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# Comparative Biochemical Effects of Natural and Synthetic Pesticides on Preserved *Phaseolus vulgaris* in Male Albino Rats

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## ABSTRACT

*The indiscriminate use of pesticides and botanicals to preserve legumes is a common practice. Phaseolus vulgaris is a legume commonly preserved to protect against pest attacks and insect infestations. This study evaluates the biochemical effect of Phaseolus vulgaris preserved with natural and synthetic pesticides in albino rats. Thirty-six (36) male albino rats weighing between 150-200 g were randomly assigned into six (6) groups A, B, C, D, E, and F with 6 rats each. Phaseolus vulgaris was preserved separately in five air-tight containers, each having 1000g of beans, and preserved with wood ash, pepper, dichlorvos, aluminum phosphide, and last beans without preservatives. The preserved beans were milled into powder and used as a dietary supplement for the experimental animals. All the animals were fed freely with animal feed and water. Group A was fed animal feed and Group B was fed Phaseolus vulgaris without preservatives. While Groups C, D, E, and F were fed with Phaseolus vulgaris preserved with wood, pepper, dichlorvos, and aluminum phosphide respectively for 60 days. The rats were euthanized and blood samples were collected after the termination of the study. The rats in group D showed a significant ($P<0.05$) increase in liver enzyme activity. A significant ($P<0.05$) decrease was observed in most of the kidney parameters in the groups except for the rats fed beans preserved with dichlorvos in sodium ion. A significant ($P<0.05$) increase in antioxidants (CAT, SOD, and GPx) was observed in the rats fed beans preserved with pepper while the rats fed beans preserved with dichlorvos showed a significant($P<0.05$) increase in malondialdehyde which shows the oxidative effect of the residual components of the preservatives. There was a significant ($P<0.05$) increase in CRP in the rats exposed to beans preserved with dichlorvos and aluminum phosphide. Also, there was a significant ($P<0.05$) decrease in CRP in the rats fed beans preserved with ash and rats fed beans preserved with pepper. This indicates that the use of these pesticides has biochemical effects that could be toxic. The result also suggests that pepper and ash which are considered a safer alternative for preservation may be harmful over a prolonged period despite their antioxidant effects.*

**Keywords:** *Pesticides, Pest, micronutrients, Aluminum phosphide, Insect infestations.*

## INTRODUCTION

Legumes are the third largest family of flowering plants belonging to the family Leguminosae also called Fabaceae that produce seeds within a pod and consisting of over 20,000 species [1]. Common legumes include kidney beans, lima beans, peas, clovers, lentils, soybeans, lupins, lotus, sprouts, tamarind green beans, and peanuts [2]. Legumes are very essential, especially in terms of nutrition. They contain the essential amino acids that are needed for building proteins, complex carbohydrates, dietary

fiber, unsaturated fats, vitamins, and essential minerals needed for human diet [3].

*Phaseolus vulgaris* also known as kidney beans is a major legume cultivated worldwide for its edible seeds and pods. It is a highly polymorphic warm-season, herbaceous annual crop. It is used in a variety of traditional dishes and is regarded as a healthy component of a well-balanced diet. It is a member of the legume family Fabaceae, most of whose members acquire the nitrogen they require through association with rhizobia, a species of nitrogen-fixing bacteria [4]. *Phaseolus vulgaris* contain several vitamins and minerals such as molybdenum, folate, iron, copper, manganese, and potassium [5]. They also contain a variety of bioactive plant compounds such as isoflavones, anthocyanins, phytic acid as well as phytohaemagglutinin (PHAs) which is a toxic lectin only found in raw or improperly cooked *P. vulgaris*. PHAs are carbohydrate-binding proteins that interact with mammalian cells [6].

