Neran Ali Thamer and Lamia A. AL-Mashhady. / International Journal of Phytopharmacology. **7(1), 2016, 13-16.**

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ACUTE TOXICITY OF GREEN SYNTHESIS OF SILVER NANOPARTICLES USING CROCUS SATIVUD L. ON WHITE ALBINO RATS

Neran Ali Thamer[1*] and Lamia A. AL-Mashhady[2]

[1]Iraqi Center for Cancer and Medical Genetic Research, University of Al-Mustansiria, Baghdad, Iraq.
[2]College of Science, University of Babylon, Babylon.

ABSTRACT

Up and down method was used to study the acute toxicity effect of green synthesis of silver nanoparticles (AgNPs)synthesized by *crocus sativus L* on white albino rats .The study involved intraperitoneal administration of silver Nanoparticles at a limited dose of 4000 mg/kg body weight. Signs of toxicity and possible death of animals were monitored for 24 hrs to calculate the median lethal dose (LD50) of AgNPs. The (LD_{50}) was found to be more than 4000 mg/kg body weight upon intraperitoneal administration in rats and no mortality were observed after single dose administration .According to Hodge and Sterner toxicity scale, the AgNPs classified as slightly toxic effect.

Key words: Acute toxicity, AgNPs, Up and Down method for LD50 calculation, Hodge and Sterner Toxicity Scale.

INTRODUCTION

Both nanomaterial science and biology Merge together with the knowledge of nanotechnology, has participate greatly to production of nanoparticles (NPs), nano-capsules nano-spheres etc. of varied sizes which have profited wide applications based on the morphological advantage features adeqate for a given application (Erick *et al.*, 2015) Sintubin *et al.*,2012 and Akhtar etal.,2013 have detected broad applications involving drug delivery, gene delivery catalysis, biological tagging, ,imaging, diagnostics, artificial implants, sensing and tissue engineering but mostly medical. Suitable characteristic features such as size, dispersion, stability and shape command their application (Sundaravadivealn *et al.*, 2012).

Though the application and advantage of these engineered nanomaterials are extensively and actually being widely used in modern technology, there is still limited information concerning human health and environmental impact (Maneawattapinyo *et al*., 2011).

Corresponding Author

Neran Ali Thamer
Email: neran1958@yahoo.com

Silver nanoparticles (AgNPs) have received fundamental attention due to their charming electronic ,chemical, and optical properties (Austin *et al*., 2014 and McConnell *et al*., 2000) and they have been openly used in many fields, including catalysis, optical sensing ,and electronics (Majdalawich *et al*., 2014 and Prarhna *et al*., 2014). AgNPs have been applied to disinfection and therapeutics such as infected burn and wound reduction, medical device sterilization, tumor therapy, and cardiovascular implants (Siripattannkul *et al*., 2014).

Despite the varied uses of these AgNPs in many fields, there is a deficiency of information on the basic toxicity of silver nanoparticles related to the health modulations,professional risk and hazards (Maneawattapinyo *et al*., 2011).

Toxicity testing is essential in the examination of newly developed drugs before it can be used on humans. It is the limitation of potential hazards a test substance may probably produced and the description of its action, most of the toxicity testing is done on experimental animals (Cunny and Hodgson 2004).Toxicity studies are divided into, acute toxicity, sub-acute toxicity and chronic toxicity studies (Arome and Chinedu 2013). Between the different types of toxicological studies, acute toxicity

studies supply input about the overall profile of magnitude of a drug toxicity, its activity and overall effects (Lee *et al*., 2003). One of the basic steps in toxicological estimation of a new substance is the account of its toxicity after a single exposure of that substance. This supply the essential evidences concerning the type of toxic effects which the substance may possess and also gives information about the lethal dose. The more routinely used acute toxicity test includes determination of the median lethal dose (LD_{50}) of the compound (Nausheen *et al*., 2014).

LD_{50} means the dose which has examined to be lethal to 50% of the tested group of animals, when entering the animalś body by a particular route, which is an initial step in the guess and estimation of the toxic characteristics of a substance and is one of the initial examination experiments carried out with all compounds (Akhila *et al*., 2007). LD_{50} values can be contrasted to other values using a toxicity scale. Disruption sometimes occurs owing to the fact that there are many different toxicity scales in use. The two most common scales used are the "Hodge and Sterner scale" and "Gosselin", "Smith and Hodge Scale" (Onwusonye *et al*., 2014).

