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Research Article

Biochemical Effects of Some Preservatives on *Vigna Unguiculata* in Adult Male Wistar Rats

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Abstract

Different studies proved the likelihood of remnant of some component of preservative on seeds and grains after a long period of time. Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate), aluminium phosphide are commonly used by local farmers and retailers in controlling insects. This research was aimed at evaluating the effect of *vigna unguiculata* treated with some preservatives on the biochemical parameters of albino wistar rats. Thirty six (36) male albino wistar rats of known body weight were assigned into six (6) groups of 5 rats each. The seed was weighed 1 kg into five sections, each treated with the selected preservative dichlorvos, aluminium phosphide, pepper and ash respectively, sealed and kept in an airtight bucket and left for six months. After preservation, the treated sample was grounded into fine powder. They rats were fed with the sample according to their groups for a period of two months. Thereafter, the rats were euthanized and blood samples were collected through throat slitting. The hematology, liver enzymes, CRP and kidney function parameters were analyzed using standard methods. A significant ($P<0.05$) increase was observed in liver enzymes in the Ash and pepper groups compared to the control. CRP increased significantly ($P<0.05$) in the rats exposed to sample preserved with ash compared to the control. No significant ($P<0.05$) difference was observed in the level of differentials in all the groups compared to control. PCV level decreased significantly ($p<0.05$) in the group exposed to sample preserved with pepper, aluminium phosphide, dichlorvos and ash compared to the control. WBC and platelet decreased significantly ($p<0.05$) in group exposed to sample preserved with ash compared to the control. There was a significant ($P<0.05$) increase in the level of urea and creatinine in dichlorvos group compared to control. A significant ($P<0.05$) difference was observed in the level of serum electrolytes in pepper and ash compared to the control. This research revealed the alteration in the biochemical parameters of adult male albino wistar rat analyzed after exposure to the treated samples, which indicates the toxic effect of the residual component of the preservative. Pepper and wood preserved samples also alters the biochemical parameters hence suggesting the possibility of presence of toxic residual components or possible contamination.

Keywords: Aluminium phosphide, Dichlorvos, Platelet, Wood preserved samples, Creatinine

INTRODUCTION

Vigna unguiculata is a grain legume which is rich in water soluble vitamins and a great source of dietary proteins. It is often called the poor man's meat because it contains a significant amount of protein, minerals, and vitamins for the rural poor who have limited access to protein from animal sources such as meat and fish. It is commonly referred as the cowpea, black eyed pea or iron beans and grown in tropics and subtropics used as food for human as well as for animal. In Nigeria it is known as "Agwa" in Igbo land, barkinkarfe wake‟ in Hausa language and "Ewa" in Yoruba language. It is a hot

weather crop. The seed ranges from 2 to 12 mm (in length) with globular shape. They are always dried before taking to market for sales. It is a valuable source of protein, vitamins mineral and dietary fiber. Beans contain about 25% protein, and also low in anti-nutritional factors. Moreover, it is also a good source of both soluble and insoluble dietary fiber with high health benefits. These features make it ideal in helping consumers to meet the dietary goals of reducing fat intake and increasing the intake of starch and other complex carbohydrates. The production and storage of this product is greatly threatened with severe insect pests in Nigeria. These insect pests damage the crop at various stages of development which will consequently affect its total production. During storage, these crops are extremely vulnerable to a wide range of viral, fungal and bacterial diseases. Huge tonnes of bean seeds are damaged by insect infestation that leads to loss of weight of about 10%. The most commonly reported insect pests of beans are weevil (*callosobruchus maculatus*) and the bean weevil (*acanthoscelides obtectus*) are the cause of damage (Ajiboso, et al., 2012).

The infestations usually originate in the field but reproduction of the weevil continues in stored seed as long as temperature is high until the entire lot of seed is eaten up and loses value.

Due to the absence of modern grain storage facilities, farmers, merchants and storekeepers are only left with using natural and synthetically made preservatives.