Pest is a major causative factor resulting in the low yields and damage of Nigeria crops. They are harmful to the tissue component and developmental stage of the plant. Pest and insect infestation have rendered stored foods worthless, creating the need for the use of pesticides and other alternatives for storage. The dire need for satisfactory results and the ignorance of farmers and traders about pesticide toxicity has led to its misuse, abusive application, and inadequate usage. Different types of pesticides such as aluminum phosphide and dichlorvos are been used to save grains and seeds from rodents as well as pests [7]. The most common insect pest usually blamable for bean damage is known as the bean weevil, *Acanthoscelides obtectus* [8]. The use of these pesticides is to increase protection against pest or insect attacks on post-harvest crops and retain the current production as well as yield level [9].

Natural preservatives such as pepper have been used for years to extend the shelf life of various foods, making them last longer and enhancing the color, taste, and aroma of food. It has also gained acceptance from consumers because of the antioxidant power of spices. Antioxidants are used as food additives to prevent and delay the negative effects of oxygen in the food matrix [10]. Also, in most climates where seeds are susceptible to microorganisms or insect infestation, storing seeds in wood ash has proven effective in preventing both rot and insect predation [11]. Wood ash is composed of the organic and inorganic residue that remains after the wood combustion, this is used as a means of biological control and is generally favored as a method of storing seeds because it does not have any of those disadvantages of chemicals and tends to be more durable in its effect [12].

Dichlorvos and Aluminum phosphide are insecticides used to control household and stored products of insects. It acts against insects as a contact and stomach poison [13]. Dichlorvos is a class of organophosphate used against household insects as well as agricultural pests [13]. Dichlorvos exerts its toxic effect like other organophosphate insecticides by irreversibly inhibiting neural acetylcholinesterase which provokes the accumulation of acetylcholine in the synapse with the destruction of nerve function [14].

Aluminum phosphide (AlP) is a solid pesticide that rapidly became one of the most commonly used grain fumigants because of its properties which are considered to be near ideal; it is toxic to all stages of insects, highly potent, and does not affect seed viability [15]. The tablets or pellets gradually lose their potency on exposure to the atmosphere as they release phosphine gas, which is the active pesticidal component, and leave behind a nontoxic residue in the form of aluminum hydroxide [16]. It emerges as a poison of suicide as this pesticide has no effective antidote and is freely available in the market [17]. The mechanism of action in the case of an oral intake causes the phosphine gas released to be absorbed by the gastrointestinal tract with simple diffusion and excreted by the kidneys and lungs. Phosphine, like cyanide, is a highly toxic gas that inhibits mitochondrial cytochrome oxidase and cellular oxygen utilization [18, 19]. It rapidly perturbs mitochondrial conformation and inhibits oxidative respiration by 70%. This situation can result in a severe decrease in mitochondrial membrane potential [20]. In presence of this gas cellular superoxide and peroxide radicals are generated, with subsequent cellular damage by lipid peroxidation [21].
The primary aim of this study is to evaluate and compare the biochemical effect of *Phaseolus vulgaris* preserved with natural preservatives and pesticides.

## MATERIALS AND METHODS

### Materials

The *P. vulgaris* was gotten from the area of cultivation in Keffi, Keffi Local Government Area, Nasarawa State, Nigeria. The sample was collected and transported to


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laboratory, where they were cleaned and sorted to remove stones and dirt. Natural preservatives used for the research were: wood ash gotten from neem tree and *Capsicum frutescens* commonly known as bird eye pepper. Pesticides used for the research were: Dichlorvos and Aluminum phosphide.

The chemicals and kits used in biochemical analysis were of analytical grade and purchased from reputable companies.

## METHODS

### Beans Preservation and Preparation

The stem from neem tree was burnt to get ash. The cooled ash was sieved to remove dirt. Then 300 g was weighed and packed into nylon bags. Fresh birds eye pepper (*Capsicum frutescens*) was purchased from the local market and dried in the sun. Then 300 g of sun dried pepper was weighed and packed in to nylon bags. The cleaned *P. vulgaris* sample was divided into five parts, each bucket contained seeds that weighed 1000 g and the buckets were tightly sealed. A bucket of *P. vulgaris* with no preservative/ treatment, the second bucket contained *P. vulgaris* preserved with 300 g of birds eye pepper, the third bucket contained *P. vulgaris* preserved with 300 g of ash, the fourth bucket contained *P. vulgaris* preserved with 2 mls of DDVP and the fifth contained *P. vulgaris* preserved with 4 tablets of Aluminum phosphide. The buckets containing the beans were properly labeled and stored for a period of six (6) months.

