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EVALUATION OF SERUM BIOCHEMICAL PARAMETERS IN MALE WISTAR RATS ADMINISTERED WITH AZADIRACHTA INDICA SILVER NANOPARTICLES

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Abstract

The excessive exposure to silver nanoparticles (AgNPs) has raised concerns about their possible risks to the human health. This study investigated the effects of administering silver nanoparticles on serum biochemical parameters in male wistar rats. Synthesis of AgNPs from AgNO3 and plant extract was achieved using standard methods. At the same time, the characteristic peak of the synthesized AgNPs was determined using a UV-VIS spectrophotometer at a resolution of 1 nM. The crystal structure of AgNPs was determined using the Fourier Transform Infrared Spectroscopy (FTIR), while kidney and liver function parameters were carried out using serum with the aid of biochemical kits. A total of sixteen male wistar rats were randomly allotted into four experimental groups of four rats per group. Group 1 served as the control and received potable water. Groups two, three and four were orally administered with varying concentrations of silver nanoparticles (AgNPs) at 100, 250 and 500 mg/kg daily for two weeks respectively. Following cessation of treatments, rats were sacrificed under anaesthetization, and serum samples were collected for analysis. The result revealed that the absorption characteristic peak of the

ultraviolet-visible spectrum of the silver nanoparticles synthesized was 450 nM. It was also observed that the urea, creatinine, and potassium ion (K+) had no significant difference (P>0.05). However, the values of total bilirubin (11.00±3.68 mg/dl) and Sodium ion (140.00±3.54) in group 4 decreased significantly (P<0.05) when compared to group 2, 3 and the control group. Also, it was observed that there is a significant increase (P<0.05) in the value of Chloride ion in all the administered group when compared to the control. Synthesized silver nanoparticles from A. indica also affected the level of liver parameters such as ALT, ALP, TP AST, and Albumin in a non-significant way. This study found no evidence of hazardous effects from silver nanoparticles, which could be attributed to the minimal dosage of AgNPs or the nanoparticles' source.

Keywords: Silver nanoparticles, Azadirachta indica, Male wistar rats, Biochemical parameters, Hazardous

INTRODUCTION

Nanoscience and nanotechnology are the greatest interdisciplinary branches that measures at the nanoscale (Arowora *et al.,* 2023). According to Boverhof *et al.* (2015), nanotechnology in the last two decades have penetrated different fields such as therapeutics, medicine, drug developmental, environment and biotechnology. Nanoparticles (NPs), which are the building blocks of nanotechnology, contains particles that are either in an unbounded, aggregated or agglomerated form, having at least 50 % distribution with a size range of 1 – 100 nm (Arowora *et al.,* 2023). These nanoparticles are broadly classified as either synthetic or natural, based on their origin (Khan *et al*., 2019). Natural nanoparticles have been present in the environment for millions of years and they have been generated by a number of natural processes including weathering, erosion, volcanic eruption, hydrolysis and biological activities (Bevers *et al.,* 2020).

A number of epidemiological studies have shown that the exposure of these nanoparticles to the body, is associated with various diseases such as anaphylaxis, heart disease and lung cancer with an expected increase of mortality (Yu *et al*., 2013; Arowora *et al.,* 2023). Silver nanoparticles (AgNPs), one of the known NPs have been reported to be among the most hazardous metal nanoparticles due to the extensive uses and the inevitable exposure to human (Mohamed *et al*., 2021). The impact of AgNPs depends mainly on their particle size.

The smaller particles can induce greater harmfulness due to increased mass diffusivity, attachment efficiency and deposition velocity of NPs over the biological or solid surfaces (Liu *et al*., 2010). However, the shape and solubility of AgNPs could also affect the cellular uptake, which in turn influence the cytotoxicity (Stoehr *et al*., 2011). AgNPs is widely used in the production of cosmetics, healthcare products and wound dressings (Yu *et al.* 2013). Interestingly, it also possess antibacterial efficacy and more recently, synergistic affects with antibiotics against resistant bacteria species (Gurunathan *et al*., 2009; Arowora *et al.,* 2023).

