

# Effect of Green Synthesized Silver Nanoparticles from Orange Peels on Growth Performance and Liver Function Parameters of Male Albino Rats

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## Abstract

It has been estimated that of all nanoparticles used in consumer products, silver nanoparticles currently have the highest degree of commercialization with a wide range of industrial and medical applications that may put human health at risk. The toxicity of nanoparticles is a subject of continued controversy and concerns about their adverse effects on human health and the environment are growing. This study investigated the effect of green synthesized silver nanoparticles from orange peels on growth performance and liver function parameters of male albino rats. Orange peel samples were collected from Federal University Wukari and air dried, ground and soaked in 80% ethanol solution for 24 hours. The orange peel extract was filtered. Silver nanoparticle was produced by boiling orange peel extract with 1mM silver nitrate solution for 30 minutes. The green synthesized silver nanoparticles were orally administered at different doses (200, 400, 600ppm) to rats in group 2-4 for two weeks. The result of growth performance elicited highest feed efficiency in group 1 (control) while the lowest value was found in group 3. Sera levels alanine transaminase (ALT) and aspartate transaminase (AST) were significantly higher in treatment groups (2-4), when compared with the control (group 1). Sera level of ALP and GGT were slightly higher in the control group when compared with that of treatments. Higher sera levels of ALT and AST were observed which could be due to slight toxicity of AgNPs administered on the liver membranes of albino rat. Based on the findings of study, it was revealed that oral administration of silver nanoparticles between 200 and 600ppm had varying effects on liver function parameters and growth performance of the experimental animals. However, higher sera levels of ALT and AST were observed which could be due to slight toxicity of AgNPs exerting its effect on liver membranes of the albino rats.

**Keywords:** Silver nanoparticles; Albino rats; Orange peel; Biochemical parameters; Growth performance

## Introduction

Nanotechnology is a branch of science and engineering devoted to designing, producing, and using structures, devices, and systems by manipulating atoms and molecules at nanoscale (Arowora et al., 2024). Nanotechnology explores a variety of promising approaches in the area of material sciences on a molecular level and silver nanoparticles (AgNPs) are of leading interest. Silver nanoparticles (AgNPs) is currently attracting great attention due to their biotechnological applications and antimicrobial activities in medical application (Abraham et al., 2020). Their applications extend to areas of cosmetics, food and feed, environmental health, mechanics, optics, biomedical science, chemical industries, electronics, space industries, drug gene delivery, energy science, optoelectronics, catalysis, single electron transistor and photo-electrochemical application. It is estimated that of all nanoparticles used in consumer products, silver nanoparticles currently have the highest degree of commercialization with a wide range of industrial and medical applications that may put human health at risk (Arowora et al., 2023). Toxicity of nanoparticles is a subject of continued controversy and concerns about their adverse effects on human health and the environment are growing. Direct exposure of organs to nanomaterials through the bloodstream is unlikely expected in medical application, hence, there is potential for nanoparticles to enter the bloodstream through inhalation (Muhleld et al., 2008), dermal contact (Korani et al., 2011) or through gastrointestinal tract (Schleh et al., 2012). The liver is one of the organs that is targeted when there is translocation of NPs through the bloodstream and previous studies have shown high accumulation of NPs in the liver after injection (Bamedi et al., 2017). Following their entry into systemic circulation, AgNPs may migrate to liver, spleen, lungs, kidneys and brain and induce toxicity (Rahman et al. 2009; Tang et al. 2009). Interactions between silver nanoparticles and living system are not as yet fully understood. However, *In vitro* studies suggest that the mechanisms of AgNPs cytotoxicity include apoptosis, inflammation, free radical production, membrane damage, and cell death (Asha et al. 2009). Several factors have been reported to influence silver nanoparticle toxicity such as particle size, shape, surface area, capping agents, and surface reactivity. In general, smaller nanoparticles have been found to be more toxic, compared with larger nanoparticles (Panda et al. 2011; Kim et al. 2012). A smaller diameter of spherical particles is related with an increased surface-to-volume ratio and, in turn, the increased surface area is accompanied by increased chemical reactivity (Charity et al., 2022).