After the storage period the sample was milled into powder, packed into clean polythene bags and used as dietary supplement for the rats.

### Experimental Animals

A total of 36 male Wistar albino rats weighing between 150-200 grams were used for this study. They were purchased from the University of Jos, animal house. The rats were housed in clean cages, maintained in an air-conditioned experimental temperature, and left to acclimatize to laboratory conditions for two weeks before the experiment. Standard poultry mash (vital growers mash) was used as a basal diet during the experimental period. The control and experimental animals were provided with drinking water.

### Experimental Design

The 36 adult male Wistar albino rats were randomly selected and divided into six (6) groups with each group having six rats. The following dietary intervention was carried out at a ratio of seventy (70) grams of preserved *P. vulgaris* to thirty (30) grams of vital growers mash for 60 days

Group A (Control): Vital growers mash + Water

Group B: Vital growers mash + *P. vulgaris* without preservatives + Water

Group C: Vital growers mash + *P. vulgaris* preserved with of ash + Water

Group D: Vital growers mash + *P. vulgaris* preserved with pepper + Water

Group E: Vital growers mash + *P. vulgaris* preserved with of dichlorvos + Water

Group F: Vital growers mash + *P. vulgaris* preserved with aluminum phosphide + Water

At the end of the experimental period, the animals were euthanized and blood serum samples collected for biochemical analysis.

### Biochemical analysis

The biochemical analysis was performed as shown below:

Serum alanine transaminase (ALT) and serum aspartate transaminase (AST) activity was carried out using teco kit according to the colorimetric method described by Reitman and Frankel [22].

Blood urea and serum creatinine were assayed using the Bartels and Bohmer [23] method as outlined in the randox kit. Sodium ion was determined according to the method of Trinder [24] and Maruna [25] as described in the teco kit. While potassium ion was determined using the method of Terri and Sesin [26]. Superoxide dismutase activity was assayed according to the method described by the International Federation of Clinical and Applied Chemistry [27] as outlined in the randox kit. Catalase activity was assayed with the randox kit according to the method described by Aebi [28]. Glutathione peroxidase was measured according to the method of Ursini *et al.* [29].

The concentration of malondialdehyde was evaluated in the serum according to the method described by

Wallin *et al.* [30].

The C-reactive protein test was carried out according to the method described by Smits *et al.* [31] using the Wellcome diagnostic kit for its qualitative and quantitative analysis.

### Data and Statistical Analysis

Statistical analysis of the data was carried out with SPSS version 25.0 using One Way Analysis of Variance (ANOVA). The statistically analyzed data were reported as Mean ± SD. A significant difference was accepted at a 95 % confidence level of probability i.e. if $p < 0.05$.

## RESULTS

### Effects of Consumption on Liver Enzymes

The result in Table 1 presents the assessment of the changes in the serum levels of liver enzymes of the experimental animals. The results show that the PV group (24.1967 ± 2.77085) had a significant ($p < 0.05$) increase in the levels of serum aspartate aminotransferase (AST) when compared with the control group (15.3767 ±1.38721). The group PVA (20.3833 ± 2.93004) and PVP (33.3433 ± 3.12085) were also revealed to be significant ($p < 0.05$) when compared with the control group. The results showed elevated levels of serum AST in PVD (18.1367 ± 1.09187) and PVAL (17.4000 ± 1.88724) groups in comparison with the control group (15.3767 ±1.38721) even though there was no significance ($p > 0.05$) in the changes. The levels of Alanine aminotransferase (ALT) were also reported in table 4.1. The control group showed a significant ($p < 0.05$) increase in the level of serum ALT (15.1167 ± 2.76841) when compared with the serum levels of ALT in groups PVP (30.8200 ± 1.74106) and PVD (18.2330 ± 2.89686). The serum ALT levels of group PV did not show any significant ($p > 0.05$) difference to any other group except group PVP (30.8200 ± 1.74106) where elevations were observed.

