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Comparative Biochemical Effects of Natural and Synthetic Pesticides on Preserved *Vigna subterranea* in Male Albino Rats

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Abstract

Preservatives both natural and synthetic have been widely used to store food in order to ensure all season availability and safety for human consumption. This study was carried out to evaluate the biochemical effects of natural and synthetic pesticides preserved *Vigna subterranea* in male albino rats. Thirty six adult male albino weighing between 150 – 200 g were randomly allocated into 6 groups A, B, C, D, E and F (n=6 each). *Vigna subterranea* were preserved for six months in 5 air tight containers: preserved with neem ash, Pepper, Dichlovors, Aluminium Phosphide and without preservative. The preserved beans were milled into powder and used as dietary supplement for the rats. All the animals freely received animal feed and water. Group A, B, C, D, E and F were fed with animal feed and water only, Vigna subterranea without preservative, neem Ash preserved *Vigna subterranea*, Pepper preserved *Vigna subterranea*, Dichlovors preserved *Vigna subterranea* and Aluminium Phosphide preserved *Vigna subterranea* respectively. The dietary intervention lasted for 2 months after which the animals were sacrificed. The blood sample of each rat was collected and analyzed using standard methods. The results from the analysis of the liver function across the groups with preservatives showed increase in liver enzymes. The serum AST shows significant increase ($p < 0.05$) in Group D compared to Group A. ALT significantly ($p < 0.05$) increased in all the test groups except the group fed with pepper compared to the control which indicates there may be possible injury to the liver. The result showed elevation ($p < 0.05$) of C-reactive protein in all the test group compared to Group A and significantly ($p < 0.05$) reduced in the group fed with neem Ash, which may indicate injury and inflammation in the test animals of the other preservatives. In the kidney, the serum urea findings showed significant increase ($p<0.05$) in all the groups fed with preservatives compared to the Group A and the concentrations of Creatinine increased significantly ($p<0.05$) in all the groups fed with the preserved *V. subterranea* compared to the control indicating there may be possible damage to the kidney. A significant decrease ($p<0.05$) in the antioxidants-CAT, SOD and GPx and increase ($p<0.05$) in MDA in the group fed with preservatives indicates there may be increase in lipid peroxidation, leading to oxidative stress and cell injury in the test animals. The results of this study suggest that the use of some of these preservatives which could be natural and synthetic may be toxic and could lead to cellular and tissue damage.

Keywords: Aluminium Phosphide; Dichlorvos; Pepper; Ash and *Vigna subterranean*

Abbreviations: ALT: Serum Alanine Transaminase; AST: Serum Aspartate Transaminase; MDA: Malondialdehyde; GST: Glutathione S-Transferase; CAT: Catalase; GSH: Reduced Glutathione; SOD: Superoxide Dismutase.

Introduction

Leguminous plants are rich source of nutrient molecules such as protein, starch, minerals and vitamins. The presence of important health protective compounds such as phenolics, inositol phosphates and antioxidants may be found in them [1].

Legumes are believed to be one of the first crops cultivated by mankind and have remained a staple food for many cultures all over the world. These seeds are valued worldwide as an inexpensive meat alternative and are considered the second most important food source after [2].

Vigna subterranea is a legume indigenous to Africa and is cultivated across the semi-arid sub-Saharan Africa region. It is a hardy crop and has been recognized as an important nutritious food source when food is scarce. This could be attributed to its climate smart features, including its ability to fix nitrogen, and to grow under adverse environmental conditions such as poor soils and drought [3].

Food is an essential thing for human survival. Except our own garden plants, all the food used today has some preservatives [1]. Food grains are the most commonly stored durable food commodities in the tropic and subtropics, they are usually stored to provide food and feed reserves as well as seed for planting [4].

A number of methods of preservation can be used that can either totally prevent delay or otherwise reduce food spoilage. Preservatives can expand the shelf life of food and lengthen the time long enough for it to be preserved, processed, sold and kept in the consumers home for a reasonable length of time [5].

Herbs and Spices have been used for thousands of years to enhance the flavor, color and aroma of food; additionally, they are known for their preservative and medicinal value. It has been shown that pepper has antimicrobial activity and some have already produced compounds, effective against antibiotic resistant strains of bacteria. They have a pungent taste and cause salivation and numbness of the mouth [6].