Orange is a fruit of various citrus species in the family Rutaceae. It is commonly referred to *Citrus sinensis* (Osbeck et al., 2011) which is also called sweet orange to distinguish it from the related *Citrus aurantium* referred to as bitter orange. The sweet orange reproduces asexually (through nucellar embryony); varieties of sweet orange arise through mutation (Cheng et al., 2013). Orange peels are known to be rich in fibre, vitamin C, folate, vitamin B6, calcium, and other essential nutrients. The skin of oranges contains ample amount of polyphenols that protects against several diseases. Orange peels have anti-cancerous properties; due to the presence of limonene, a naturally occurring chemical, and the essential oils present have anti-irritation properties (David et al., 2014). Several eco-friendly approaches have been used to synthesize nanoparticles since they have several advantages such as cost effectiveness, simplicity, and compatibility for antibacterial and antifungal activities. Many scientists are focused on green synthesis of nanoparticles from plant extracts (Huma et al., 2020). AgNPs have reportedly been synthesized from various plant parts such as roots, seeds, leaves, stems, and flowers for different applications, especially to the biomedical applications (Georgii et al., 2020). This synthesis is majorly by the reduction of silver ions to neutral silver atoms. Positive is achieved by the reduction of silver ions by a reducing agent (Jain, 2009). This study investigated the effect of green synthesized silver nanoparticles from orange peels on the growth performance and liver function parameters of male albino rats.

## Materials and Method

### Chemicals Used

All the reagents purchased and used for this study were of analytical grade and needed no further purification. Silver nitrate ( $\text{AgNO}_3$ ) and ethanol were purchased from Bayero University, Kano State, Nigeria laboratory with  $\geq 99.5\%$  purity.

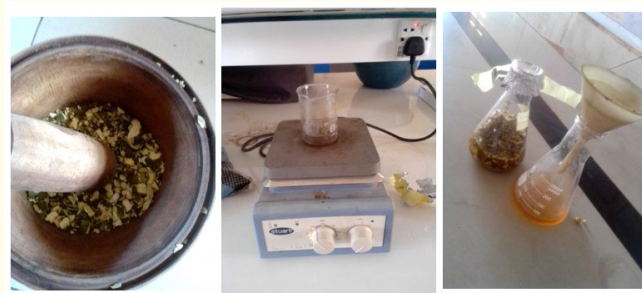
### Collection and Preparation of Orange Peels Extract

Sufficient amount of orange peels was collected from the premises of Federal University Wukari, Taraba State, Nigeria. Orange peel extract was prepared using the method described Arowora et al. (2024). 80% of ethanol solution was prepared by mixing 80ml of absolute ethanol and 20 ml of distilled water. 20g of the orange peels was weighed and soaked in the prepared ethanol solution for 24 hours. The mixture was then filtered and stored at  $4^\circ\text{C}$  after covering the beaker with aluminum foil for further analysis.



**Figure 1:** Image of collected orange peel samples.

Source: Snapshot.



**Figure 2:** Images showing the preparation of orange peels extract.

Source: Snapshot.

### **Synthesis of Silver Nanoparticles**

The method described in SHESTCO's laboratory manual (2010) was used for the synthesis of nanoparticles. A stock solution of silver nitrate was prepared by dissolving 0.170g of silver nitrate ( $\text{AgNO}_3$ ) in 1000ml (1 liter) of distilled water. 100 ml of ( $\text{AgNO}_3$ ) solution in a beaker was placed on hot plate with mild stirring for 30 minutes and then 50ml of the orange peel filtrate or extract was added and boiled for 30 minutes. The conical flask was covered with aluminum foil to prevent the solution from evaporating and reacting with light. Colour change was observed and the change in colour from brown to light brown and finally to reddish brown indicated the formation of silver nanoparticles.