**Table 1:** Effect of Consumption on Serum Liver Enzymes.

| Groups | AST (IU/L) | ALT (IU/L) |
|---|---|---|
| Control | 15.38±1.39$^{a}$ | 15.12± 2.77$^{a}$ |
| PV | 24.20±2.77$^{c}$ | 17.17±1.13$^{ab}$ |
| PVA | 20. 38±2.93$^{b}$ | 17.05±2.07$^{ab}$ |
| PVP | 33.34±3.12$^{d}$ | 30.82±1.74$^{c}$ |
| PVD | 18.14±1.10$^{ab}$ | 18.23±2.90$^{b}$ |
| PVAL | 17.40±1.89$^{a}$ | 17.86±2.28$^{ab}$ |

Figures with the same alphabets as superscripts do not have a statistically significant difference at $p < 0.05$.Data is presented as mean ± SD. AST= Aspartate amino transferase, ALT= Alanine amino transferase, PV = Rats fed *Phaseolus vulgaris* without preservatives, PVA= Rats fed *Phaseolus vulgaris* preserved with wood ash, PVP= Rats fed *Phaseolus vulgaris* preserved with pepper, PVD= Rats fed *Phaseolus vulgaris* preserved with dichlorvos, PVAL= Rats fed *Phaseolus vulgaris* preserved with aluminum phosphide

### Effects of Consumption on Kidney Function Test

The results for the analysis of kidney function showed values that are reported in Table 2. The result shows that the levels of $Na^+$ in the control group showed a significant ($p < 0.05$) decrease in groups PV and PVP. There was no significant ($p > 0.05$) difference between the control group and the PVA, PVD, and PVAL groups. The levels of $K^+$ in the groups PVA, PVAL, and PVD showed a significant ($p < 0.05$) decrease when compared with the control group. The results did not reveal any significant ($p > 0.05$) difference between the control group and PV. The results for urea analysis recorded a significant ($p < 0.05$) increase when the PV was compared to the control group. The PVP also recorded a significant ($p < 0.05$) decrease when compared with the control group. When compared to the control group, PVD and PVAL respectively showed no significant ($p > 0.05$) difference. Creatinine levels revealed that the control group was significant ($p < 0.05$) to only PV. The other groups, PVA, PVP, PVD, and PVAL showed no significant ($p > 0.05$) difference when compared with the control group.

**Table 2:** Effect of Consumption on Kidney Function Parameters

| Groups | $Na^+$ (mmol/L) | $K^+$ (mmol/L) | Urea (mg/dl) | Creatinine (mmol/L) |
|---|---|---|---|---|
| Control | 128.41 ± 1.78$^{b}$ | 7.02 ± 2.23$^{b}$ | 5.40 ± 0.95$^{b}$ | 2.01 ± 1.17$^{a}$ |
| PV | 121.17 ± 1.81$^{a}$ | 7.08 ± 0.99$^{b}$ | 7.27 ± 1.40$^{c}$ | 4.59 ± 0.80$^{b}$ |
| PVA | 125.85± 4.80$^{ab}$ | 5.32 ± 1.40$^{a}$ | 6.19 ± 0.73$^{b}$ | 1.91 ± 1.47$^{a}$ |
| PVP | 120.62 ± 2.18$^{a}$ | 7.49 ± 0.28$^{b}$ | 4.35 ± 0.50$^{a}$ | 1.98 ± 0.28$^{a}$ |
| PVD | 131.40 ± 8.99$^{b}$ | 5.20 ± 0.23$^{a}$ | 5.41 ± 0.47$^{b}$ | 2.23 ± 0.37$^{a}$ |
| PVAL | 126.24 ± 5.88$^{b}$ | 5.18 ± 0.13$^{a}$ | 5.54 ± 0.24$^{b}$ | 2.12 ± 0.62$^{a}$ |