Wood-ash is composed of the organic and inorganic residue remaining after the combustion of wood, this is used as a means of biological control which is generally favored as a method of storing seeds because it does not have any of those disadvantages of chemicals and tend to be more durable in its effect [7].

Aluminium phosphide is a highly toxic inorganic compound with the chemical formula AlP used as a wide band gap semiconductor and a fumigant. Aluminum phosphide is used as a fumigant to protect stored grain from insects and rodents. In the presence of moisture, aluminum phosphide releases phosphine, which is highly toxic [8].

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is one of the classes of insecticides referred to as organophosphates used to control households and food pest insects [9]. It is the most commonly used organophosphate pesticide in developing countries [10]. Therapeutically, dichlorvos is used as a fumigant or to treat a variety of parasitic worm infections in dogs and livestock and it acts against insects as both a contact and a stomach poison [9].

The primary aim of this study is to evaluate and compare the biochemical effects of natural and synthetic pesticides preserved *Vigna subterranea* in male albino rats.

Materials and Methods

Freshly harvested *Vigna subterranea* was be purchased from the area of cultivation at Keffi, Keffi Local Government Area, Nasarawa State, Nigeria. The natural pesticides used for the research were: wood ash gotten from neem tree and *Capsicum frutescens* commonly known as bird eye pepper. The synthetic pesticides used for the research were: Dichlovors or 2, 2-dichlorovinyl dimethyl phosphate and Aluminium Phosphide. The chemicals and kits used in biochemical analysis were of analytical grade and purchased from reputable companies.

Stem from a neem tree were burnt to ashes, the cooled ash was sieved to remove the dirt. About three hundred (300) gram was weighed and packed into a polythene bag.

Fresh birds eye pepper (*Capsicum frutescens*) was dried under the sun. Three hundred (300) gram of the dried pepper was weighed and packed into a polythene bag.

Vigna subterranea was cleaned and sorted to remove stones and dirt. 1kg of *Vigna subterranea* was weighed and placed into five buckets each which were tightly sealed and labelled. The content of the buckets are as follows; first bucket contained *Vigna subterranea* with no preservative, second bucket contained *Vigna subterranea* with neem Ash (300g), third bucket contained *Vigna subterranea* with pepper (300g), fourth bucket contained *Vigna subterranea* with Dichlovors (2ml), and fifth bucket contained *Vigna subterranea* with Aluminium Phosphide (2g). The *Vigna subterranea* were stored for a period of six (6) months. The

preserved *Vigna subterranea* were milled into powder and used as dietary supplement for the rats.

A total of 36 male albino rats weighing between 150-200 grams were used for this study. They were purchased from 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 condition for two weeks prior to experiment. Standard poultry mash (vital growers mash) were used as a basal diet during the experimental period. The control and experimental animals were provided with drinking water.

The 36 adult male 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 preserved *Vigna subterranea* to thirty (30) grams vital growers mash for duration of 60 days.
Group A: (Control) Animal feed and water.
Group B (*V.S*): Animal feed and water + *Vigna subterranea* without preservative.
Group C (*V.S*A): Animal feed and water + *Vigna subterranea* preserved with Ash.
Group D (*V.S*P): Animal feed and water + *Vigna subterranea* preserved with Pepper.
Group E (*V.S*D): Animal feed and water + *Vigna subterranea* preserved with Dichlovors.
Group F (*V.S*Al): Animal feed and water + *Vigna subterranea* preserved with Aluminium Phosphide.

The dietary intervention lasted for 2 months after which the animals were sacrificed. The blood sample of each rat was collected for 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 [11].

Blood urea and serum creatinine was assayed using the Bartels and Bohmer [12] method as outlined in the randox kit.

Sodium ion was determined according to the method of Trinder [13] and Maruna [14] as described in the teco kit.

While potassium ion was determined using the method of Terri and Sesin [15].

The C-reactive protein test was carried out according to the method described by Smits, et al. [16] using the Wellcome diagnostic kit for its qualitative and quantitative analysis.