### **Characterization of Silver Nanoparticles Using UV-Visible Spectroscopy**

UV-Visible spectra of synthesized AgNPs were recorded at different time intervals with spectrophotometer with the slit width and spectral bandwidth of 1.0 nm. The periodic scans of the optical absorbance between 300 and 600nm with a UV-Vis Spectrophotometer at a resolution of 1nm were performed to characterize the silver nanoparticle synthesized from orange peel extract.

### **Characterization of Silver Nanoparticles Using FTIR**

For FTIR characterization of silver nanoparticles the samples were placed in a holder in the path of the IR source. A detector read the analog signal and converted the signal to a spectrum. A computer was used to analyze the signals and identify the peaks. An IR beam went through a partially silvered mirror, which splitted the beam into two beams of equal intensity.

### **Experimental Animals**

Sixteen (16) healthy male albino rats were purchased from the Department of Biochemistry, Federal University Wukari animal farm. They were maintained under standard environmental conditions of temperature, relative humidity and light (12 hours of light and 12 hours of darkness) and fed on standard rat feed and water. They were randomly allotted into four treatments, Group 1(Control group), Group 2, Group 3 and Group 4 using varying levels of silver nanoparticles as follows: 200, 400 and 600ppm respectively. The animals were acclimatized for one week. The rats received humane care and later treated for two weeks (14 days). The rats were then starved overnight. On the following day, they were anaesthetized using 10% chloroform before sacrificing them.

### **Animal Grouping and Treatments**

Animals were randomly assigned into four experimental groups of four rats per group. Group 1 served as the control and received potable water daily. Groups 2, 3 and 4 were orally administered daily with 200, 400 and 600 ppm AgNPs respectively for 14 days.

### **Liver Function Parameters**

At the end of two weeks of administration of the silver nanoparticles, the animals were sacrificed under anaesthetization in slight chloroform. Blood samples were obtained by cardiac puncture into plain bottles and liver function tests were carried out. The serum samples of the rats were analyzed for various liver function parameters, including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total protein (TP), and Albumin (ALB) using a UV/visible spectrophotometer.

#### **Assessment of Aspartate Aminotransferase (AST) Activity**

Aspartate Aminotransferase activity in the serum samples was measured using the procedures described by Reitman and Frankel (1957). A Randox reagent kit was used for this analysis. The amino group was enzymatically transferred by AST present in the sample from L-aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate. AST activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The reaction involved in the assay system is presented as follows:



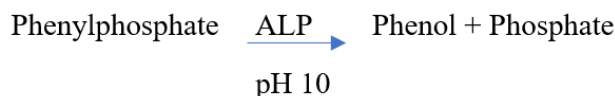
#### **Assessment of Alanine Aminotransferase (ALT) Activity**

Alanine Aminotransferase activity in the serum samples was investigated using the method described by Reitman and Frankel (1957) using a Randox reagent kit. The amino group was enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate. The reaction involved in the assay system is presented as follows:



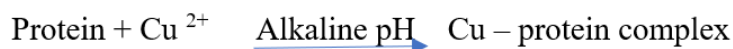
#### **Assessment of Alkaline Phosphatase (ALP) Activity**

Alkaline Phosphatase serum activity in the serum samples was assessed using the Agappe reagent kit, following the procedure by Schlebusch et al. (1974). Colorimetric determination of alkaline Phosphatase activity was according to the following reaction: The phenol liberate was measured in the presence of 4-aminoantipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stopped the enzymatic reaction. The reaction involved in the assay system is presented as follows:



#### **Determination of Total Protein (TP)**

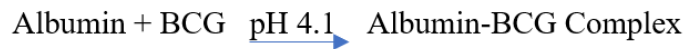
Randox reagent kit was utilized to determine the total protein concentration following the method outlined by Ayo et al. (2023). In alkaline medium copper reacted with the peptide bonds of proteins to form the characteristic pink to purple biuret complex. Sodium potassium tartarate prevented copper hydroxide precipitation, and potassium iodide prevented the autoreduction of copper. The color intensity which is directly proportional to the protein concentration was determined by measuring the increase in the absorbance at 546nm. The reaction involved in the assay system is as follows:



### **Determination of Albumin (ALB)**

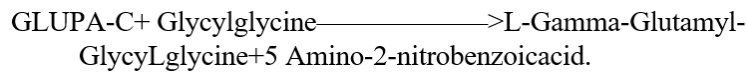
Agappe kit was used to determine the serum albumin concentration following the method described by Doumas et al. (1971). Measurement of albumin was based on its binding to the indicator dye bromocresol green (BCG) in pH 4.1 forming a blue-green colored complex. The intensity of the blue-green color was directly proportional to the concentration of albumin in the sample. It was determined by monitoring the increase in absorbance at 623 nm, or 578 nm. The reaction involved in the assay system is as follows:

Albumin + BCG pH 4.1 Albumin-BCG Complex



### **Determination of Gamma- Glutamyl Transferase (GGT)**

The serum activity determination of GGT was carried out by the method described by Szasz (1976) using Agappe reagent kit. GGT catalyzes the transfer of the gamma-glutamyl group from the donor substrate (GLUPA-C) to the glycylglycine acceptor to yield 3-carboxy-4-nitroanilide. Kinetic determination of Gamma GT according to the following reaction.



### **Calculation**

Gamma GT activity (U/L) = (OD/min) x 1158.

### **Growth Performance**

Growth performance was carried out using feed intake and body weight gain which represented the feed efficiency. The following formula was used to calculate the feed efficiency.

$$\text{Feed efficiency} = \frac{\text{Body weight gained}}{\text{Feed intake (g)}}$$

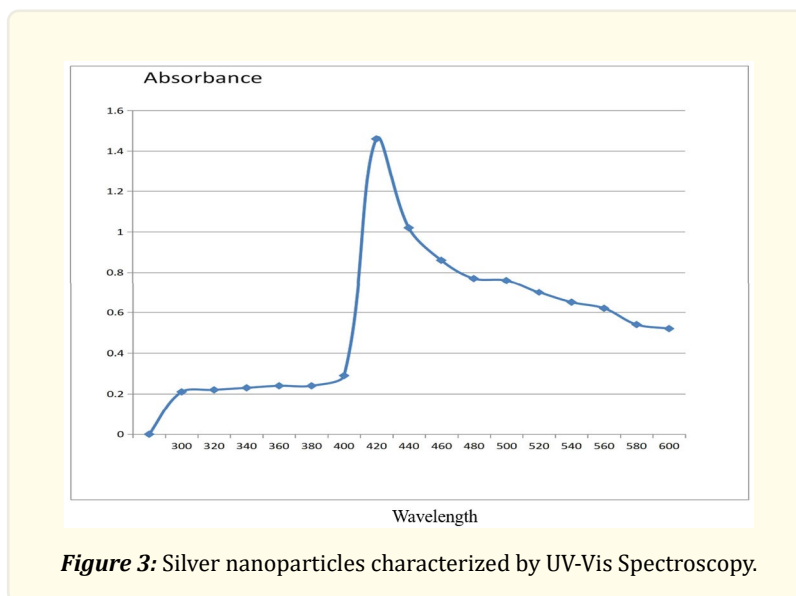
### **Statistical Analysis**

All values were presented as Mean  $\pm$  Standard deviation (SD). The significant differences in the means of all parameters determined using analysis of variance (ANOVA) and using statistical package for social sciences (SPSS) version 25. Group means were compared for significance at  $P < 0.05$ . Means along the same row having different superscripts are statically significant.