Among the different methods employed in the synthesis of AgNPs, the plant-mediated green synthesis of silver nanoparticles, which is a well-known substitute for conventional techniques, has gained prominence due to its eco-friendly and cost effective, lesser toxicity when associated with chemical hazards (Arowora *et al.,* 2023). It has also grown into a new and important branch of nanotechnology (Chanel *et al*., 2017). This method utilizes plant extracts in the reduction of silver ions to biosynthesize silver nanoparticles (Arowora *et al.,* 2023). Plant-mediated silver nanoparticles have higher biological functions than those synthesized by chemical methods (Choudhury *et al*., 2016)

Nanoparticles synthesized from plant extracts have enhanced antioxidant activity, which maybe attributed to the excellent absorption of antioxidant from plant extracts on the surface of nanoparticles (Bunchez *et al*, 2012). Salari *et al.* (2019) illustrated how AgNPs synthesized using an aqueous *Prosopis farcta* fruit extract were excellent free radical cleaners that had similar effect to that obtained from aqueous apple extract (Nagaich *et al*., 2016), *Indigofera hirsuta* extract (Netala *et al*., 2018), and leaf extracts of *Elephantopus scaber* (Kharat and Mendhulkar, 2016). Silver nanoparticles were also found to stimulate the apoptotic pathway by creating free oxygen radicals, implying that they possess antitumor, antiproliferative and antiangogenic activities *in vitro* (Gurunathan *et al*., 2009). Silver nanoparticles disturb normal cellular function and affect the influence of membranes by persuade various apoptotic signaling genes in mammalian cells, leading to planned cell death (Sanpui *et al*., 2011). Taking into account the enormous potentiality of plants as a source, this work is aimed at determining the effect of administering AgNPs synthesized from aqueous leaf extract of *Azadirachta indica* on some biochemical parameter in male Wistar rats.

Azadirachta indica, usually called neem, is a species from a family called Meliaceae, which was used to bioconvert silver ions into nanoparticles. This plant is widely distributed in

India, and from ancient times, all parts of the tree have been employed as natural remedies for a variety of human maladies, including viral infections (Arowora *et al.,* 2023). The plant leaves contain β-sitosterol (a polyphenolic flavonoid) and quercetin with anti-fungal and antibacterial activities (Srivastava et al. 2020). According to reports, neem leaves and their constituents have immuno-modulatory, anti-inflammatory, anti-hyperglycemic, ulcer-healing, antioxidant, anticarcinogenic, anti-mutagenic, antimalarial, antibacterial, anti-fungal, and antiviral properties (Girish and Shankara 2008).

METHODS

Chemicals and Plant Material Collection

All the reagents purchased were of analytical grade and used without any further purification. Silver nitrate ($AgNO_3$) was purchased from Merck with a ≥99.5% purity. *Azadirachta indica* leaf were collected from Wukari environs into a polyethylene zipper bag and transported to the Biochemistry Research Laboratory, Federal University Wukari, Taraba State, Nigeria.

Preparation of Plant Sample

The extraction of the plant sample was done using the method described by David et al. (2014). The *Azadirachta indica* leaf extract was prepared with distilled water. It was thoroughly washed with tap water and then with distilled water and cut into small pieces. The chopped leaves were then ground using mortar and pestle and 10g was weighed and heated in 100 ml of distilled water for 30 minutes at 60°C. The leaf broth was then cooled and filtered using Whatman No. 1 paper.

Synthesis and characterization of Silver Nanoparticles

The biosynthesis of Silver Nanoparticles was done using plant materials as described by Sahayaraj and Rajesh (2011). A stock solution of 1 mM silver nitrate ($AgNO_3$) was prepared with distilled water. 10 ml of aqueous extract of *Azadirachta indica* leaf was added to 200 ml of 1 mM $AgNO_3$ solution placed on a hot plate with mild stirring. After heating the solution at 60°C for 30 minutes, there was a change in colour from green to dark brown which indicates the formation of silver nanoparticles. The characteristic peaks were determined using a UV-VIS spectrophotometer at a resolution of 1 nm, by periodically scanning the optical absorbance between a range of 300-600 nm to investigate the

reduction rate of silver ions by the leaf extract. The crystals of green synthesized silver nanoparticles produced were subjected to FTIR spectroscopy for characterization.