Malondialdehyde was evaluated in the serum according to the method described by Wallin, et al. [17].

Superoxide dismutase activity was assayed according to the method described by the International Federation of Clinical and Applied Chemistry [18] as outlined in the randox kit.

Catalase activity was assayed with the randox kit according to the method described by Aebi [19]. Glutathione peroxidase was measured according to the method of Ursini, et al. [20].

Results Analysis

The data obtained were analyzed using one-way ANOVA in IBM SPSS version 23.0 to get the means and standard deviations. Further test for levels of significance across the groups was done using Duncan test and the acceptable level of significance was set at $p<0.05$.

Effect of Aluminium Phosphide, Dichlorvos, Pepper and Ash on Serum Liver Function Enzymes (AST and ALT)

The result obtained from the analysis of the serum levels of liver enzymes of the different groups of experimental animals is shown in Table 1. The serum AST shows significant increase ($p < 0.05$) in group *V.S* P (19.50±3.25) compared to the control group (15.37±1.38). Even though there was increase in other groups, there was no statistical significance in the changes in comparison with the control group. ALT significantly ($p < 0.05$) increased in all the test groups except the group fed with *V.S* P compared to the control.

Group	AST (IU/L)	ALT (IU/L)
CONTROL	15.37±1.38^a	15.11±2.76^a
V.S P	17.39±2.84^a	19.50±3.25^b
V.S A	16.01±3.09^a	18.40±2.386^b
V.S D	16.58±1.17^a	18.98±1.38^b
V.S Al	18.22±1.84^a	19.52±2.42^b
V.S	18.79±5.53^a	23.70±3.21^c

Table 1: Effect of Aluminium phosphide, Dichlorvos, Pepper and Ash on Serum Liver Function Enzymes (AST and ALT)

Results are presented in Mean ± SD, mean values with different letters as superscripts are considered at $P < 0.05$. AST= Aspartate amino transferase, ALT= Alanine amino transferase. *V.S* = Animal feed + *Vigna subterranea* without preservative, *V.S*A = Animal feed + *Vigna subterranea* preserved with Ash, *V.S*P = Animal feed + *Vigna subterranea* preserved with Pepper, *V.S*D = Animal feed + *Vigna*

subterranea preserved with Dichlovors, *V.S*Al = Animal feed + *Vigna subterranea* preserved with Aluminium Phosphide.

Effect of Aluminium Phosphide, Dichlorvos, Pepper and Ash on Some Kidney Function Indices

Table 2 shows a significant increase ($p<0.05$) in sodium ion in the groups fed with *V.S* Al and *V.S* P and *V.S* D compared to the compared to the control. Potassium ion was significantly ($p<0.05$) higher in the group fed with *V.S* Al, *V.S* D and *V.S* P compared with the control. Urea was significantly ($p<0.05$) higher in the groups fed with *V.S* Al, *V.S* D, *V.S* P, and *V.S* A compared to the control. The concentrations of Creatinine increased significantly ($p<0.05$) in all the groups fed with the preserved *V. subterranea* compared to the control.

Group	Na^+ (mmol/L)	K^+ (mmol/L)	Urea (mg/dL)	Creatinine (mmol/L)
CONTROL	128.41 ± 1.77^a	7.02 ± 2.2^a	$5.40\pm.94^a$	2.01 ± 1.16^a
V.S P	139.78 ± 1.62^c	14.47 ± 1.97^b	8.99 ± 1.74^b	5.70 ± 2.56^c
V.S A	125.92 ± 2.33^a	$8.07\pm.79^a$	12.20 ± 2.47^c	4.64 ± 1.75^c
V.S D	135.01 ± 5.19^b	15.08 ± 2.47^c	11.75 ± 2.44^b	12.11 ± 1.76^c
V.S Al	145.98 ± 28.74^b	14.59 ± 2.80^b	10.48 ± 1.54^b	6.13 ± 2.25^b
V.S	123.51 ± 3.71^a	8.27 ± 1.80^a	5.43 ± 1.53^a	6.11 ± 2.57^c

Table 2: Effect of Aluminium phosphide, Dichlorvos, Pepper and Ash on some Kidney Function Indices.