## **Results**

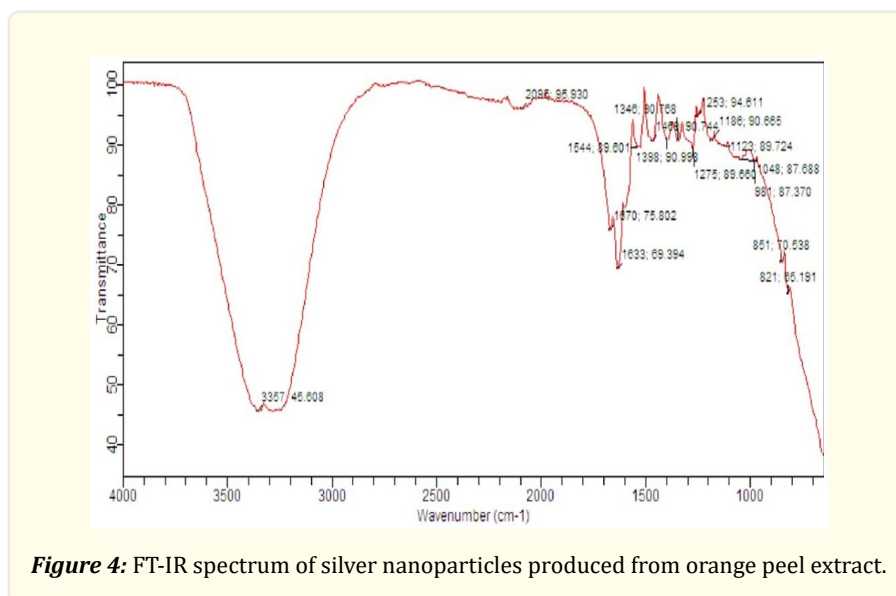
### **Characterization of Silver Nanoparticles Using UV-Visible Spectroscopy**

The result for characterization of silver nanoparticles using UV-visible spectroscopy is presented in Figure 3 below. The absorption characteristic peak of the ultraviolet-visible spectrum of the silver nanoparticles synthesized was observed at 420nm. The lowest wavelength at which silver nanoparticles were synthesized was 300nm.



#### Characterization of Silver Nanoparticle Using FTIR Spectroscopy

The changes observed in the reaction converting  $\text{AgNO}_3$  to AgNPs using FTIR analysis is represented below (Figure 4). Table 1 reveals various functional groups present upon subjecting the silver nanoparticles to FTIR spectroscopy. Alkyl or aryl halides were observed to be the most abundant functional groups present followed by alkenes.





S/N	Wavelength	Functional Groups	Inferences
1	3357	N-H Amines O-H stretch	Primary and secondary amine and amide Alcohols
2	2095	C≡C stretch	Alkynes
3	1670	C=C stretch (unsat.)	Alkenes
4	1663	C=C stretch (unsat.)	Alkenes
5	1544	NO <sub>2</sub> stretch	Nitro-compounds
6	1469	CH <sub>2</sub> stretch	Alkanes
7	1398	C-F stretch	Alkyl or Aryl halides
8	1346	C-F stretch	Alkyl or Aryl halides
9	1275	C=O stretch	Alcohols, Esters, Carboxylic Anhydride acid
10	1253	C-F stretch	Alkyl or Aryl halides
11	1186	C-F stretch	Alkyl or Aryl halides
12	1123	C-F stretch	Alkyl or Aryl halides
13	1048	C-F stretch	Alkyl or Aryl halides
14	981	Disubstituted trans	Alkenes
15	851	Disubstituted para	Phenols
16	821	C-CL	Alkyl or Aryl halides

**Table 1:** FTIR spectroscopy of silver nanoparticles showing the wavelength, functional groups and inference.

#### **Effect of Green Synthesized Silver Nanoparticles on Growth Performance of Experimental Animals After 14 days of feeding**

The result for the effect of green synthesized silver nanoparticles on growth performance of experimental animals after 14 days of being fed is captured in Table 2 below. The growth performance results showed that group 1 had the highest value of feed efficiency ratio. The lowest value of feed efficiency was recorded for group 3 that received 400ppm of AgNPs orally. The average weekly feed intake was observed to be highest for group 4 animals that were administered 600ppm of silver nanoparticles. Group 2 animals had the lowest average weekly feed intake. Experimental rats in the control group were observed to have the highest average weekly body weight gained whereas group 3 experimental animals that received 400ppm silver nanoparticles orally showed the lowest average weekly body weight gained.