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Figures with the same alphabets as superscripts do not have a statistically significant difference at $p < 0.05$. Data is presented as mean ± SD. $Na^+$= Sodium ion, $K^+$= Potassium ion, PV = Rats fed *Phaseolus vulgaris* without preservatives, PVA= Rats fed *Phaseolus vulgaris* preserved with wood ash, PVP= Rats fed *Phaseolus vulgaris* preserved with pepper, PVD= Rats fed *Phaseolus vulgaris* preserved with dichlorvos, PVAL= Rats fed *Phaseolus vulgaris* preserved with aluminum phosphide

**Effects of Consumption on Antioxidant activity and Malondialdehyde Concentration**

The results of the analysis of antioxidants are presented in Table 3. The results in the table revealed changes in the levels of CAT, even though only the values in group PV (7.73 ± 1.70) and PVP (9.03 ± 1.45) showed a significant ($p < 0.05$) increase to the control group (5.78 ± 0.81). The serum levels of CAT in PV (7.73 ± 1.70) showed no significant ($p > 0.05$) difference to the serum levels of CAT in PVP (9.03 ± 1.45). The GPx activity was only significant ($p < 0.05$) in group PVA when compared with the control group. There was a significant ($p < 0.05$) increase in SOD activity in only groups PV (19.60 ± 2.19) and PVP (19.45 ± 1.84) when compared with the control group (15.86 ± 1.84). The levels of MDA in the control group (3.45 ± 1.25) showed a significant ($p < 0.05$) increase to only the PVD group (4.98 ± 0.57).

**Effects of Consumption on C-reactive protein**

Table 4 shows the results for the mean values of the levels of CRP. The table records changes in the values of the CRP in the test groups when compared to the control group. There was a significant ($p < 0.05$)

**Table 3:** Effect of Consumption on Serum Levels of Antioxidants and Malondialdehdyde

| GROUPS | CAT (IU/L) | GPx (IU/L) | SOD (IU/L) | MDA (mg/dl) |
|---|---|---|---|---|
| Control | 5.78 ± 0.81$^{a}$ | 26.78 ± 4.44$^{bc}$ | 15.86 ± 1.84$^{a}$ | 3.45 ± 1.25$^{ab}$ |
| PV | 7.73 ± 1.70$^{b}$ | 25.08 ± .15$^{abc}$ | 19.60 ± 2.19$^{b}$ | 3.82 ± 0 .73$^{b}$ |
| PV A | 5.50 ± 0.49$^{a}$ | 28.64 ± 1.26$^{c}$ | 17.19 ± 1.19$^{ab}$ | 3.73 ± 0.88$^{b}$ |
| PV P | 9.03 ± 1.45$^{b}$ | 24.69 ± 4.17$^{ab}$ | 19.45 ± 1.84$^{b}$ | 2.37 ±0 .84$^{a}$ |
| PV D | 5.33 ± 1.15$^{a}$ | 22.94 ± 1.31$^{a}$ | 15.13 ± 3.77$^{a}$ | 4.98 ± 0.57$^{c}$ |
| PV AL | 5.72 ± 0.87$^{a}$ | 23.70 ± 2.42$^{ab}$ | 16.27 ± 1.01$^{a}$ | 4.26 ± 1.78$^{bc}$ |

Figures with the same alphabets as superscripts do not have a statistically significant difference at $p < 0.05$. Data is presented as mean ± SD. CAT= Catalase, GPx= Glutathione peroxidase, SOD= Superoxide dismutase, MDA= Malondialdehyde, PV= Rats fed *Phaseolus vulgaris* without preservatives, PVA= Rats fed *Phaseolus vulgaris* preserved with wood ash, PVP= Rats fed *Phaseolus vulgaris* preserved with pepper, PVD= Rats fed *Phaseolus vulgaris* preserved with dichlorvos, PVAL= Rats fed *Phaseolus vulgaris* preserved with aluminum phosphide decrease in the levels of CRP in groups PV (57.134 ± 3.16), PVA (56.05 ± 3.20), and PVP (57.08 ± 1.74) respectively when compared with the control group (62.98 ± 4.74). The PVAL group (66.65 ± 2.86) and PVD group (71.35 ± 1.99) respectively recorded significant ($p < 0.05$) increases in CRP levels when compared with the control group (62.98 ± 4.74).