Results are presented in Mean ± SD, mean values with different letters as superscripts are considered at $p < 0.05$. *V.S* = Animal feed + *Vigna subterranea* without preservative, *V.S*A = Animal feed + *Vigna subterranea* preserved with Ash, *V.S*P = Animal feed + *Vigna subterranea* preserved with Pepper, *V.S*D = Animal feed + *Vigna subterranea* preserved with Dichlovors, *V.S*Al = Animal feed + *Vigna subterranea* preserved with Aluminium Phosphide.

Effect of Aluminium Phosphide, Dichlorvos, Pepper and Ash on C-Reactive Protein

Table 3 shows the results for CRP in the test groups when compared to the control group. CRP was significantly ($p < 0.05$) higher in the test groups *V.S* Al, *V.S* P, *V.S*D and *V.S* compared to the normal control group and significantly ($p < 0.05$) reduced in the group fed with *V.S* A.

GROUP	CRP (ug/m
CONTROL	62.97 ± 4.73^a
V.S P	69.88 ± 2.12^b
V.S A	60.91 ± 1.55^a
V.S D	67.82 ± 4.55^b
V.S Al	66.88 ± 4.55^b
V.S	66.52 ± 3.39^b

Table 3: Effect of Aluminium phosphide, Dichlorvos, Pepper and Ash on C-reactive protein.

Results are presented in Mean ± SD, mean values with different letters as superscripts are considered at $p < 0.05$. CRP= C reactive protein. *V.S* = Animal feed + *Vigna subterranea* without preservative, *V.S*A = Animal feed + *Vigna subterranea* preserved with Ash, *V.S*P = Animal feed + *Vigna subterranea* preserved with Pepper, *V.S*D = Animal feed + *Vigna subterranea* preserved with Dichlovors, *V.S*Al = Animal feed + *Vigna subterranea* preserved with Aluminium Phosphide.

Effect of Aluminium Phosphide, Dichlorvos, Pepper and Ash in Antioxidant Indices

Table 4 shows a significant ($p<0.05$) increase in MDA in the group fed with *V.S*Al, *V.S* P and *V.S*D compared to the control. Catalase increased significantly ($p<0.05$) in the group fed with *V.S* compared to the control and reduced significantly ($p<0.05$) in the groups fed with *V.S*Al, *V.S*D, and *V.S*P compared to the control. The activities of GPx were found to be significantly ($p<0.05$) lower in the groups fed with *V.S*D, *V.S*A and *V.S* preserved compared to the control. The activities of SOD significantly ($p<0.05$) increased in the groups fed with *V.S* Al and *V.S*D compared to the control and significantly reduced in the groups *V.S* P and *V.S* compared to the control.

Group	MDA (mg/dL)	CAT (IU/L)	GPx(IU/L)	SOD (IU/L)
CONTROL	3.45±1.25[a]	5.78±.81[a]	26.78±4.44[a]	15.86±1.84[a]
V.S P	6.44±1.19[c]	2.12±.20[c]	21.97±3.26[a]	10.93±4.44[c]
V.S A	2.74±1.035[a]	5.22±.92[a]	18.51±6.91[c]	12.92±3.59[a]
V.S D	13.85±3.96[b]	4.62±1.03[b]	8.39±1.48[b]	22.45±4.66[b]
V.S Al	5.94±.093[c]	4.67±.75[b]	22.40±2.87[a]	23.79±4.30[b]
V.S	4.68±1.40[a]	8.930±1.00[d]	19.23±1.32[c]	11.78±1.314[d]

Table 4: Effect of Aluminium phosphide, Dichlorvos, Pepper and Ash in antioxidant indices.

Results are presented in Mean ± SD, mean values with different letters as superscripts are considered at $p < 0.05$. *V.S* = Animal feed + *Vigna subterranea* without preservative, *V.S*A = Animal feed + *Vigna subterranea* preserved with Ash, *V.S*P = Animal feed + *Vigna subterranea* preserved with Pepper, *V.S*D = Animal feed + *Vigna subterranea* preserved with Dichlovors, *V.S*Al = Animal feed + *Vigna subterranea* preserved with Aluminium Phosphide.