Most of these preservatives are poisons specifically produced to get rid of pests and other related insects. They are applied prior to preservation or storage of seeds. This serves as a preventive measure against fungal and insect infections on the dried-beans. Some of the preservatives include natural preservatives, such as sugar, salt, acids, ash, pepper etc., as well as synthetic preservatives e.g 2, 2-dichlorovinyl dimethyl phosphate (dichlorvos), aluminium phosphide etc. Yusuf, et al., revealed abusive application of preservatives on stored cowpea grains at Dawanau grains market (largest grains market in West Africa) in Kano State, the Northern part of Nigeria. Consumption of synthetic preserved cowpea grains had resulted in bioaccumulation and several health related problems (e.g cancer). Analysis carried out in a laboratory and reported by daily trust newspaper Nigeria, 2020 revealed the presence of some residual component of these preservatives on the seeds and consumers of grains treated with pesticides including beans are at risk due to their harmful effects.

The aim of this research is to evaluate the effects of *Vigna unguiculata* treated with some preservatives on the biochemical parameters of male albino wistar rats (Ashaye, et al., 2006).

Studies had been done and results had revealed residues of different preservatives used grain storage, while biochemical effects of consuming such crop products through toxicological examination of feeding to wistar rats have a dearth of information. Dichlorvos (DDVP), aluminium phosphide, dried pepper and wood ash were chosen for this experiment because they are being widely used in Nigeria by farmers and retailers. Therefore, there is need to assess the biochemical effects of these preservatives using *Vigna unguiculata* grains on wistar rats in ensuring high food quality and safety.

MATERIALS AND METHODS

Sample collection

The sample (*Vigna unguiculata*) was obtained from place of cultivation in Keffi, Keffi local government, Nasarawa state. The sample was then collected in bags and transported to laboratory where they were cleaned and sorted to remove stones.

The sample was identified in the department of plant science and biotechnology, faculty of science, Nasarawa university Keffi, Nigeria (Banjo, et al., 2010).

Chemicals/Reagents/Glass wares

Dichlorvos (2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) and Aluminum phosphide was obtained from standard agro allied store in Keffi, Nasarawa state. 2,4-dinitrophenylhydrazine, phosphate buffer, α-oxoglutarate, Picric acid, Sodium hydroxide, Urease, Sodium Nitroprusside, Phenol, Sodium hypochlorite, Sodium Tetraphenylboron, Uranyl Acetate, Magnesium Actate, Drapkin's solution, Glacial Acetic Acid, Latex reagents. Test tubes (pyrex), pipettes (pyrex), Beakers (PYREX), Surgical glass (PYREX).

Equipment

UV-spectrophotometer (Shimdazn), refrigerator (Thermo cool), C-Gen incubator (SS304. India), pH meter (HI-2210 India), centrifuge (Gallen Kamp), colorimeter (Haracus Christ), PCV hematocrit reader (OHAUS), weighing balance (OHAUS), microscope (CH20i, Kolkata). Surgical blades purchased from a standard pharmaceutical store drug field, Nigeria.

Methods

Seed preservation: The sorted *Vigna unguiculata* was divided into five parts, each having a bucket with tight lids that contain seeds weighing about 1 kg.

The bucket containing *V. unguiculata* with no preservative served as control, the second bucket contained *V. unguiculata* mixed with bird eye pepper, the third bucket contained *V.* unguiculata mixed with ash. The fourth bucket contained *V. unguiculata* mixed with DDVP and the fifth contained *V. unguiculata* mixed with four tablets aluminiunm phosphide.

The seeds was stored for a period of six (6) months and properly labeled. All during the storage period the seed was checked periodically (Brown, et al., 2015).

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Sample preparation

Each treatment including the control was milled into powder, packed in a clean polythene bag, labeled and sealed. The powdered sample was kept for analysis.

The stem from neem tree (*Azadirachta indica*) 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 market and dried in the sun. 300 g of the sundried pepper was weighed and packed in nylon bags.

