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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. 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 1kg 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 a fine powder and fed to albino rats according to their respective groups. After two months of dietary intervention, the rats were euthanized and a blood sample was collected through throat slitting. The hematology, liver enzymes, CRP, and kidney function parameters were analyzed using standard methods. The residual content of the preservatives was also analyzed and was discovered to exceed the international permissible standard across the treated samples except for the pepper group in which there was contamination. A significant ($P<0.05$) increase was observed in liver enzymes in the Ash and pepper groups compared to the control. There was a significant ($P<0.05$) increase in CRP 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 the control. PCV level decreased significantly ($p<0.05$) in the group exposed to a sample preserved with pepper, aluminum phosphide, dichlorvos and ash compared to the control. WBC and platelet decreased significantly ($p<0.05$) in the group exposed to a sample preserved with ash compared to the control. There was a significant ($P<0.05$) increase in the level of urea and creatinine in the dichlorvos group compared to the 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 rats analyzed after exposure to the treated samples, which indicates the toxic effect of the residual component of the preservative. Pepper and Wood ash-preserved samples also alters the biochemical parameters hence suggesting the possibility of the presence of toxic residual components or contamination observed in the pepper group.

INTRODUCTION

Vigna unguiculata is a grain legume that 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 [1]. It is commonly referred to as the cowpea, black-eyed pea, or iron beans and grown in the tropics and subtropics and used as food for humans as well as for animals [2]. In Nigeria it is known as "Agwa" in the Igbo land, barkinkarfe wake" in the Hausa language, and "Ewa" in the Yoruba language. It is a hot weather crop. The seed ranges from 2 to 12 mm (in length) with a globular shape. They are always dried before taking to market for sale. It is a valuable source of protein, vitamins, minerals, and dietary fiber. Beans contain about 25% protein, and are also low in anti-nutritional factors [2]. Moreover, it is also a good source of both soluble and insoluble dietary fiber with high health benefits. These features make it ideal for helping consumers to meet the dietary goals of reducing fat intake and increasing the intake of starch and other complex carbohydrates [2]. The production and storage of these products are greatly threatened by 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 which leads to a loss of weight of about 10% [2]. The most commonly reported insect pests of beans are weevil (*Callosobruchus maculatus*) and the bean weevil (*Acanthoscelides obtectus*) are the cause of the damage [3].

The infestations usually originate in the field but reproduction of the weevil continues in stored seed as long as the temperature is high until an entire lot of seed is eaten up and loses value [4].

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 [5]. They are applied prior to the 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-dichloro vinyl dimethyl phosphate (dichlorvos), aluminum phosphide, etc. Study [6] revealed the abusive application of preservatives on stored cowpea grains at the Dawanau grains market (the 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.

Studies had been done and results revealed residues of different preservatives used in 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), aluminum 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 a 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 method

Sample Collection

The sample (*Vigna Unguiculata)* was obtained from a place of cultivation in Keffi, Keffi local government, Nasarawa state. The sample was then collected in bags and transported to the 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.

Chemicals/Reagents/Glass-wares.

Dichlorvos (2, 2-dichloro vinyl dimethyl phosphate (DDVP) and Aluminum phosphide were obtained from the 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), pippettes (pyrex), Beakers (PYREX), Surgical glass (PYREX).

Equipment

UV-spectrophotometer (SHIMDAZN), Refrigerator (THERMOCOOL), 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 1kg.

The bucket containing *V. unguiculata* with no preservative served as control, the second bucket contained *V. unguiculata* mixed with birds eye pepper, and 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 of aluminum phosphide.

The seeds were stored for a period of six (6) months and properly labeled. During the storage period the seed was checked periodically.

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 *(Azadirachtaindica)* was burnt to get ash. The cooled ash was sieved to remove dirt. Then 300g was weighed and packed into nylon bags.

Fresh bird's eye pepper (*Capsicum frutescens*) was purchased from the market and dried in the sun. 300g 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* + 300g of ash

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

Group V: Animal feed + *Vigna unguiculata* + 2ml 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.