Discussion

The liver has a significant role in metabolism, digestion, detoxification, and elimination of substances from the body. Aminotransferase includes AST and ALT are markers of hepatocellular injury [21]. Injury to the liver, whether acute or chronic, eventually results in an increase in serum concentrations of aminotransferases [22].

From the Table 4.1, the findings in group *V.S* P shows a significant increase in serum levels of AST. This agrees with the study that treated animals suffered impaired liver function as both levels of ALT and AST enzymes were increased about 45%, when compared to the control rabbits, these scientific evidences show that hot red pepper under study possesses some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing liver damage. This may indicate that the preservation of beans with pepper causes liver injuries [23].

From the result, the group fed with only *V.S* shows no significant increase ($p<0.05$) in serum AST in comparison with the AST level of the control group. However, a significant increase ($p<0.05$) in serum ALT was observed in comparison with the ALT level of the control group which indicates there may be injury to the liver. This agrees with the study that AST is also diffusely represented in the heart, skeletal muscle, kidneys, brain and red blood cells, and ALT has low concentrations in skeletal muscle and kidney, hence an increase in ALT serum levels is, therefore, more specific for liver damage [22].

The findings in group *V.S* A, *V.S* D and *V.S*Al showed a significant increase ($p<0.05$) in serum level of ALT indicating injury to the liver. The elevation of the liver enzyme in group *V.S*D agrees with the study on adult male domestic rabbits which observed elevation of the liver function biomarkers as a result of organophosphate exposure could be attributed to their release from the cytoplasm into the blood circulation indicating a necrosis and inflammatory reactions and reflect the alteration of the membrane permeability of the hepatocytes [24]. The result showed that aluminium phosphide may cause liver damage due to increase in ALT level which agrees with the study on Liver histopathology of fatal phosphine poisoning, that phosphine can cause liver dysfunction, especially after the first day of poisoning [25].

The aim of renal function tests is to detect impairment of renal function as early as possible. The kidney function assay showed that the control has the lowest levels of urea and creatinine. The low serum levels of urea and creatinine in the control group is an indication that there may be no impairment of renal function hence, no damage in the kidney. The Urea level in the group *V.S* showed significant increase ($p\leq0.05$) recorded in the creatinine level of the *V.S*. This may indicate a renal impairment as stated that at all stages of renal insufficiency, the creatinine is a much more reliable indicator of renal function than the BUN because the BUN is far more likely to be affected by dietary and physiologic conditions not related to renal function [26]. There is significant increase ($P\leq0.05$) in urea and creatinine level of all the other groups fed with preservatives. This shows that there may be possible damage of the kidney based on the increase in urea and creatinine level. The significant increase ($p\leq0.05$) in urea and creatinine level in *V.SD* may be due to the exposure to dichlovors which agrees with a study on dichlovorovinyl dimethyl phosphate in rabbits that dichlorvos exposure caused significant elevation in the mean urea and creatinine from day 30-90 [27]. The elevation in blood urea and creatinine in the study agrees with the study on Aluminium Phosphide-induced Hepato-nephrotoxicity and Oxidative Damage in Rats: The Protective Effect of -lipoic Acid that there was increase in blood urea and creatinine due

to aluminium phosphide treatment [28].

There was a significant increase (p≤0.05) in Na^+ and K_+ in groups *V.S*P, *V.S*D and *V.S*Al. This may signify that there are imbalance in the electrolytes that can disrupt normal bodily functions in test animals fed with *V. subterranea* preserved with Aluminium phosphide, Dichlorvos and Pepper. The increase (p≤0.05) in *V.SD* agrees with a study on dichlovorovinyl dimethyl phosphate (sniper) in rabbits that Dichlorvos exposure caused significant increase in all the measured electrolytes. The elevation in the level of electrolyte ions may be due to the inhibitive effect of dichlorvos on the tubular cells reabsorption of the ions [27]. Inhibition in the reabsorption of the ions may be due to serious nephrotic damage that is caused by the toxic metabolites of the dichlorvos [27].

C-reactive protein forms an integral component of innate immunity and serves primarily to recognize potential pathogens and damaged cells. Regardless of the nature and location of cellular/tissue damage, a non-specific, systemic acute-phase response is initiated [29].

