. In dichlorvos-induced rats, the activities of kidney GSH, SOD and CAT as well as GPx decreased significantly relative to the control **(Table 4)**.

**TABLE 3: CHANGES IN THE LEVELS OF LIPID PEROXIDATION IN DICHLORVOS-INDUCED NEPHROTOXICITY RATS TREATED WITH ETHANOLIC EXTRACT OF *ALSTONIA BOONEI***

| Treatments | KIDNEY (µmol MDA/mg protein) | SERUM (µmol MDA/mg protein) |
|---|---|---|
| Control | 6.05 ± 0.02 | 6.82 ± 0.08 |
| Dichlorvos Untreated | 8.89 ± 0.15* | 8.38 ± 0.02* |
| Dichlorvos + 200 mg/kg | 5.54 ± 0.04** | 5.90 ± 0.36** |
| Dichlorvos + 400 mg/kg | 6.02 ± 0.05** | 6.25 ± 0.05** |

Values are means ± S.E.M. of 5 animals per group, dichlorvos Treated = *Alstonia boonei* at 200 mg/kg, dichlorvos treated = *Alstonia boonei* at 400 mg/kg, *significantly different from control ($p < 0.05$), ** significantly different from dichlorvos untreated ($p < 0.05$).

**TABLE 4: CHANGES IN THE LEVELS OF KIDNEY ANTIOXIDANT PARAMETERS IN DICHLORVOS-INDUCED RATS TREATED WITH ETHANOLIC EXTRACT OF *ALSTONIA BOONEI***

| Treatment | Kidney | | | |
|---|---|---|---|---|
| | GSH | GPx | SOD | CAT |
| | (mg /g tissue) | | (U/mg protein) | |
| Control | 40.85 ± 0.05 | 47.55 ± 0.73 | 48.46 ± 1.21 | 45.78 ± 1.06 |
| Dichlorvos untreated | 22.25 ± 0.51* | 25.47 ± 0.83* | 21.42 ± 0.48* | 22.35 ± 0.22* |
| Dichlorvos + 200 mg/kg | 36.42 ± 0.25** | 38.51 ± 0.45** | 40.12 ± 0.28** | 39.13 ± 0.51** |
| Dichlorvos + 400 mg/kg | 38.25 ± 0.17** | 44.21 ± 0.21** | 45.38 ± 1.39** | 42.34 ± 0.72** |

Values are means ± S.E.M. of 5 animals per group, dichlorvos Treated = *Alstonia boonei* at 200 mg/kg, dichlorvos treated = *Alstonia boonei* at 400 mg/kg, *significantly different from control ($p < 0.05$), ** significantly different from dichlorvos untreated ($p < 0.05$).

Excellent performance of extract at (400 mg/kg) reversed the adverse effect of dichlorvos by normalizing this enzymic antioxidant. *Alstonia boonei* treatment to dichlorvos treated groups caused a significant increase in GPx activities as well as a noticeable increase in GSH level. In dichlorvos-induced rats, serum ALT and AST were significantly increased **(Table 5)** relative to the control. Treatment with *Alstonia boonei* resulted in significant protection of the kidney, as indicated by reductions in the elevated levels of ALT and AST; however, there was evidence of amelioration in the treated group.

**TABLE 5: CHANGES IN THE ACTIVITIES OF SERUM AND KIDNEY ALANINE AND ASPARTATE AMINOTRANSFERASES IN DICHLORVOS-INDUCED NEPHROTOXICITY RATS TREATED WITH ETHANOLIC EXTRACT OF *ALSTONIA BOONEI***

| Treatments | KIDNEY (U/L) | | SERUM (U/L) | |
|---|---|---|---|---|
| | AST | ALT | AST | ALT |
| Control | 78.58 ± 0.03 | 75.22 ± 2.56 | 4.54 ± 1.77 | 6.37 ± 1.46 |
| Dichlorvos untreated | 22.42 ± 2.45* | 21.11 ± 1.78* | 8.39 ± 0.56* | 9.89 ± 2.24* |
| Dichlorvos + 200 mg/kg | 58.60 ± 1.25** | 57.20 ± 1.44** | 4.13 ± 1.50** | 5.78 ± 1.38** |
| Dichlorvos + 400 mg/kg | 63.02 ± 0.32** | 62.79 ± 1.22** | 4.01 ± 1.42** | 6.01 ± 1.28** |