Biochemical Analysis

The biochemical parameters were analyzed using standard methods

Determination of the concentration of preservative residues on *Vigna unguiculata* Extraction

Extraction was done according to the methods of Pang., *et al*., 1999. Fifty grams per sample were blended with 5 ml water and 100 ml acetone in a high-speed chemical-resistant blender (National Analytical Corporation, Mumbai, India) for two minutes. The extract was collected in an Erlenmeyer flask and filtered through a fast-rate filter paper (Whatman No.1) in a Buchner funnel. The blender jar was rinsed with a few mls of water and acetone and filtered as above. The combined filtrates were collected in a clean Erlenmeyer flask for partitioning. Extracts from each sample were transferred into a 250 ml separation funnel. Fifty ml of dichloromethane and 10 ml of saturated NaCl solution were added. The mixtures were carefully shaken for two minutes with the frequent opening of the valve to release the pressure. The separating funnel was left to stand for a few minutes to allow the separation of layers. The dichloromethane layer was collected in a clean conical flask. The aqueous layer was re-extracted with 50, 30, and 20 ml dichloromethane, respectively. The combined dichloromethane extracts were mixed with 25g of anhydrous Na_2SO_4, filtered through cotton wool and collected in 500ml round-bottom flask. Extracts were again re-filtered through cotton wool, with a 3cm layer of anhydrous Na_2SO_4 in a funnel. The solvent was removed to dryness by a rotary evaporator (Buchi, Postfach, Switzerland) operating under a vacuum at 40^0C. Dried extracts were reconstituted in 5ml of n-hexane and kept in closed vials at -10^0C for clean-up and residue analysis using an atomic absorption spectrophotometer.

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 were expressed at $p<0.05$.

Results

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]	5.40±1.06[a]
Standard	18.46±2.64 [a]	18.07±2.63[a]	2.01±1.31[a]
V. u + ALP	19.08±2.35[b]	16.31±1.15[a]	Standard 136.03±7.56[a] 7.36±0.65[c] 3.03±0.84[a]
V. u + D	14.39±2.51[a]	17.20±2.16[a]	2.45±1.26[a]
V. u + P	25.51±1.90[c]	34.63±2.97[b]	V. u + ALP 130.67±3.97[a] 9.02±2.16[c]
V. u + A	39.57±1.97[d]	36.17±3.79[b]	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]

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.

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

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 thegroup exposed to the sample preserved with aluminum 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 a sample preserved with dichlorvos (17.20±2.16) and aluminum phosphide (16.31±1.15) compared to the control (15.12±3.10).

Table 2. Effect of *V. Unguiculata* preserved with different preservatives on kidney function parameters of albino Wistar rats

Groups	Na^+(mmol/L)	K^+ (mmol/L) Urea (mmol/L) Creatinine(mmol/L)
Control	128.41±1.99[a]	7.02±2.5b[c]

The results of the changes in the level of kidney function parameters— in albino rats fed with *V. Unguiculata* preserved with some selected preservatives are shown in table 2.

Here, the table shows a significant (p<0.05) increase in sodium ions in the groups exposed to the sample treated with pepper (156.90±3.26) and ash (171.28±3.03) compared to the control (128.41±1.99). There was a significant (p<0.05) increase in potassium ions in the group fed with a 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 the sample preserved with dichlorvos (9.99±2.51) and aluminum phosphide (9.02±2.16) while the 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 a significant (p<0.05) increase in urea level in groups fed with a 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.

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	7216.33±537.64[a]	205.67±4.46[b]
59.91±3.55[a]					
V. u + ALP				22.03±4.32[a]	43.00±4.36 [b]
V.	u	+	D	7247.00±124.82[a]	200.44±17.02[b]
63.01±2.40[a]					
V. u + D				19.37±1.2[a]	44.67±1.53[b]
V.	u	+	P	7059.00±141.17[a]	188.49±16.47[b]
57.01±2.64[a]					
V. u + P				13.41±2.85[a]	36.45±2.70[a]
V.	u	+	A	6915.33±380.40[a]	179.75±26.07[b]
67.08±2.29[b]					
V. u + A				8.43±1.96[a]	43.33±3.05[b]
				6523.33±840.31[a]	100.82±3.59[a]

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.