**Table 4:** Effect of Consumption on Serum C - reactive protein

| Groups | CRP (ug/ml) |
|---|---|
| Control | 62.98 ± 4.74$^{b}$ |
| PV | 57.13 ± 3.16$^{a}$ |
| PVA | 56.05 ± 3.20$^{a}$ |
| PVP | 57.08 ± 1.74$^{a}$ |
| PVD | 71.35 ± 1.99$^{d}$ |
| PVAL | 66.65 ± 2.86$^{c}$ |

Figures with the same alphabets as superscripts do not have a statistically significant difference at $p < 0.05$. Data is presented as mean ± SD. CRP= C-reactive protein, PV = Rats fed *Phaseolus vulgaris* without preservatives, PVA= Rats fed *Phaseolus vulgaris* preserved with wood ash, PVP= Rats fed *Phaseolus vulgaris* preserved with pepper, PVD= Rats fed *Phaseolus vulgaris* preserved with dichlorvos, PVAL= Rats fed *Phaseolus vulgaris* preserved with aluminum phosphide

## DISCUSSION

The preservation of food and food crops has come a long way. Preservatives are used to keep microorganisms and/or insects that would otherwise pose a danger to stored food and food crops, thereby reducing the integrity of the material. The screening of blood constituents of experimental animals for research plays a vital role in studying the biochemical effect of preservatives [32]. The liver plays a primary role in the detoxification process of whatever gains access to the body [34]. This role contributes to

exposing the liver to danger. A set of tests exists to check suspected hepatic disorder, measure the outcome of disease, or simply evaluate the status of liver function. These tests involve the screening of blood, which gives information about a wide range of disease conditions [35]. It is well understood that any destruction or damage to the liver causes the release of enzymes from hepatocytes into the serum and later on the condition advances to necrosis or leads to alteration in cell membrane permeability [36]. The present study reveals the changes in the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), two common aminotransferases. In the group that was fed with beans preserved in pepper, PVP showed an even higher level of AST (33.34 ± 3.12) and ALT (30.82 ± 1.74) when compared to the control group with values of 15.38 ± 1.39 and 15.12 ± 2.77 respectively at $p > 0.05$. This suggests that pepper may be hepatotoxic on its own or in combination with the effect of lectins found in *Phaseolus vulgaris*. The increase in serum AST and ALT levels suggests leakage of these enzymes in the blood which could be from the liver damage. This finding is in agreement with the work by Mohammed & Saleh [36], on the biochemical effect of pepper on rabbits which shows that capsaicin the active compound of pepper, possesses some chemical and pharmacological properties similar to the classes of drugs that may cause liver damage. Although, this is also contrary to the study by Alam *et al.* [37] which suggested that capsaicin possesses therapeutic potential against cyclophosphamide---induced liver damage. The results of the group PVA shows lower levels of serum AST (20. 38 ± 2.93) than PV (24.20 ± 2.77). This suggests that wood ash that was used to preserve the beans has some therapeutic effects. Wood ash has potash which contains potassium [38]. In liver damage, there is a decrease in the level of potassium. Hence wood ash contains, potassium, it is hypothesized that the potassium supply ameliorates the shortage of potassium alongside other micro-nutrients. The results also revealed an insignificant increase in the serum activities of AST in the groups PVD and PVAL when compared to the control group. This non-significant increase of the liver enzymes in the PVAL group may be due to the consumption period as several studies have also shown that aluminum phosphide increases the activity of aspartate aminotransferase and alanine aminotransferase in rats, making the liver one of the important target organ of phosphine poisoning in the body [39]. While a significant increase in ALT for PVD indicates hepato-toxicity. Dichlorvos residues could cause hepato-toxicity through the generation of free radicals and lead to the degeneration of the liver cells [40]. Animal studies have reported that dichlorvos induces autoimmune hepatitis which leads to the degeneration and necrosis of hepatocytes. This immune response causes inflammation of the liver [41].