Treatments	Group 1	Group 2	Group 3	Group 4
Average weekly feed intake (g)	449	356	418	502.5
Average weekly body weight	95.5	54	24	62
Gained (g)	0.213	0.151	0.057	0.123
Feed efficiency ratio				

**Table 2:** Growth performance of the experimental animals at the end of the 14 days feeding experiment.

#### **Effect of Green Synthesized Silver Nanoparticles on Biochemical Parameters of Experimental Animals After 14 days of feeding**

The results for the effect of green synthesized silver nanoparticles on the biochemical parameters of experimental animals after 14 days of feeding is presented in Table 3 and Table 4 below. The values gotten in ALT were observed to have a definite trend with group 1 (control) having lowest value while other groups increased proportionately to their doses i.e groups 2-4 respectively. ALP seemed to be normal compared to the control group. However, there is a significant difference ( $p \leq 0.05$ ) between control and treatments groups.



The range of GGT observed in this study was 124.8- 132.2(mM/l) with the highest value recorded for group 1 while the lowest was recorded for group 4. The other serum biochemical parameters such as the total protein, albumin and globulin had no significant difference  $p < 0.05$  (Table 4) and as such were not affected by the AgNPs in the blood.

Parameters	Group 1 (Control)	Group 2 200ppm	Group 3 400ppm	Group 4 600ppm
AST (u/l)	30.310 <sup>a</sup>	36.008 <sup>b</sup>	36.810 <sup>b</sup>	37.310 <sup>b</sup>
ALT (u/l)	22.324 <sup>a</sup>	51.9 39 <sup>b</sup>	57.008 <sup>c</sup>	62.008 <sup>d</sup>
ALP (u/l)	46.407 <sup>b</sup>	43.908 <sup>a</sup>	44.016 <sup>a</sup>	44.308 <sup>a</sup>
GGT(u/l)	132.209 <sup>b</sup>	129.616 <sup>ab</sup>	127.535 <sup>ab</sup>	124.845 <sup>a</sup>

**Table 3:** Effect of oral administration of silver nanoparticles on liver function enzymes of experimental rats.

\*Values are represented as mean S.D for duplicate determinations and values with different superscript on the same row are significantly different ( $P < 0.05$ ).

Parameters	Group 1 (Control)	Group 2 200ppm	Group 3 400ppm	Group 4 600ppm
Total Protein (g/dl)	49.01 <sup>a</sup>	49.07 <sup>a</sup>	49.03 <sup>a</sup>	50.04 <sup>a</sup>
Albumin (g/dl)	24. 01 <sup>a</sup>	25.07 <sup>a</sup>	25. 08 <sup>a</sup>	25. 09 <sup>a</sup>
Globulin (g/dl)	25. 01 <sup>a</sup>	24. 02 <sup>a</sup>	24. 02 <sup>a</sup>	24. 02 <sup>a</sup>

**Table 4:** Effects of oral administration of silver Nanoparticles on Total Protein, Albumin and Globulin of experimental rats.

\*Values are represented as means S.D for duplicate determination and values with different superscript on the same rows are significantly different ( $P < 0.05$ ).

## Discussion

Several eco-friendly approaches have been used to synthesize nanoparticles since they have several advantages such as cost effectiveness, simplicity, and compatibility for antibacterial and antifungal activities. Many scientists are focused on green synthesis of nanoparticles from plant extracts (Huma et al., 2020). AgNPs have reportedly been synthesized from various plant parts such as roots, seeds, leaves, stems, and flowers for different applications, especially to the biomedical applications (Georgii et al., 2020). This study investigated the effect of green synthesized silver nanoparticles from orange peels on the growth performance and liver function parameters of male albino rats.

FTIR Spectroscopy was used to identify the functional groups and biomolecules available in the crystal structure of silver nanoparticles. These biomolecules were identified based on the wavelength of their absorption and transmittance. The peak at which 3357nm could be due to the absorption by hydroxyl group that are found in flavonoids and polyphenolics. This band was observed for AgNPs at transmittance of 45.608, the shift occurred is indicative of the chelative between silver nanoparticles via OH group (alcohols) aromatic alcohol unsaturated hydrocarbons which could be present within the crystal structure of Ag-NPs. Furthermore, the alkyne ( $C \equiv C$ ) and alkene ( $C = C$ ) vibration peaks were observed at 2095 and 1663 respectively for AgNPs.