Values are means ± S.E.M. of 5 animals per group, dichlorvos untreated group = at 50 mg/kg dichlorvos Treated = *Alstonia boonei* at 200 mg/kg, dichlorvos treated = *Alstonia boonei* at 400 mg/kg, *significantly different from control ($p < 0.05$), ** significantly different from dichlorvos untreated ($p < 0.05$).

**Effects of *Alstonia boonei*stem barkon serum protein, urea and creatinine in Dichlorvos-induced nephrotoxicity in rats**

There was a significant decrease in the levels of serum total protein in the dichlorvos group when compared with the control group **(Table 6)**. However, levels of this compound in the serum were significantly increased in dichlorvos treated groups when compared with the dichlorvos control group. Levels of urea and creatinine in the serum of the dichlorvos group were significantly increased when compared with the control group **(Table 6)**. However, levels of serum urea and creatinine were significantly decreased in dichlorvos treated groups when compared with the dichlorvos group. The ameliorative effect treatment of *Alstonia boonei* on the levels of serum urea and creatinine was more prominent.

**TABLE 6: LEVELS OF TOTAL PROTEIN, UREA AND CREATININE IN THE SERUM OF CONTROL AND EXPERIMENTAL GROUPS OF RATS**

| Treatments | Protein (g/dl) | Urea (mg/dl) | Creatinine (mg/dl) |
|---|---|---|---|
| Control | 12.28 ± 0.49 | 4.89 ± 0.01 | 5.84 ± 0.02 |
| Dichlorvos untreated | 4.48 ± 0.02* | 7.33 ± 0.78* | 8.24 ± 0.04* |
| Dichlorvos + 200 mg/kg | 8.89 ± 0.55** | 2.89 ± 0.55** | 3.58 ± 0.54** |
| Dichlorvos + 400 mg/kg | 10.21 ± 0.22** | 3.76 ± 0.24** | 4.68 ± 0.04** |

Values are means ± S.E.M. of 5 animals per group, dichlorvos untreated group = at 50 mg/kg dichlorvos Treated = *Alstonia boonei* at 200 mg/kg, dichlorvos treated = *Alstonia boonei* at 400 mg/kg, *significantly different from control ($p < 0.05$), ** significantly different from dichlorvos untreated ($p < 0.05$).

**Effects of *Alstonia boonei*stem bark on the histology of kidney**

The histology of kidney slide of dichlorvos untreated rats showed mononuclear cell infiltration, mild spongiosis, severe congestion and glomerular atrophy **(Figure 1)**. Treatment with ethanolic extract of stem bark *Alstonia boonei* (200 and 400 mg/kg) confirmed the nephroprotective activity as a significant recovery of damage and decreased necrosis was evident against dichlorvos induced oxidative damage in the kidney of the rats, which is similar to their control. The histological results further corroborated the biochemical findings suggesting the useful effects of *Alstonia boonei*stem bark in dichlorvos-induced nephrotoxicity in rats.