Experimental Animals

Male rats of Wistar strain weighing between 120 and 180 g were obtained from National Veterinary Research Institute Vom, Plateau state, Nigeria. Animals were housed in a hygienic environment and allowed to acclimatize for a week before the commencement of treatments. Animals were fed with commercial rat chows and given potable water *ad libitum*.

Animal Groupings and Treatments

Animals were randomly assigned into four experimental groups 1–4 of four rats per group. Group 1 served as control and received potable water daily. Groups 2, 3, and 4 were daily administered with 100, 250, and 500 mg/kg AgNPs respectively for 14 days. All treatments were orally administered with the aid of a cannula.

Serum Biochemical Parameters

At the end of two weeks administration, rats were sacrificed under anaesthetization in slight chloroform. Blood samples was obtained by cardiac puncture into plain bottles and allowed to stand for 15 minutes for coagulation and centrifuged 10 min at 3,000 rpm, Serum was obtained by aspiration of the clear yellowish liquid. Estimation of kidney function parameters such as Serum urea, Creatinine, Total bilirubin, Sodium ion, Potassium ion and Chloride ion; and liver function tests, such as AST (Aspartate Transaminase), ALT (Alanine Transaminase), Alkaline phosphatase (ALP), Total Protein (TP), and Albumin (ALB), were carried out using serum with the aid of biochemical kits.

Statistical analysis

All the analyses were carried out in duplicates in a completely randomized design. The data were subjected to analysis of variance using Statistical Package for Social Science (SPSS) software. Means that were significantly different were separated by the Least Significant Difference (LSD) test. Significance was accepted at $P<0.05$.

RESULTS

Synthesis of AgNPs from $AgNO_3$ and *Azadirachta indica* leaf extract

The biosynthesis of Silver Nanoparticles was done using plant materials, as described by Sahayaraj and Rajesh (2011). After heating the solution at 60°C for 30 minutes, there was a change in color from dark green to yellow, indicating the formation of silver nanoparticles (Figure 1).

Spectrophotometric evaluation of $AgNO_3$ reduction

In this study, silver nanoparticles were synthesized from plant material. The absorption characteristic peak of the ultraviolet-visible spectrum of the silver nanoparticles synthesized was observed at 445 nM (Figure 2).

FTIR spectroscopy characterization of green synthesized silver nanoparticles: The crystals of green synthesized silver nanoparticles produced were subjected to FTIR spectroscopy for characterization. The changes observed in the reaction converting $AgNO_3$ to AgNPs using FTIR analysis are represented below (Figure 3).