In the study, with the exception of group *V.SA* preserved with Ash, other test groups showed a significant increase (p<0.05) compared to the control group. This would suggest injury and inflammation in the test animals as CRP shows increase in concentration during the occurrence of an injury, inflammation or tissue death [30].

Malondialdehyde (MDA) is a useful biomarker for lipid peroxidation and oxidative stress. MDA levels in different biological systems can be used as an important indicator of lipid peroxidation both in-vitro and in- vivo for various heath disorders [31]. Determination of MDA in blood plasma or tissue homogenates is one of the useful methods to predict the oxidative stress levels [31].

The serum MDA level from Table 4 showed statistically significant increase (p≤0.05) in the groups *V.SA, V.S* P and *V.S* D. This claims that there may be oxidative stress in groups as stated in a study that high MDA level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients [32]. The elevation of serum MDA level in test group *V.S* D which agrees with study that have reported that dichlorvos induce oxidative stress, as shown by enhanced MDA production [33]. In group *V.S* Al, the mechanism of phosphine induced lipid peroxidation could involve ROS generated from inhibition of cellular respiration, or a direct reaction between phosphine and H_2O_2 and agrees with the study on Aluminium Phosphide-induced Hepato-nephrotoxicity and Oxidative Damage in Rats: The Protective Effect of -lipoic Acid that Phosphine can trigger iron release from storage protein, increasing lipid peroxidation, leading to cell injury and/or cell death [28].

The CAT level showed a significant decrease (p≤0.05) in all the test groups compared to the control group except in the test group *V.S*. This signifies that there are high levels of H_2O_2 as stated that CAT is abundant in cells, where it continuously scouts for hydrogen peroxide molecules [34]. The decrease in CAT level in the test groups *V.S*Al, *V.SD* and *V.SP* may indicate presence of oxidative stress as catalase is one of the crucial antioxidant enzymes that mitigates oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen [35].

The levels of GPx revealed in table 4 shows statistically significant decrease (p<0.05) in *V.S* D, *V.S* A and *V.S* groups compared to the control group. This suggests that consuming the beans preserved in ash and Dichlovors might have effect on the oxidative integrity of the body as low GPx was observed. The levels of SOD revealed in Table 4.4 shows a significant decrease (p<0.05) in groups *V.S* and *V.S*P compared to the control group. The increase in levels of MDA and decrease in CAT, GPx and SOD in group *V.S* P may indicate that the preservation of beans with pepper may be a problem to the oxidative integrity of the test animals. The decrease in some serum antioxidant in groups *V.SD*, *V.S* Al may indicate oxidative stress in the test animals which agrees with the study that pesticide chemicals induce oxidative stress, leading to generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzyme system [36]. Organophosphate has also been reported to also induce oxidative stress in different parts of the brain, liver through increased levels of reactive oxygen species [37]. Treatment with aluminium phosphide significantly increased SOD and decreased CAT and GPx which agrees with the study on Aluminium Phosphide-induced Hepato-nephrotoxicity and Oxidative Damage in Rats: The Protective Effect of -lipoic Acid that treatment with Aluminium phosphide significantly decreased glutathione S-transferase (GST), catalase (CAT) and reduced glutathione (GSH) while superoxide dismutase (SOD) were increased in the rats [28].

Conclusion

From the results of this study, *V. Subterranea* preserved with the selected preservatives for a prolonged duration of time when consumed over time showed significant increase or decrease in the biochemical effect on the rats. The results from the analysis of the liver function across the groups with preservatives showed increase in liver enzymes which indicates there may be possible injury to the liver. The result showed elevation of C-reactive protein in all the test group except the group preserved with Ash which may indicate injury and inflammation in the test animals of the other preservatives. The Kidney function parameters findings

showed significant increase in the parameters indicating there may be possible damage to the kidney. A decrease in antioxidants and increase in MDA in the preservatives indicates there may be increase in lipid peroxidation, leading to oxidative stress and cell injury in the test animals. The results of this study suggest that the use of some of these preservatives which could be natural or synthetic may be toxic and could lead to cellular and tissue damage.

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