Experimental design

A total of 36 rats were used for the study. The rats were grouped into six groups of six rats each as follows:

Group I (normal control): Animal feed.

Group II (positive control): Animal feed+Vigna unguiculata without preservatives.

Group III: Animal feed+Vigna unguiculata+300 g of ash.

Group IV: Animal feed+Vigna unguiculata+300 g pepper.

Group V: Animal feed+Vigna unguiculata+2 ml of dichlorvos.

Group VI: Animal feed+Vigna unguiculata+4 tablets of aluminum phosphide.

Collection of blood sample

After two (2) months of dietary intervention, the blood sample of each rat was collected into respective plain capped tubes. The blood samples in the plain tubes were allowed to stand for two hours at room temperature to clot before centrifugation at 1000 rpm for 10 minutes using a bench top centrifuge to separate cells from serum. Aliquots of the serum were carefully obtained into correctly labeled dry plastic tubes. The samples were carefully stored in the refrigerator and used for various biochemical analyses (Chaudhry, et al., 2014).

Biochemical analysis

The biochemical parameters was analyzed using standard methods.

Statistical analysis

The data collected was analyzed using means, standard deviation and standard error of the means. One way analysis of variance (Anova) and duncan's new multiple range test were used to separate and compare differences between means. Significant differences was expressed at $p<0.05$.

RESULTS

Effect of *V. Unguiculata* preserved with different preservatives on the liver enzymes of albino wistar rats: The results of the changes in the level of liver function enzymes in albino rats fed with *V. Unguiculata* preserved with some selected preservatives is shown in **Table 1**. As shown in table, AST showed a significant increase ($p<0.05$) and higher in the group fed with ash (39.571.97) and pepper (25.51 ± 1.90) compared to the control (15.38 ± 1.55). There is no significant ($p<0.05$) difference in group exposed to sample preserved with aluminium phosphide (19.08 ± 2.35) and dichlorvos (14.39 ± 2.51) compared to the control. ALT showed a significant increase ($p<0.05$) in the group fed sample preserved with ash (36.17 ± 1.97) and pepper (34.63 ± 2.97) compared to the control (15.12 ± 3.10). ALT showed no significant ($p<0.05$) change in the group fed with sample preserved with dichlorvos (17.20 ± 2.16) and aluminium phosphide (16.31 ± 1.15) compared to the control (15.12 ± 3.10).

Table 1. Effect of *V. Unguiculata* preserved with different preservatives on the liver enzymes of albino wistar rats.

Groups	AST (IU/L)	ALT (IU/L)
Control	15.38 ± 1.55^{a}	15.12 ± 3.10^{a}
Standard	18.46 ± 2.64^{a}	18.07 ± 2.63^{a}
V. u+ALP	19.08 ± 2.35^{b}	16.31 ± 1.15^{a}
V. u+D	14.39 ± 2.51^{a}	17.20 ± 2.16^{a}
V. u+P	25.51 ± 1.90^{c}	34.63 ± 2.97^{b}
V. u+A	39.57 ± 1.97^{d}	36.17 ± 3.79^{b}

Note: Results are presented in Mean ± SD, (N=3), mean values with different letters as superscripts are statistically significant ($p<0.05$) *i.e.,* null hypothesis: Rejected; AST: Aspartate amino Transferase; ALT: Alanine amino Transferase; AlP: Aluminium Phosphide; D: Dichlorvos; P: Pepper; A: Ash; V. u: *Vigna unguiculata*

Effect of *V. Unguiculata* preserved with different preservatives on kidney function parameters of albino wistar rats: The results of the changes in the level of kidney function parameters in albino rats fed with *V. Unguiculata* preserved with some selected preservatives is shown in **Table 2**.