Figure 1. Synthesis of silver nanoparticles

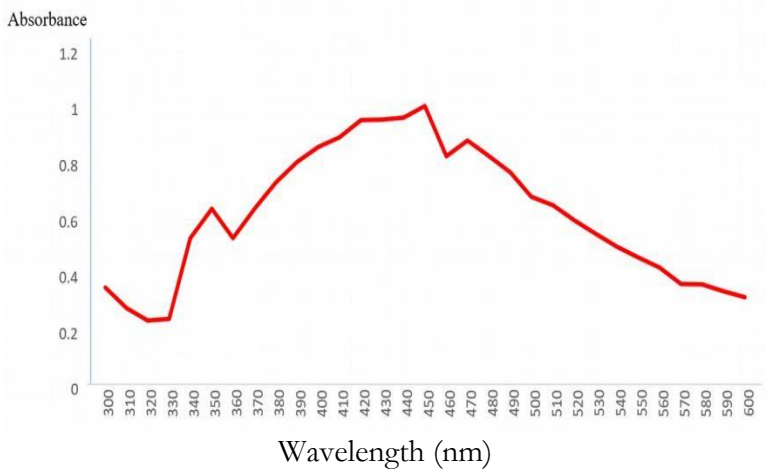

Figure 2. Characteristic peak of silver nanoparticles absorption

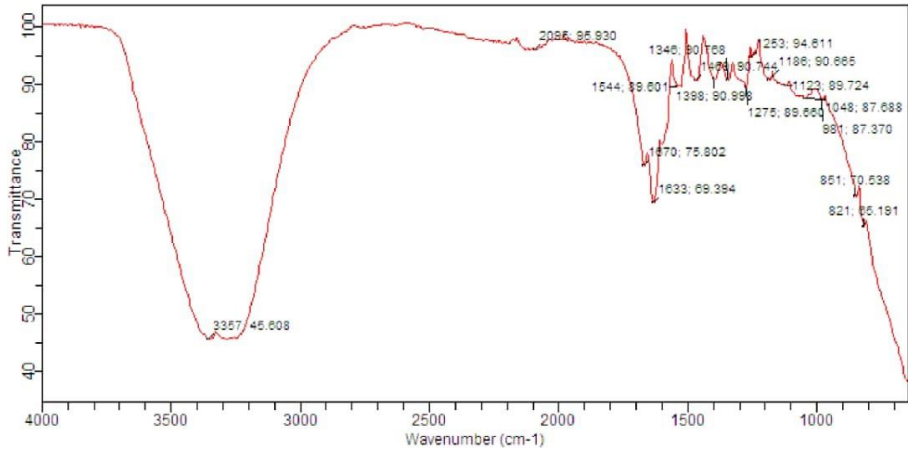

Figure 3. FTIR spectrum of synthesized AgNPs

Table 1. Effects of orally Administered Silver nanoparticles on kidney function parameters

Group	**UREA mmol/l**	**CREA µmm/l**	**TB mg/dl**	**Na^+ mEq/l**	**K^+ mEq/l**	**Cl^- mEq/l**
1	4.75±0.64[a]	91.50±9.19[a]	15.40±4.53[a]	129.50±0.71[b]	5.25±0.49[a]	104.50±0.71[b]
2	6.25±1.48[a]	104.00±2.83[a]	18.20±0.57[a]	133.00±1.41[b]	4.30±0.42[a]	112.00±1.41[a]
3	7.05±1.91[a]	120.00±0.00[a]	16.85±9.26[a]	134.50±0.71[b]	5.20±0.28[a]	113.00±1.41[a]
4	6.15±0.78[a]	108.50±27.58[a]	11.00±3.68[b]	140.00±3.54[a]	5.00±0.00[a]	111.50±3.54[a]

KEY: Values are presented as means ± SD, duplicate determinations and values with different superscripts within the same row are significantly different ($p<0.05$). Group 1 served as control and received potable water daily. Groups 2, 3, and 4 were administered daily with 100, 250, and 500 mg/kg AgNPs for 14 days. Creatinine (CREA), Total Bilirubin (TB), Sodium (Na^+), Potassium (K^+) and Chloride (Cl^-).

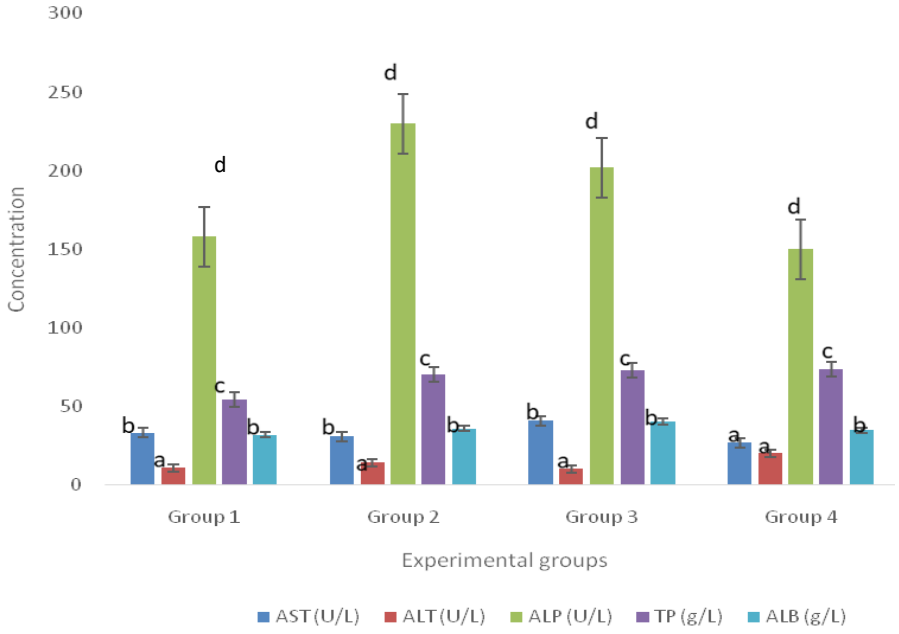

Figure 4. Effects of experimental diets on serum biochemical parameters of albino rats at the end of 14 days feeding experiment. Note: Values are presented as means ± SD for duplicate determinations, and values with different superscripts within the same column have no significant difference (P>0.05). Group 1 served as control and received potable water daily. Groups 2, 3, and 4 were administered daily with 100, 250, and 500 mg/kg AgNPs for 14 days. Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP), and Albumin (ALB).