The results of the changes in theCRP 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 the group fed with a sample preserved with Ash (67.08±2.29) and no significant change in other groups compared to the control group (62.97±5.30).

Effect of *V. Unguiculata* preserved with different preservatives in hematological parameters of albino Wistarrats

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 table 4.4 and 4.5. In the table, there is no significant (p<0.05) differences in hemoglobin level across the groups except those fed with asample with no preservative (35.48±28.19) compared to the control group (25.34±9.85). But a non-significant reduction was observed across the group. PCV level decreased significantly (p<0.05) in the group exposed to a sample preserved with pepper, aluminum 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 the group exposed to a 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 a slight reduction was observed.

Table 4. Effect of *V. Unguiculata* preserved with different preservatives in hematological parameters of albino wistar rats

Groups	HB (g/dl)	PCV	WBC(x10^3/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]

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. Effect of *V. Unguiculata* preserved with different preservatives in hematological parameters of albino Wistar rats continued.

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]

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.

The concentration of preservative residues on *Vigna unguiculata* samples compared to the international permissible standard and FAO

The result of the concentration of preservative residues on *Vigna unguiculata* samples is shown in table 4.1. As shown in the table, Dichlorvos showed a slight increase (0.015 µg/g) compared to the standard (0.01 µg/g). Aluminum phosphide was discovered to be 0.34µg/g which is slightly above the international permissible standard (0.30). The sample preserved with pepper showed no presence of residue but during preservation, weevil infestation was observed. Heavy metal (Cd, Pb, Cr, AS) concentration was analyzed in samples preserved with ash and their concentration was found to be above the recommended standards.

Table 6. The residual concentration of preservative on *Vigna unguiculata* samples compared to the international permissible standard and FAO

Preservatives	**Residue (1 & 2)**	
Average	**FAO/IPS (**μg/g)	
Dichlorvos	0.014	0.016
0.015	0.01	
Aluminium phosphide	0.36	0.32
0.34	0.3	
Pepper	-	
-	-	
Ash (Cd, Pb, Cr,AS)		
Cd	0. 26	0.28
0.27	0.2	
Pb	0.47	0.49
0.48	0.4	
Cr	0.021	0.018
0.019	0.02	
As	0 .048	0.056
0. 052	0.05	

Cd: Cadmium, Pb: Lead, Cr: Chromiun, As: Arsenic.

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 [7]. The study thus revealed that DDVP-treated samples fed to male rats showed a reduction in some of the hematological parameters. Several studies have shown the adverse effect of dichlorvos on the hematological parameters of albino Wistar rats. According to a similar study [8] 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 a non-significant reduction in WBC, platelet and Hb values recorded in this study. Also, this is consistent with the findings by [9][10] who reported a 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, 2002. Also, no significant difference was observed in the value of differentials and this is consistent with the report by [8].

In the liver, AST and ALT are often used as biomarkers of injury because they are released by hepatocytes into the extracellular space [11]. In this study, a non-significant increase was observed in the serum levels of ALT and AST in rats fed with a sample preserved with dichlorvos. This is contrary to earlier observations by [12][13] 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 in hepatotoxicity in rat [14], which in turn will increase the enzymes [15][10]. The non-significance might be dependent on the dosage and the liver is the site for biotransformation could have transformed the toxic compound into an inactive metabolite thus reducing the toxicity.

Serum CRP level showed a slight but non-significant increase after exposure to a 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. This increase could be attributed to increased inflammatory responses. Inflammation is an initial response of the immune system to irritations [16]. This process is stimulated by factors released from exposed cells to form a physical barrier against the spread of irritations or infections The vasodilatation of blood vessels caused by inflammation attracts phagocytes. This might have resulted to a systemic inflammatory response where IL-1, IL-6and 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.

The kidney is one of the main targets of organophosphate compounds [17]. Continuous exposure to dichlorvos significantly disrupts 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 remarkably significant ($p<0.05$) increase in urea and creatinine levels– in the group exposed to dichlorvos preserved samples compared to the control. This finding is consistent with a study carried out by Tela and Sagir, 2016 [17], 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 [18].