Here, the table shows a significant ($p<0.05$) increase in sodium ion in the groups exposed to sample treated with pepper (156.90 ± 3.26) and ash (171.28 ± 3.03) compared to control (128.41 ± 1.99). There was a

significant (p<0.05) increase in potassium ion in the group fed with sample preserved with pepper (15.99 ± 1.12). No significant (p<0.05) difference in potassium and sodium ion was observed in groups exposed to sample preserved with dichlorvos (9.99 ± 2.51) and aluminium phosphide (9.02 ± 2.16) while group fed with sample preserved with Ash (A) showed a significant (p<0.05) decrease with value 2.52 ± 1.1 compared to the control (7.02 ± 2.5). There was significant (p<0.05) increase in urea level in groups fed with sample preserved with dichlorvos (9.53 ± 2.89) compared to the control (5.40 ± 1.06). Other groups showed no significant difference in the level of urea.

The level of creatinine increased significantly (p<0.05) in the group exposed to sample treated with dichlorvos (5.17 ± 1.56) compared to the control (Celestina, et al., 2021).

Table 2. Table shows a significant (p<0.05) increase in sodium ion in the groups exposed to sample treated with pepper and ash compared to control.

Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Control	128.41 ± 1.99^a	$7.02 \pm 2.5b^c$	5.40 ± 1.06^a	2.01 ± 1.31^a
Standard	136.03 ± 7.56^a	7.36 ± 0.65^c	3.03 ± 0.84^a	2.45 ± 1.26^a
V. u+ALP	130.67 ± 3.97^a	9.02 ± 2.16^c	7.99 ± 1.31^b	4.82 ± 1.42^a
V. u+D	133.79 ± 5.78^a	9.99 ± 2.51^c	9.53 ± 2.89^c	5.17 ± 1.56^b
V. u+P	156.90 ± 3.26^b	15.99 ± 1.12^b	7.12 ± 1.22^b	2.78 ± 1.86^a
V. u+A	171.28 ± 3.03^c	2.52 ± 1.11^a	6.41 ± 0.37^b	2.51 ± 1.36^a

Note: Results are presented in Mean ± SD; (N=3); mean values with different letters as superscripts are statistically significant (p<0.05) null hypothesis: Rejected; Na^+: Sodium; K^+: Potassium; AlP: Aluminium Phosphide; D: Dichlorvos; P: Pepper; A: Ash; V. u: Vigna unguiculata

Effect of *V. unguiculata* preserved with different preservatives on C-reactive protein level of albino wistar rats: The results of the changes in CRP level of albino rats fed with *V. unguiculata* preserved with some selected preservatives is shown in **Table 3**. CRP showed a significant (p<0.05) increase in group fed with sample preserved with Ash (67.08 ± 2.29) and no significant change in other groups compared to the control group (62.97 ± 5.30).

Table 3. Effect of *V. unguiculata* preserved with different preservatives on C-reactive protein level of albino wistar rats.

Groups	CRP (ml/L)
Control	62.97 ± 5.30^a
Standard	60.10 ± 7.06^a
V. u+ALP	59.91 ± 3.55^a
V. u+D	63.01 ± 2.40^a
V. u+P	57.01 ± 2.64^a
V. u+A	67.08 ± 2.29^b

Note: Results are presented in Mean ± SD; (N=3); mean values with different letters as superscripts are statistically significant (p<0.05) null hypothesis: Rejected; CRP=C-reactive Protein; AlP: Aluminium Phosphide; D: Dichlorvos; P: Pepper; A: Ash; V.u: Vigna unguiculata

Effect of *V. unguiculata* preserved with different preservatives in hematological parameters of albino wistar rats: The results of the changes in the level of hematological parameters in albino rats fed with *V. unguiculata* preserved with some selected preservatives is shown in **Tables 4 and 5**. In the table, there is no significant (p<0.05) differences in hemoglobin level across the groups except those fed with sample with no preservative (35.48 ± 28.19) compared to the control group (25.34 ± 9.85). But non-significant reduction was observed across the group. PCV level decreased significantly (p<0.05) in the group exposed to sample preserved with pepper, aluminium phosphide, dichlorvos and ash compared to the control. And no significant difference in the dichlorvos treated group compared to the control. WBC and platelet decreased significantly (p<0.05) in group exposed to sample preserved with ash compared to the control. And non-significant reduction was observed in other groups. Differential showed no significant (p<0.05) difference in all the groups but slight reduction was observed (Desai, et al., 2008).