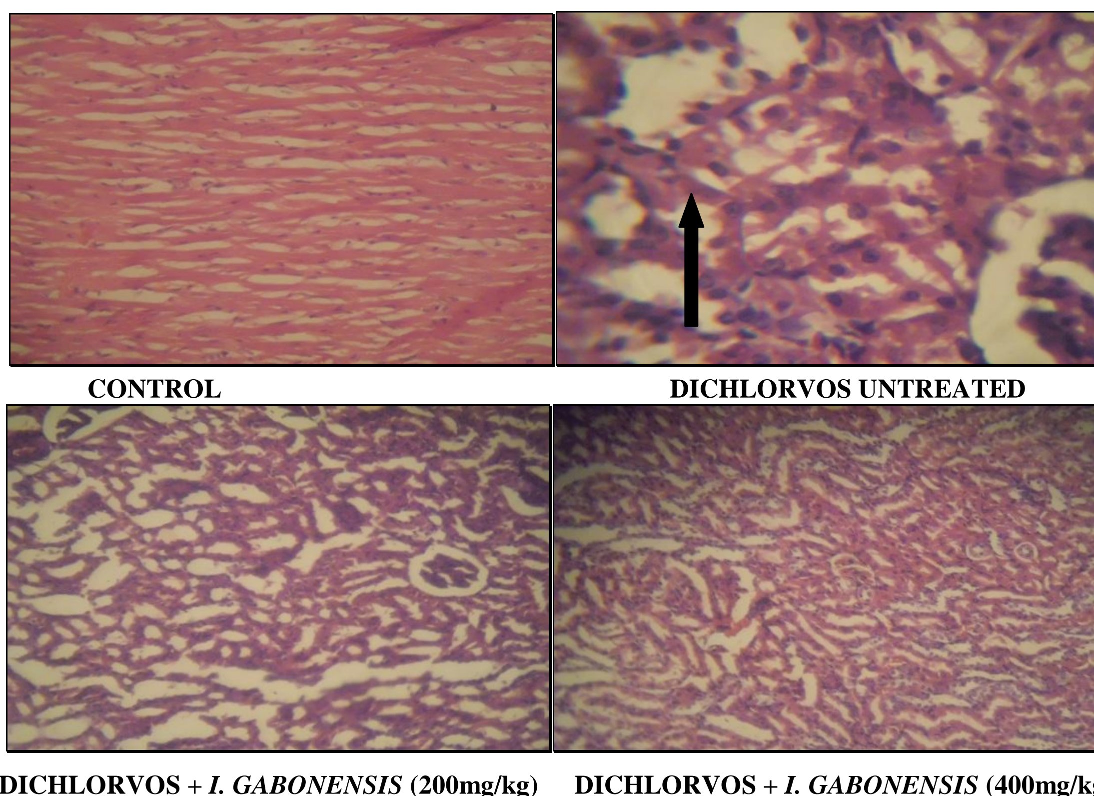


**FIGURE 1. CHANGES IN HISTOLOGY OF KIDNEY SAMPLES OF DICHLORVOS-INDUCED NEPHROTOXICITY IN RATS TREATED WITH *ALSTONIA BOONEI* ETHANOLIC STEM BARK EXTRACT**

**Black arrows**: mononuclear cell infiltration, tubular degeneration, necrosis and severe renal cortical congestion

**DISCUSSION:** Dichlorvos is one of the environmental pollutant which showed a broad spectrum toxicological effects and biochemical dysfunctions constituting serious hazards to health. Histological examination of kidney of dichlorvos-intoxicated rats revealed many alterations such as tubular degeneration, atrophy of glomeruli, leucocytic infiltrations and congestion of renal blood vessels. Pesticides cause various histopathological changes in kidney tissues of experimental animals [27, 28, 29, 30].

Regarding the histopathological observation, *Alstonia boonei* treated dichlorvos induced nephrotoxicity rats the observed pathological impairments by dichlorvos have been recovered significantly which indicates that *Alstonia boonei* is capable of preventing the nephron damage induced by dichlorvos. The phytochemical study of *Alstonia booneistem* bark extracts revealed the presence of polyphenol-rich compounds. Polyphenols have been suggested to decrease the oxidative stress in human. Flavonoids found in the extract may inhibit the oxidative stress by scavenging free radicals by acting as reducing agent, hydrogen atom donating molecules or singlet oxygen quenchers; chelating metal ions and sparing other antioxidants (e.g. carotene, vitamin C and E) [31].

Literature reveals that, the carbonyl groups present in the flavonoids and phenolic compounds were responsible for antioxidant activity [32]. This investigation revealed that the *Alstonia boonei* contain pharmacologically active substance (s) such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the antioxidant activity.