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 results in cell shrinkage and degeneration of the glomerular tuft and the renal

tubule diameters. This in turn, might have affected the glomerular function of ultrafiltration and selective re-absorption thereby leading to a higher concentration of virtually all the serum electrolytes, urea, and creatinine [17].

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 [19]. Aluminum phosphide is a highly toxic, low cost and freely available pesticide. The fatal dose for a 70 kg man is 0.5 g [12]. Toxicity of phosphine is related to oxidant free radicals and associated inhibition of enzymes of metabolism, such as cytochrome c oxidase [20].

In this study, aluminum 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 a similar study by Morteza *et al.*, 2013 who reported an elevated level of liver enzymes in patients upon exposure to aluminum phosphide poisoning. Also Iniobong et al. [19] 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.

The kidney is another major target of phosphine poisoning in humans [21]. A non-significant increase in urea and creatinine levels was observed in rats fed with a sample preserved with ALP. The present finding is closely related to those stated by Mohamed, 2017 [22] 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 kidney function and renal injury [3]. 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 renal functions, which is attached to increases in serum creatinine and urea levels [23].

A slight increase in the level of Na^+ and K^+ was confirmed in this study and this can be attributed to renal injury after exposure to s 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 the promotion of lipid peroxidation, and Cholinesterase inhibition [24]. 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 [25]. This is in contrast with the result of this present study, where no significant difference was recorded in the CRP level.

In the hematological studies, there is a significant ($p<0.05$) decrease in PCV and a slight reduction in other parameters across the groups compared to the control group. Other studies have reported distortion in hematological parameters may be 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 hemichrome, a derivative of methemoglobin and Heinz bodies [26] [27] with the concomitant induction of free radicals [27]. Leukopenia is associated with rice tablet poisoning and has been considered a symptom of acute poisoning [28]. Thrombocytopenia has also been considered one of the late symptoms of poisoning in previous studies [28]. This might be linked to the reduction in the level of white blood cell and platelet observed in this study.

Pepper has been shown to possess antimicrobial activity and some have already produced compounds, effective against antibiotic-resistant strains of bacteria [3]. It has a pungent taste and according to studies posses active ingredients capable of retarding the infestation of pests [3]. However, the safety of the seeds over a long period of time has a dearth of information. In this research, during the preservation period of six months, a number of weevils were observed. Studies have revealed the role of weevils in seed contamination by fungi and molds. Bhusal and Khanal, 2019 [29] reported the highest mean contamination found under the presence of weevils while it was lower under the absence of weevils. In presence of weevil, the infestation of the fungus increased and in their absence, the infestation was low which signifies the role of weevil in fungal spread [29]. The overall effect of the sample preserved with pepper on the biochemical parameters might be attributed to possible contamination by the weevils detected on the sample after a period of six months.

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. Studies have revealed the presence of heavy metals in wood ash. According to Katarina Pastircakova, 2004 [30] the ashes from wood samples have generally higher As, Cd, Pb, and Hg contents than those of agricultural residues. Ten

elements of primary concern are arsenic, boron, cadmium, copper, mercury, molybdenum, nickel, lead, selenium, and zinc. Of these cadmium has the most– dangerous long-term effects on human health [30].

Conclusion

In this study, the biochemical effect of *Vigna Unguiculata* treated with preservatives (dichlorvos, aluminum 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 being* treated with both synthetic and natural preservatives, using matured adult male albino rats. The level of liver enzymes and kidney function parameters were analyzed, and a significant alteration was recorded majorly in the groups exposed to samples treated with pepper and ash which is suggestive of possible organ damage by the residual content of the preservatives as well as contamination as observed in the pepper group during the storage period. 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 a sample treated with wood ash. This suggests that there might be a possibility of inflammation by the components of wood ash used in preservation.

Pepper and wood ash usually considered safe alternative also alters the biochemical parameters hence suggesting the possibility of the presence of toxic residual components and contamination due to long periods of storage.

Recommendations for further studies

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

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