The kidney is a very important organ in the detoxification of the body, its major work being the filtration of wastes. This exposes it too dangerous compounds. Knowledge of the health of the kidney gives an insight into the overall well-being of the biochemical activities in the body as well the overall health of the organism [42]. The results emanating from the present study revealed the analysis of some markers of kidney health. The results show that the rats fed with *P. vulgaris* alone had a higher amount of urea. This implies the utilization of protein as suggested by Nwaigwe *et al.* [43]. This could also imply that the kidneys may not able to excrete enough amount of urea. Animals in groups PVA and PVP did not show any significant difference in the levels of creatinine groups, this suggests that wood ash and pepper may not have any negative effect on the kidney's ability to clear urea. The significant decrease of urea in group PVP suggests that the amount of protein in beans preserved with pepper made available for digestion is small. Overall this may not stress the kidneys for clearance of urea. In comparison with the control group, the PVP group showed a significant decrease in sodium. The osmolarity of body fluids is determined by the serum sodium ion concentration which has a regulatory effect on the blood volume. This means that low levels of sodium ions may result to reduced blood pressure. In the case of potassium, PVA, PVD, and PVAL groups revealed a significant ($p < 0.05$) decrease when compared to the control group. The decrease in the PVD group is contrary to the study by Nwankoe *et al.* [44] which observed an increase in potassium ions in rats due to prolonged exposure to toxic dichlorvos.

Stress is a collective occurrence that attracts a cascade of events and is implicated as a cofactor in the severity and development of numerous diseases, and threatens cellular homeostasis by neutralizing oxidants in various tissues of the body. Stress also plays a vital role in aggravating several ailments, particularly hepatic inflammation. An excess in the reactive oxygen species production according to AbdulSalam *et al.* [45], can affect different tissues following excess lipid peroxidation, oxidation, and


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different lesions generated in DNA. Aprioku [46] maintains that deviations within the human system from the normal physiological ranges of antioxidants may result in the progression of several diseases in respective tissues. Antioxidant systems function by minimizing the over-production of oxygen-derived free radicals released in excess during stress to protect them from oxidative damage [47]. Stress response could be perceived as a compensatory mechanism to the disturbances in the living system caused by a stressor by increasing corticosterone levels [48]. The antioxidant system differs from tissue to tissue and cell- type to cell-type. Most important in-vivo enzymatic antioxidant defense system include; SOD, CAT, GPx, and GST. They act as antioxidant by inactivating pro-oxidants to scavenge the activities of the free radicals [49]. Antioxidant protection system can also include; dietary antioxidants, endogenous enzymatic and non-enzymatic constituents that regulates' the overproduction of these ROS [47]. Perhaps, one of the markers of lipid peroxidase (end products of unsaturated fats) widely used in determining oxidative stress measurement in experimental animals is Malondialdehyde (MDA) [50]. Most important in-vivo enzymatic antioxidants of focus in experimental research are; Catalase (CAT) and Superoxide Dismutase (SOD) activities of the different tissues considering that they are vital ROS-neutralizing antioxidant and index of peroxidation. The enzyme, catalase (CAT) is important in the elimination of hydrogen peroxide ($H_2O_2$) from tissues. Several suggestions have been made according to Yamada *et al*. [51] that catalase is the major enzyme responsible for the elimination of $H_2O_2$. The results from the present study revealed elevated levels of CAT in group PVP when compared to the control group at $p < 0.05$. This suggests the consumption of PVP increases the antioxidant activity in the blood. This agrees with the claims made by Kim *et al.* [52] that the consumption of pepper may increase antioxidant activity. The Levels of MDA shows a significant increase between the PVD and control. The elevated levels of MDA recorded in PVD suggest the possible degradation of $H_2O_2$, possibly by CAT. This is in line with the claims made by Chinedu *et al.* [53] which suggested that the levels of MDA are elevated to confirm the possibility of oxidative stress suggested by decreased levels of CAT. This agrees with the study by Sharma *et al.* [54] that the consumption of kidney beans preserved with dichlorvos may induce oxidative stress which may lead to lipid peroxidation. Lipid peroxidation plays a vital role in arthritis and inflammatory diseases. Glutathione peroxidase plays a crucial role of inhibiting lipid peroxidation process and protecting the cell from oxidative stress [40]. The level of GPx from the study shows that there is a statistically significant difference between the control group and the PVA group. This suggests that the consumption of kidney beans preserved with ash does not affect the level of lipid peroxides. The PVD group had a significant decrease when compared with the control. The GPx decrease in the PVD group indicates the ability of dichlorvos to generate free radicals which may lead to oxidative stress. This is in line with the increase of MDA, a lipid peroxidation marker [40]. Superoxide dismutase is the primary defense against oxidative stress. The levels of SOD significantly increased in the PVP group against the control group. This suggests the antioxidant potential of pepper as earlier noted. The results in the table revealed a decrease in SOD levels in group PVD despite it not being significant. This is in line with the results of the other antioxidants in this study which suggest that the preservation of beans with dichlorvos may lead to oxidative stress in the body.