There are several processes to calculate the LD_{50} values like graphical methods of Miller and Tainter, arithmatical method of Karber and statistical process (Deora *et al*., 2010). Generally the smaller the LD_{50} value, the more toxic the substance is and vice versa (Onwusonye *et al*., 2014).

Three alternative methods was adopted which use fewer animals than the LD_{50} test. They register toxicity sings instead of death. These three alternative tests are fixed dose procedure, acute toxic class and up and down procedure.

In this method animals are doses one at a time .If the animals stay alive, the dose for the next animal is increased; if it dies, the dose is decreased. Each animal remain under surveillance for 1 or 2 days before dosing the next animals (Deora *et al*., 2010).

The present study was performed to evaluate the acute toxicity effects of AgNPs intraperitoneally. The Acute toxicity was determined through performing the lethal dose $_{50}$ (LD_{50}). Using the up and down methods.

MATERIALS AND METHOD

Materials

Silver nitrate, $AgNO_3$, was obtained from Sigma Aldrich Co. All glassware's were properly washed with distilled water and oven dried before use. *Crocus sativus L* (Saffron) has been collected from the market.

Green synthesis silver nanoparticles using Crocus sativus L.

Different concentration of colloidal AgNPs solution was synthesized according to method previously (Thamer and Al-Mashhedy, 2014) described using a green bio synthesis methods for aqueous extract of *Crocus sativus L* act as a reducing and capping agents.

Experimental animals

Healthy waster albino rats weighting (150-200) mg were purchased from Iraqi Center for Cancer and Medical Genetic Research (ICCPR), Baghdad/Iraq. The animals had been bred under conventional condition for research purposes. They were separated in plastic cages with stainless steel mesh lids in a ventilated room. The room was maintained at around 25 °C and 50% to 60% relative humidity with a 12 h light- dark cycle .They were provided with pellet food and tap water *ad libitum* through the experiment. The animals were kept in their cages for at least 7 days prior to dosing, to allow for their acclimatization to the laboratory condition .All animals experiment were conducted in accordance with the Institute of Laboratory Animal Resources, the guidelines of the Guide for the Care and Use of Laboratory Animals, National Research Council and in accordance with the guidelines of the international guidelines for animal experimentation.

AgNPs were intraperitoneally administrated as single different selected doses. For each dose, rat was weighed and each dose of AgNPs was calculated in accordance to the animal weight. Then rats were weighed again at the end of the study. At day 14.Animals were observed individually after dosing with special attention given during the first 4 hours and periodically during the first 48 hours, and at least once daily for 14 days for any deviation in their behavior.

Calculation of LD_{50} of AgNPs using up –and – down method.

The acute intraperitoneal toxicity of colloidal AgNPs was evaluated in rats using the up and down procedure using the equation

$$(LD_{50} = Xf + Kd).$$

Where ,Xf ; is the final experimental dose.
K; is the value from Dixon's table of maximum likelihood solutions for LD_{50}(Table 1) (OECD1998)
d; is the equal spacing between doses.

Doses of AgNPs were increased and decreased gradually .The starting dose was 200mg/Kg body weight and should not normally exceed 4000 mg/ Kg body weight. If the animal remained a live, we increase the dose (200 mg/Kg body weight), but if it died reduce does with the same way. The trials were carried out using the rule: increase the dose following a negative response [i. e. Survived (O)], and decrease the dose following a positive response [i.e. dead(X)].

Hodge and Sterner scale (Table 2) was used for the evaluation of toxicity with the help of LD_{50} (Hodage and Sterner, 2005)

Table 1. Represents estimate of potential lethal dose of the values of moderation tabular LD_{50} Dixon table 1980

	K; represented serial test started with;				
	OOOO	**OOO**	**OO**	**O**	
OXXX	0.154-	0.154-	0.154-	0.157-	XOOO
OXXO		0.860-	0.861-	0.860-	XOOX
OXOX	0.741	0.741	0.747	0.740	XOXO
OXOO	0182	0.181	0.169	0.084	XOXX
OOXX	0.381	0.380	0.372	0.305	XXOO
OOXO	0.142-	0.144-	0.169	0.305-	XXOX
OOOX	1.549-	1.544	1.500	1.288	XXXO
OOOO	1.000	0.985	0.0897	0.555	XXXX
	XXXX	XXX	XX	X	
	K represented serial test started with;				