Table 4. Level of hematological parameters in albino rats fed with V. unguiculata preserved with some selected preservatives.

Groups	HB (g/dl)	PCV	WBC (x 103/µl)	Platelet
Control	25.34 ± 9.85[a]	49.92 ± 5.48[c]	7468.33 ± 389.80[b]	213.31 ± 32.32[b]
Standard	35.48 ± 28.19[b]	40.00 ± 1.00[a]	7216.33 ± 537.64[a]	205.67 ± 4.46[b]
V. u+ALP	22.03 ± 4.32[a]	43.00 ± 4.36[b]	7247.00 ± 124.82[a]	200.44 ± 17.02[b]
V. u+D	19.37 ± 1.2[a]	44.67 ± 1.53[b]	7059.00 ± 141.17[a]	188.49 ± 16.47[b]
V. u+P	13.41 ± 2.85[a]	36.45 ± 2.70[a]	6915.33 ± 380.40[a]	179.75 ± 26.07[b]
V. u+A	8.43 ± 1.96[a]	43.33 ± 3.05[b]	6523.33 ± 840.31[a]	100.82 ± 3.59[a]

Note: Results are presented in Mean ± SD; (N=3); mean values with different letters as superscripts are statistically significant (p<0.05) null hypothesis: Rejected; HB: Hemoglobin; PCV: Packed Cell Volume; WBC: White Blood Cell; AlP: Aluminium Phosphide; D: Dichlorvos; P: Pepper; A: Ash; V. u: *Vigna unguicuata*

Table 5. Differences in hemoglobin level across the groups except those fed with sample with no preservative (35.48 ± 28.19) compared to the control group.

Groups	Lymph	Eosi	Monocyte	Basophil	Neutrophil
Control	27.54 ± 5.15[a]	3.33 ± 1.53[a]	1.67 ± 1.16[a]	0.33 ± 0.58[a]	67.25 ± 5.93[a]
Standard	30.67 ± 1.53[a]	3.67 ± 0.58[a]	2.33 ± 0.58[a]	0.67 ± 0.58[a]	63.33 ± 4.16[a]
V. u+ALP	28.00 ± 7.00[a]	2.00 ± 1.00[a]	1.67 ± 1.16[a]	1.00 ± 0[a]	67.25 ± 5.41[a]
V. u+D	28.33 ± 5.51[a]	3.00 ± 1.00[a]	2.67 ± 1.16[a]	0.67 ± 0.58[a]	65.33 ± 5.51[a]
V. u+P	31.33 ± 1.53[a]	3.00 ± 1.00[a]	2.67 ± 1.16[a]	0.33 ± 0.58[a]	65.33 ± 4.93[a]
V. u+A	27.34 ± 6.42[a]	3.67 ± 3.05[a]	1.67 ± 1.16[a]	0.67 ± 1.16[a]	66.11 ± 0.99[a]

Note: Results are presented in Mean ± SD; (N=3); mean values with different letters as superscripts are statistically significant (p<0.05) null hypothesis: Rejected; HB: Hemoglobin; PCV: Packed Cell Volume; WBC: White Blood Cell; AlP: Aluminium Phosphide; D: Dichlorvos; P: Pepper; A: Ash; V. u: *Vigna unguicuata*