Literature survey indicates that there is no scientific evidence to support the nephroprotective effect of ethanolic extract of *A. boonei*. Therefore, the present study is undertaken to investigate nephrotoxicity effect of dichlorvos and the effects of *A. boonei* ethanol leaf extract in ameliorating it. In dichlorvos induced rats treated with *Alstonia boonei*, the changed body weight and kidney weight parameters recovered to near normal levels due to the antioxidant effects of found in *Alstonia boonei* stem bark. *A. boonei* has been shown to possess

many pharmacological and physiological activities such as antioxidants [16]. Hence, the probable mechanism of nephroprotection by *Alstonia boonei* may be attributed to its antioxidant and free radical scavenging property.

Administration of dichlorvos resulted in a significant increase in the renal content of MDA indicating increased lipid peroxidation which implicates the renal oxidative stress. Moreover, dichlorvos caused a significant decrease in the activities of SOD and CAT. Antioxidant enzymes, mainly SOD and CAT are the first line of defense against free radical induced oxidative stress. SOD is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide, and CAT is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water [33].

A decrease in the level of antioxidant enzymes and an increase in lipid peroxidation level were recorded after dichlorvos intoxication [34, 35, 36]. Damage of renal tissue observed in the present study may be resulted from the increase in lipid peroxidation and decrease of antioxidant enzymes in the kidney following exposure to dichlorvos. Dichlorvos exposure leads to decrease in GPx activity in our study, which may be due to the depleted level of GSH.

A decrease in SOD, CAT, and GPx depletion was significant in the dichlorvos untreated group indicating that dichlorvos induced nephrotoxicity. In treatment groups, *Alstonia boonei* showed protective role by decreasing the LPO level in animals exposed to dichlorvos. *Alstonia boonei* worked as an antioxidant and increased the level of non-enzymatic antioxidant GSH, enzymatic antioxidants CAT, SOD, and GPx, and the AST and ALT in animals exposed to dichlorvos. *Alstonia boonei* reduces the oxidative stress in the animals, by its high ROS scavenging capacity and protecting the antioxidant enzymes from being denatured.

Well known biomarkers (ALT and AST) were examined to evaluate the nephroprotective effects of *Alstonia boonei*. Consistent with previous studies, our study confirmed that dichlorvos exposure damaged the kidney, as shown by elevation of the serum aminotransferase activities and morphological changes observed in the kidney sections.

Many pesticides can cause some toxic and adverse effects on the kidney tissues [37]. Kidney is one of the targets organs of experimental animals attacked by organophosphate compounds [38, 39]. Urea, uric acid and creatinine levels are kidney function parameters [38, 40]. Pesticides can alter plasma urea, and creatinine levels [38, 41, 42]. In this study, dichlorvos exposure increased in the urea and creatinine levels when compared to control rats. This increase may be due to kidney damage caused by dichlorvos. Urea is the end product of protein catabolism. Increased blood urea is correlated with an increased protein catabolism in mammalian body and/or referred to kidney dysfunction [42, 43]. The levels of urea in the plasma of rats are tested as indicators for kidney functions [44]. Also, the high levels of blood urea results caused from increased breakdown of tissue or impaired excretion [42].

In this study, this increased urea level is due to toxic effects of dichlorvos. The creatinine excretion is dependent almost on the process of glomerular filtration. Previous study reported that significant rise in the serum creatinine level may due to the impairment of the glomerular function and tubular damage in the kidneys [38]. Creatinine level is a good risk marker for chronic renal insufficiency [41, 45].

Increased creatinine level shows that damage of the glomerular function and tubular damage in the kidneys [46, 47]. The obtained results in the current study showed that the ethanol extract of *Alstonia boonei* had a protective effect against dichlorvos induced kidney damage. This was obvious as there was a significant decrease in urea and creatinine levels in the *Alstonia boonei* treated group when compared with the dichlorvos group. To conclude, our studies have shown that the entire plant of *Alstonia boonei*, possesses marked nephroprotective activity and thus has a promising role in the treatment of acute renal injury induced by nephrotoxins, especially dichlorvos (DDVP).

**CONCLUSION:** It can be concluded that our studies have shown that ethanolic extract of *Alstonia boonei* possess marked nephroprotective activity which is already reported in the literature. Further, isolation of active components and its nephroprotective activity in chronic renal failure model have to be evaluated.

## CONFLICT OF INTEREST:

The authors declare that they have no conflict of interests.

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