DISCUSSION

An expanding field of study is the green production of nanoparticles, a well-known substitute for conventional techniques. This method utilizes plant extracts for the biosynthesis of nanoparticles. The advantages of green syntheses over chemical and physical approaches include environmental friendliness, cost-effectiveness, and ease of scaling up for large-scale nanoparticle production. In addition, there is no need to utilize harmful compounds or high temperatures, pressures, or energies (David et al. 2014). Considering the enormous potential of plants as a source, this work synthesized AgNPs using *A. indica* aqueous leaf extract.

In this study, silver nanoparticles were synthesized from plant material. The absorption characteristic peak of the ultraviolet-visible spectrum of the silver nanoparticles synthesized was observed at 445 nM (Figure 2). FTIR spectroscopy was used to identify the functional

groups and biomolecules available in the crystal structure of silver nanoparticles (Figure 2). These biomolecules were identified based on the wavelength of their absorption and transmittance. The peak at this wavelength (3357 nM) was due to the absorption by the hydroxyl group that could be found in flavonoids and polyphenolics. This band appeared for AgNPS at a transmittance of 45.608; the shift indicates the chelating between silver nanoparticles via the OH group, which could be alcohols, i.e., aromatic alcohol. Unsaturated hydrocarbons could be present since the stretches at 2095 nM could be due to the alkyne (c≡c) bond absorption, while 1670 and 1663 nM could be due to alkene (c═c) and alkanes within the crystal structure of Ag-NPs.

Therefore, to study the effects of silver nanoparticles on kidney and liver function parameters, the nanoparticles synthesized were administered to the experimental rats at different concentrations for 14 days. It was observed from the result of serum kidney function parameters (table 1) that the values of urea, creatinine, and potassium ion (K^+) had no significant difference ($P>0.05$). However, the values of total bilirubin (11.00±3.68 mg/dl) and Sodium ion (140.00±3.54) in group 4 decreased significantly ($P<0.05$) when compared to group 2, 3 and the control group. Also, it was observed that there is a significant increase ($P<0.05$) in the value of Chloride ion in all the administered group when compared to the control. However, there were no significant differences ($P>0.05$) among the groups in the parameters estimated for serum liver function parameters (Figure 4).

Urea nitrogen is a normal waste nitrogen product found in blood that comes from the breakdown of protein from foods. According to Rusul Arif *et al.* (2014) healthy kidneys remove urea nitrogen from blood, but the level of urea in blood rises when kidney failure occurs. The quantity of creatinine in serum depends on their generation, glomerular filtration and tubular secretion of serum creatinine. Gavanji et al. (2014) reported that Calculations based on serum creatinine and the age groups of the patients are used to estimate more precisely the degree of kidney function. However, there is significant difference ($P<0.05$) observed between groups 1 and 2 and groups 3 and 4 of sodium (Na^+). It has been reported that aqueous extract of *Hibiscus sabdariffa* is rich in sodium ions (Na^+), implying that its oral consumption may increase plasma sodium (Na^+) levels. A mechanism for the sodium increase could be due to the action of the flavonoid content of *Hibiscus sabdariffa* (Emelike *et al.*, 2013). The values of chloride ion (Cl^-) indicated a significant variation ($P<0.05$) between group 1 and the other three groups 2, 3 and 4

respectively. Arowora et al. (2023) reported that normal ranges of electrolytes, creatinine and urea are evidences that the kidney of wistar rats are without any forms of disorders. Increase and abnormal creatinine and blood urea nitrogen levels have been reported as evidence of kidney disorders and adrenal dysfunction. Furthermore, Saratale et al. (2019) explained that low or high total protein is an indication of liver disorders and malfunction.