C-reactive protein is an acute-phase inflammatory protein, a highly conserved plasma protein that was initially discovered by Tillet and Francis [55] while investigating the sera of patients suffering from the acute stage of Pneumococcus infection and was named for its reaction with the capsular polysaccharide of *Pneumococcus* [55]. CRP has been classed as an acute inflammation marker [56]. The present study yielded results of the levels of CRP, the rats in groups PVA and PVP were fed with *Phaseolus vulgaris* preserved in wood ash and bird pepper recorded values of CRP to be $56.05 \pm 3.20$ and $57.08 \pm 1.74$ respectively. This decrease in CRP is significant in the control group and suggests no sign of persistent inflammation. The groups that were fed with beans preserved with pesticides, PVD and PVAL showed very high levels of CRP, $71.35 \pm 1.99$ and $66.65 \pm 2.86$ respectively. These values are significantly higher than the values recorded in the control and PV group. This suggests events that would increase the activity of CRP. The purpose of CRP is to detect inflammation in the body [56]. Pesticides may induce oxidative stress, leading to the generation of free radicals and alteration in antioxidants. The most prominent clinical effects of poisoning with pesticides result from their inhibition of acetylcholinesterase (AchE) [14]. Several studies have demonstrated oxidative stress induced by pesticides like dichlorvos and aluminum phosphide in rats and humans.

## CONCLUSION

Despite the effectiveness of these pesticides as preservatives in the storage of legumes to prevent pest attacks and the benefits associated with the usage of wood ash and pepper. This research suggests that the selected preservatives used in the experiment possess some toxic effects that may be lethal over some time. According to the findings from the study *P.vulgaris* preserved with pepper for a prolonged duration of time when consumed in excess showed some level of toxicity in the liver when compared to the control group. Despite the increase in liver enzymes, this effect did not reflect on their other biochemical parameters when compared with the control, as there was an increase in antioxidant and a decrease in C-reactive protein in the group-fed beans preserved with pepper. This suggests that the residual content on the beans may only be harmful to the liver. There was no significant damage to the kidney by the preservatives when compared to the control group. The group fed dichlorvos preserved beans and the animals fed aluminum phosphide preserved beans showed a decrease in antioxidant activity and an increase in malondialdehyde concentration which may cause oxidative damage. Also, the C-reactive protein from the *P.vulgaris* preserved with dichlorvos and the *P.vulgaris* preserved with aluminum phosphide showed that there may be severe health implications as it increased significantly when compared to the control group. This is to say that, although these preservatives have been useful and often used in small quantities by farmers, they may lead to cellular damage.

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