The growth performance results showed that group 1 had the highest value of feed efficiency. This could be due to the fact that the animals in this group were fed with rat cubes and potable water *ad-libitum*. More so, when they were not administered silver nanoparticles that could have effects on metabolic processes and consequently weight gained by animals. The lowest value of feed of efficiency was recorded for group 3 that received 400ppm of AgNPs orally administered. According to Haytham and Ibrahim (2015), the low weight gained by rats may be due to the poor absorption of the nutrients in their feed which could have resulted from mild toxicity of

silver nanoparticles. Available information showed that nanoparticles are often used due to their antimicrobial effects. Deposition in vital organs or tissues could induce cellular damage.

In this study, the oral administration of rats with AgNPs could have had impact on the tissues of the rats which were fed with different doses ranging from 200- 600ppm. The effects of the AgNPs in the liver tissues could lead to liver damage. ALT as an important liver marker of hepatocyte injury when seen in the blood indicate chronic liver disease such as cirrhosis, heart failure, damage to red blood cells e.t.c (Lain et al., 2020). The values gotten in ALT were observed to have a definite trend with the group 1 (control) having lowest values while other groups increased proportionately to their doses i.e groups 2-4 respectively. It is pertinent to mention that there were significant differences, ( $p < 0.05$ ) among treatments. This observation is in line with the previous studies carried out by (Allen and Gurrin, 2008).

AST which is also a marker of hepatocyte injury, as elevated level in the liver indicates chronic liver disease and also kidney disease such as viral hepatitis, cirrhosis and kidney failure (Asrani et al., 2019). There is also an increasing trend between group 1 and other groups which were significantly different  $p < 0.05$  (Table 3) could have been due to accumulation of nanoparticles in the liver which affected liver functionality and integrity. According to Larue (2014), ALP is an isoenzyme found in the liver, bone, intestine and placenta; its production is increased when the biliary tract is damaged. In this study the levels of ALP seem to be normal compared to the control group. However, there is a significant difference ( $p \leq 0.05$ ) between control and treatments groups. The range of GGT observed in this study was 124.8- 132.2 (mM/l) with the highest value recorded for group 1 while the lowest was recorded for group 4. However, it is pertinent to mention that there was a significant difference between treatment 1 and 4 (Larue, 2014). The other serum biochemical parameters such as the total protein, albumin and globulin had no significant difference  $p < 0.05$  (Table 4.3) and as such were not affected by the AgNPs in the blood. This indicates that the liver was able to maintain the production of these components.

## Conclusion

Based on the findings of study, it was revealed that oral administration of silver nanoparticles between 200 and 600ppm had varying effects on liver function parameters and growth performance of the experimental animals. However, higher sera levels of ALT and AST were observed which could be due to slight toxicity of AgNPs exerting its effect on liver membranes of the albino rats.

## Acknowledgments

We acknowledge all the authors of this work for their contributions.

## Conflicts of Interest

All authors declare that they have no conflict of interest associated with this research work.

## Funding

No special funding was received for this research work.

## Justification of the Study

It is true that silver nanoparticles are of greater importance; their applications are generally in the manufacture of medical, household and industrial products. As antibacterial agents silver nanoparticles were used for wide range of applications from disinfecting medical devices and home appliances to water treatments. AgNPs promisingly used in drastic field such as health care products, food storage, textile and medicinal devices. Despite all this aforementioned advantages, silver nanoparticle has been implicated in the toxicity range of up to 5000ppm, Hence there is a need to investigate effects of green synthesized nanoparticles from orange peels on the growth performance and liver function parameters of male albino rats.

### ***Aim and objectives of the study.***

The aim of this study is to investigate the effects of green synthesized silver nanoparticle from orange peels on growth performance and liver function parameters in male albino rats.

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