The activity of AST and ALT enzymes is the first stage in examining liver damage; normally, an increase in AST and ALT indicates a liver problem (Gavanji et al. 2014). The most sensitive and the most practical recognizing enzymes in the liver are aminotransferases; the enzymes normally exist within the liver cells. When the liver gets damaged, the cells flow the enzymes to the blood, and the increase in the level of the enzymes indicates liver damage (Reitman and Frankel 1957). ALP is a hydrolytic enzyme whose activity is observed in alkaline pH; different forms of these enzymes exist in the blood. ALP serum levels in blood increased in pathologic conditions and bone and liver damage (Soochan et al. 2012).

The average amounts of AST, ALT, and ALP were observed to be 33.50±3.54, 11.00±9.90, and 158.00.0±31.11 U/L, respectively, in the Control Group. The highest amount of AST (41.00±25.46 U/L), ALT (20.50±0.7), and ALP (230.00±0.00) were observed to be non-significant with a silver nanoparticle concentration of 250 mg/kg (Group 3), 500 mg/kg (Group 4), and 100 mg/kg (Group 2) respectively. These findings are similar to research by Gavanji et al. (2014), who reported no meaningful difference in AST and ALP levels in all groups. However, the level of ALT leads to a meaningful effect when compared with the control. This result differs from that reported by Parang and Moghadamnia (2018), who observed a significant increase in the mean serum concentrations of AST, ALT, and ALP with an increase in silver nanoparticle concentrations. That could be attributed to the type of synthesis used and the dosage of administered AgNPs. Also, the change in the mean of albumin and total protein levels in the experimental groups was insignificant. However, Parang and Moghadamnia (2018) reported a significant increase in all the treated groups compared to the Control Group.

Al Gurabi and associates in 2015 have looked into the possible impacts of silver nanoparticles on DNA damage and apoptotic cell death in albino mice in vivo (Ali et al. 2012). Silver nanoparticles have been demonstrated to significantly worsen liver injury symptoms, resulting in elevated ALP, ALT, and AST enzyme levels. Silver nanoparticles

were experimented with mice, and it was discovered that the particles caused DNA damage and cell death in lymphocytes and the liver. Furthermore, 7.8 mg/kg of silver nanoparticles significantly damaged DNA and resulted in cell death (Ali et al. 2012).

According to Guo et al. (2015), intravenous administration of silver nanoparticles causes the liver and kidneys to become toxic by weakening endothelial connections associated with intracellular ROS (Guo et al. 2015). Additionally, through intravenous exposure, alterations of endothelial cells brought on by silver nanoparticles influence widespread peripheral inflammation in the liver and kidneys (Guo et al. 2015). Also, systemic exposure to silver nanoparticles was demonstrated to cause liver damage and NLRP3-dependent inflammation in a study by Ramadi et al. (2016). These nanoparticles also raised the concentrations of AST, ALT, and LDH. The findings of this study, which indicate that silver nanoparticles increase blood serum levels in a non-significant manner, agree with some researchers' investigations and differ slightly from other studies.

In conclusion, synthesized silver nanoparticles from *A. indica* altered the level of some selected biochemical parameters in a non-significant pattern. This study also found no evidence of hazardous effects from silver nanoparticles, which could be attributed to the minimal dosage of AgNPs or the nanoparticles' source.

CONCLUSION

This study confirmed the successful synthesis of AgNPs with characteristic peaks observed in UV-VIS and FTIR spectroscopy, indicating the presence of functional biomolecules and crystal structures typical of AgNPs.

Administration of AgNPs to Wistar rats showed no significant adverse effects on kidney parameters such as urea and creatinine, indicating normal kidney function. A decrease in bilirubin and sodium ion levels at higher concentrations of AgNPs was noted, along with an increase in chloride ions in all treated groups. Liver function parameters remained largely unaffected, with non-significant changes in ALT, ALP, AST, and albumin levels, aligning with some previous research but differing slightly from others that reported more significant effects.

Overall, this study concluded that synthesized silver nanoparticles from A. indica did not produce hazardous effects on selected biochemical parameters in male Wistar rats, which

could be due to the controlled dosages or the plant-based synthesis method. This finding supports the potential safety of AgNPs at low dosages and highlights the importance of further research to confirm these results across different conditions and concentrations.

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