DISCUSSION

Hematology examination is very important in analytical research and environmental monitoring because it serves as a pointer to physiological or pathological changes under investigation. Various alterations in the blood parameters occur in warm blooded animals due to damages to some tissues or organs which could have led to their dysfunctions (Ebeye, et al., 2007). The study thus revealed that DDVP treated sample fed to male rats showed reduction in some of the hematological parameters. Several studies have shown the adverse effect of dichlorvos on the hematological parameters of albino wistar rats (Edem, et al., 2012). According to similar study by olajumoke, et al., on dichlorvos toxicity on histological organs of wistar rats fed on treated Cowpea grains, a reduction in PCV, RBC, WBC and Hb was observed. This finding is related to the significant decrease in PCV and non-significant reduction in WBC, platelet and Hb values recorded in this study. Also this is consistent with the findings by Edem, Idowu, et al., who reported significant decrease in hematological parameters in rats exposed to dichlorvos by inhalation. The reduction in WBC might be due to the destruction of white blood cell as reported by Haratym-Maj. Also no significance difference was observed in the value of differentials and this is consistent with the report by olajumoke and Olaoye, et al.

In the liver, AST and ALT are often used as biomarkers of injury because they are released by hepatocytes into the extracellular space (Farshid, 2015). In this study a non-significant increase was observed in the serum levels of ALT and AST in rats fed with sample preserved with dichlorvos. This is contrary to earlier observations by wahab and Kingsley, et al., who reported a significant increase in the level of AST and ALT in rats exposed to dichlorvos. Dichlorvos at cholinergic junctions of the central nervous system irreversibly inhibits the enzyme acetylcholinesterase which induces oxidative stress and results to hepatotoxicity in rat, which in turn will increase the enzymes (Friday, et al., 2015). The report on the non-significant increase of liver enzymes is supported by Yang et al. The non-significance might be dependent on the dosage and liver being the site for biotransformation could have transformed the toxic compound into an inactive metabolite thus reducing the toxicity (Haratym-Maj, 2002).

Serum CRP level showed a slight but non-significant increase after exposure to sample preserved with Dichlorvos. This finding is similar to other studies where CRP was significantly raised in farm workers occupationally exposed to DOP compared with the controls (Hassan, et al., 2010). This increase could be attributed to increased inflammatory responses. Inflammation is an initial response of the immune system to irritations. This process is stimulated by factors released from exposed cells to form a physical barrier against the spread of the irritations or infections the vasodilatation of blood vessels caused by inflammation attracts phagocytes. This might have resulted to systemic inflammatory response where IL-1, IL-6 and TNF-α would have acted on the liver to increase the production of CRP. This may partly explain the slight increase in the level of CRP in this study (Hend, et al.,

2014).

The kidney is one of the main targets of organophosphate compounds. Continuous exposure to dichlorvos significantly disrupts the renal parameters as well as electrolyte concentrations. The finding in this study showed a slight but non-significant increase in the Na^+ and K^+ levels and a remarkable significant ($p<0.05$) increase in urea and creatinine levels in the group exposed to dichlorvos preserved samples compared to the control (Idowu, et al., 2016). This finding is consistent with study carried out by Tela and Sagir, where prolonged exposure of experimental animals to dichlorvos significantly increases the mean serum renal electrolytes, urea and creatinine concentration in the treated groups compared to the control. The reason for this increase might be due to the fact that DDVP like other organophosphates are generally eliminated through urine and are likely to affect nephrons (Iniobong, et al., 2019).

This was assumed to be due to oxidative stress resulting from Reactive Oxygen Species (ROS) generated by DDVP. These species might have as well caused cellular damage that result in cell shrinkage and degeneration of the glomerular tuft and the renal tubules diameters. This in turn might have affected glomerular function of ultrafiltration and selective re-absorption thereby leading to higher concentration of virtually all the serum electrolytes, urea and creatinine (Kingsley, et al., 2016).

Aluminum Phosphide serves as a fumigant used by local farmers in preserving grains such as maize and seeds such as beans. Aluminum phosphide is toxic to both target and non-target organisms. Aluminum phosphide is a highly toxic, low cost and freely available pesticide. Fatal dose for a 70 kg man is 0.5 g. Toxicity of phosphine is related to oxidant free radicals and associated inhibition of enzymes of metabolism, such as cytochrome c oxidase (Madhumathi, et al., 2020).