Table 2. Hodge and Sterner Toxicity Scale

Toxicity rating	Commonly used term	LD50
1	Extremely Toxic	Less than mg/ Kg
2	Highly Toxic	1-50 mg /Kg
3	Moderately toxic	50-500 mg/ Kg
4	Slightly Toxic	500-5000 mg/Kg
5	Practically non-Toxic	5000 -15000 mg/ Kg

RESULTS AND DISCUSSION

Acute toxicity is a short time estimation and evaluation of probable risk by a single dose of a test substance. The best way to study the acute toxicity by determines the median lethal dose (Aroma and Chinedu, 2013). The determination of the 50% of lethal dose (LD50) of the studied compound *in vivo* was detected in the rats by using the "up-and-down" procedure described by (Dixon, 1980) (Dixon, 1980)

Clinical and general signs; In all acute toxicity doses , no death was recorded in the 14 days of observation period in all treated animals .The animals did not show any change in the general appearance during the observation periods , no change in water/ food consumption, so the colloidal Ag NPs in this study can be classified as a slightly toxic(Table2) substance when taken for single dose .According for the result of the animal the code which formed as being (XXXX) and according for Dixon value was get and LD_{50} was determined according to the formula employed by Dixon 1980.

$$\mathbf{LD50 = X}f \mathbf{+ Kd}$$

LD50 = 4000 + 1.000 x200= 4200 mg/ kg body weight

In oral route the LD50 of silver nanoparticles was greater than5000 mg/kg body weight (Maneewattanapinyo *et al*., 2011). Intraperitoneal route is more often applied to animals than human, its rapid absorption; large volume of drug may be injected and used frequently in lab animals.

However the whole, in vivo studies, intraperitoneal is less frequently used route compared with the other route such as oral ,inhalation and intratracheal installation in the administration of Ag NPs (Kim *et al*., 2008; Kim *et al*., 2009; Tkenaka *et al*., 2001; Tang *et al*., 2009).

In the present study intraperitoneal route was preferred; it has the advantage of getting substances into the circulation faster than oral route (OECD, 2001). There has been only one study used intraperitoneal route to administration for different sizes of AgNPs (Eliham *et al*., 2015).

CONCLUSION

In the present study was, firstly, to investigate in vivo toxic effects, and secondly to find acute toxic dose (LD50) of green synthesis of colloidal silver. There results suggest that colloidal AgNPs is relatively slightly toxicologically when administrated intraperitoneal, and these nanoparticles could be used with some degree of safety to extend the researches for the in vivo similarity estimation, concerning the effect of this product on biochemical parameters, immune system, and oxidative stress in lab animals. The obtained LD_{50} value classifies the study of AgNPs as slightly toxic according to Hodge and Sterner toxicity scale. We determined the low toxic dose at a rate of 4200 mg of body weight for the green synthesis of silver nanopartices.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

Akhila J S, Deepa S, and Alwar MC. Acute toxicity studies and determination of median lethal dose. *Current Science*, 93 (7), 2007, 917- 920.

Akhtar MS, Panwar J, Yun YS. Biogenic synthesis of metallic nanoparticles by plant extracts. *ACS Sustainable Chemistry & Engineering*, 1, 2013, 591-602

Arome D, Chinedu E, The importance of toxicity testing. *J .Pharm.Bio Sci*, 4, 2013, 146-148.

Austin L A, Mackey M A, Dreaden E C, El-Sayed MA. The optical, photothermal, and facile surfacechemical properties of gold and silver nanoparticles inbiodiagnostics, therapy and drug delivery. *Arch. Toxicol*, 88, 2014, 1391–417

Cunny H, Hodgson E. A test book on modern toxicity3rd edition. A John Wiley and Son, Inc., Hoboken, NJ, USA, 2004, 353-484.

Deora PS, Mishra C K, Mavani P,Asha R, Shrivastava B, Rajesh KN. Effective alternative of LD50 help to save number of experimental animals. *JOCPR*, 2(6), 2010, 450-453.

Dixon WJ. Efficient analysis of experimtal observation. *Ann.Res.Pharmacol.Toxicol*, 20, 1980, 441-462.

Eliham A. E Mahmoud. M, Maha F. M. 2015Acute toxicity of different sizes of silver nanoparticles intraperitonally injected in Balb/ c mice using two toxicological methods. *Int J Pharm Pharm Sci*, 7(1), 2015, 94-99.

Erick ON, Padmanabhan MN. Green chemistry focus on optimization of silver nanoparticles using response surface methodology (RSM) and mosquitocidal activity: *Anopheles stephensi* (Diptera: Culicidae). *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 149, 2015, 978-984.

Hodge, A and Sterner B. Toxicity classes .In Canada Center for occupational health and safety, 2005.

Kim WY, Kim J, Park JD, Ryu HY, Yu IJ. Histological study of gender differences in accumulation of silver nanoparticles in kidneys of fischer 344 rats. *J Toxicol Environ Health Part A*; 72, 2009, 1279-84

Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, Park JD. Twenty eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague–Dawley rats. *Inhal Toxicol*, 20, 2008, 575-83.

Lee CJ, Lee LH, Lu CH. Development and evaluation of drugs from laboratory through licensure to market. 2nd ed., USA; CRC Press, 2003 37.

Majdalawieh A, Kanan M C, El-Kadri O and Kanan S M. Recent advances in gold and silver nanoparticles: synthesisand applications. *J. Nanosci. Nanotechnol*, (14), 2014, 4757–80.

Maneewattanapinyo W, Banlunara C, Thammacharoen S, Ekgasit T, Kaewamatawong .An evaluation of acute toxicity of colloidal silver nanoparticles. *J Vet Med Sci*, 73, 2011, 1417–1423.

McConnell W P, Novak J P, Brousseau L C, Fuierer R R, Tenent R C, Feldheim D L .Electronic and opticalproperties of chemically modified metal nanoparticles and molecularly bridged nanoparticle arrays. *J. Phys. Chem. B*, 104, 2000, 8925-30.

Nausheen H S , Arshad A , Zafar .S.S , Iqbal A , Shazia H , Zafar A M, Acute toxicology and neurobehavioral studies on a new 7- Azaindole derivative. *World Journal of Pharmaceutical Research*, 3(5), 2014, 77-87.

OECD. Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 32nd Joint Meeting of the Chemicals, Pesticides and Biotechnology, 2001.

OECD. Organization for Economic Co-operation and Development, Acute oral toxicity-modified up and down procedure, No.425 Paris 1998.

Onwusonye J C, Uwakwa AA, Iwuanyanwu P , Iheagwam U. Oral acute toxicity (LD_{50}) study of methanol extract of *Annona senegalensis* leaf in albino mice. *Sky Journal of Biochemistry Research*, 3(5), 2014, 46-48.

Prathna T, Raichur A M, Chandrasekaran N ,Mukherjee A, Recent developments on biosynthesis of noble metal nanoparticles: synthesis, characterization and potential applications. *Rev. Adv. Sci. Eng*, 3, 2014, 239–49

Sintubin L, Verstraete W, Boon N,Biologically produced nanosilver: current state and future perspectives. *Biotechnol .Bioeng*, 109(10), 2012, 2422-36.

Siripattanakul-Ratpukdi S, Fürhacker M. Review: issues of silver nanoparticles in engineered environmental treatment systems. *Water Air Soil Poll*, 225, 2014, 1–18

Sundaravadivealn C, Nalini, MP, Sivaprasarth, Kishmu L. .Efficacy of fungus mediated silver and gold nanoparticles against *Aedes aegypti* larvae. *Parasitology Research*, 110(11), 2012, 175-184.

Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, Heyder J. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ Health Perspect*, 109, 2001, 547-51.

Tang J, Xiong L, Wang S, Wang J, Liu L, Li J, Yuan F. Distribution, translocation and accumulation of silver nanoparticles in rats. *J Nanosci Nanotechnol*, 9, 2009, 4924-32.

Thamer NA and AL-Mashhedy LA. Green synthesis optimization and characterization of silver nanoparticles using aqueous extract *crocus sativus L*. *Int J Pharm Bio Sci*, 5(4), (B)**,** 2014, 759-770.