In this study, aluminium phosphide was used to preserve a certain portion of the sample and a non-significant increase in liver enzymes was discovered after exposure to the preserved samples. This is closely related to similar study by Morteza, et al., who reported an elevated level of liver enzymes in patients upon exposure to aluminium phosphide poisoning. Also Iniobong, et al., evaluated the impact of aluminum phosphide on the transferases of muscles and liver of Parophiocephalus obscure and it was shown that aluminum phosphide initiates critical alteration on the transferases (ALT and AST) in the liver and muscle of parophiocephalusobscurus (Mohamed, 2017).

The kidney is another major target of phosphine poisoning in the human. A non-significant increase in urea and creatinine levels was observed in rats fed with sample preserved with ALP. The present finding is closely related to those stated by Mohamed who investigated the effect of melatonin against aluminum phosphide-induced renal toxicity in rats, he reported that rats administered ALP, revealed signs of toxicity and elevated levels of urea and creatinine. The slight increase in serum creatinine and urea level in this study might be due to impairment in the kidney function and renal injury. Phosphine has been reported to induce inhibition of mitochondrial cytochrome c oxidase leading to the generation of reactive oxygen species and cellular peroxides. The overproduction of ROS diminishes the renal functions, which is attached to increases in serum creatinine and urea levels.

A slight increase in level of Na^+ and K^+ was confirmed in this study and this can be attributed to the renal injury after exposure to sample preserved with ALP. Electrolyte abnormalities upon ALP poisoning include high or low sodium, potassium, and magnesium. The mechanisms of toxicity include; Inhibition of oxidative phosphorylation, free radical production with promotion of lipid peroxidation and Cholinesterase inhibition.

C-reactive protein is described as a reactive substance in acute lesions, and elevated plasma levels of CRP are a result of inflammation and trauma. Organophosphates may cause lesions in tissues and organs in the body, leading to increased plasma CRP levels. This is in contrast with the result of this present study, where no significant difference was recorded in CRP level.

In the hematological studies, there is a significant ($p<0.05$) decrease in PCV and slight reduction in other parameters across the groups compared to the control group. Other studies have reported distortion in hematological parameters maybe connected with oxidative stress induced by AlP because of its implication in AlP induced hematotoxicity. Phosphine reacts with free hemoglobin and hemoglobin in normal red blood cells to produce hemi chrome, a derivative of met hemoglobin and Heinz bodies with the concomitant induction of free radicals. Leukopenia is associated with rice tablet poisoning and has been considered as a symptom of acute poisoning. Thrombocytopenia has also been considered as one of the late symptoms of poisoning in the previous studies. This might be linked to the reduction in the level of white blood cell and platelet observed in this study (Morteza, et al., 2013).

Pepper has been shown to possess antimicrobial activity and some have already produced compounds, effective against antibiotic resistant strains of bacteria. It has a pungent taste and according to studies possess active ingredients capable of retarding the infestation of pests.

The liver enzymes, ALT and AST increased significantly after exposure to sample preserved with pepper. Black pepper produced side effects such as irritation, scaring and tissue necrosis and may be responsible for cell necrosis in the liver. This might be the reason for liver enzyme increase observed in this study. The result here is contrary to similar study by Friday, et al., on effect of aqueous extract of piper guineese seeds on some liver enzymes, antioxidant enzymes and some hematological parameters in albino rats, who reported a decrease in the level of liver enzymes after treatment. The significant increase in the level of liver enzymes in this study might be indicative of an injury to the liver.

On the hematological studies of the effect of sample preserved with pepper, there is a significant ($p \leq 0.05$)

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decrease in PCV values and a slight but non-significant reduction in the other parameters, after exposure to sample preserved with pepper. This result is similar with the report by Obembe, et al., on the effect of aqueous extract of xylopiaaethiopica (nigro pepper) on some hematological parameters in albino rats, where WBC was significantly lower ($p<0.05$) in the low dose treated group and also lower in the high dose treated groups. Chili pepper decreased significantly the level of RBC, Hb and PCV in the study by Nwangwa, et al., on comparative effect of chilli pepper (*Capsicum frutescens*) extract and capsaicin on some hematological parameters and serum electrolytes in albino wistar rats. This result suggests that the pepper on the preserved sample has an effect on erythropoiesis which is marked by the decreased PCV. It is also likely that heme biosynthesis was impaired along with erythropoiesis which led to decreased Hb concentration. However, this result is contrary to the report of Hassan, et al., who discovered an increased level of RBC, WBC, PCV and MCHC on the short and long term administration of *P. guineese* on albino wistar rats.

Furthermore, the sample preserved with pepper has no significant difference in the value of CRP in this study. Only a slight reduction was recorded. This is contrary to the report by Hend, et al., who reported an elevated CRP level in his study on the Potential anti-inflammatory effect of lemon and hot pepper extracts on adjuvant induced arthritis in mice.

On kidney studies of the effect of sample preserved with pepper, there is a significant increase in the level of sodium and potassium after exposure to pepper treated sample and slight increase in urea and creatinine. The increase in the level of K^+ here is in agreement with the report of Nwangwa et al. The increase in the electrolyte might be attributed to damage to the kidney. Creatinine and urea level showed no significant difference after treatment. This is contrary to the report by Nwangwa, et al., on the effect of chilli pepper on the kidney function parameters. However, histopathological studies on the effect piper guineese on wsitar by Ebeye, et al., revealed the hypercellularity of the glomerulus as well as increase in size, due to high doses of pepper extract.

Wood ash is another natural seed preservative used by farmers in local areas for storage. Wood ash contains natural salts that repel pests. In moist climates where seeds are susceptible to insect infestation, studies have shown that storing seeds in wood ash is effective in both preventing rot and insect predation.

In the analysis of liver enzymes after exposing albino wistar rats to sample preserved with ash, there is a significant increase in the level of ALT and AST after exposure. Also a rise was observed in the level of sodium after exposure. The rise in sodium electrolyte could be attributed to the presence of salts in wood ash or toxicity of chemical substances (like cadmium, lead and mercury) in wood ash on the liver and kidney.

On CRP and hematology analysis, there is a significant increase recorded in the level of C-reactive protein after exposure to sample treated with ash. C-reactive protein is described as a reactive substance in acute lesions, and elevated plasma levels of CRP are a result of inflammation and trauma. The increase observed here could be linked to inflammatory response caused by toxic chemicals in wood ash. PCV, WBC and platelet decreased significantly after exposure to the treated sample.

CONCLUSION

In this study, the biochemical effect of *Vigna unguiculata* treated with preservatives (dichlorvos, aluminium phosphide, ash and pepper) on adult albino wistar rats was assessed. The findings in this study has further implicated the adverse effects of both synthetic and natural preservative following the distortion of biochemical parameters analyzed. This research was conducted to affirm the possibility of *Vigna unguiculata* treated with both synthetic and natural preservative, using matured adult male albino rats. The level of liver enzymes and kidney function parameters was analyzed, and a significant alteration was recorded across all the treated groups which are suggestive of possible organ damage by the remnant of both the synthetic and natural preservatives used on the sample. A significant change in hematological parameters was observed across the treated groups, which shows the suppressive effect of the residual components of the preservatives on the blood parameters. There is no significant change detected in the level of differentials. The level of CRP was altered in the group exposed to sample treated with wood ash. This suggests that there might be possibility of inflammation by the components of wood ash used in preservation.

Pepper and wood ash usually considered as safe alternative also alters the biochemical parameters hence suggesting the possibility of presence of toxic residual components.

Recommendations for further studies

- Further studies are required to investigate chemical constituents and mechanisms responsible for the effect of toxicity in wood ash.
- More means of preservation will be considered in future studies.
- It is recommended that dosage of the synthetic chemicals be increased in further studies.
- It is recommended that grain be washed properly before and after parboiling in order to remove any possible remnant of the preservatives on them.

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