Effects of *Annona muricata* Biofunctionalized Gold Nanoparticles on Erythrocyte Osmotic Fragility and Hematological Profile in Rats Model

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Introduction

Nanomedicine is making huge impact in the healthcare sector for the treatment of various chronic diseases. Hence, eco-friendly synthesis of nanoparticles is considered as building blocks of the forthcoming generations to cure or manage various diseases (Mieszawska *et al*., 2013).

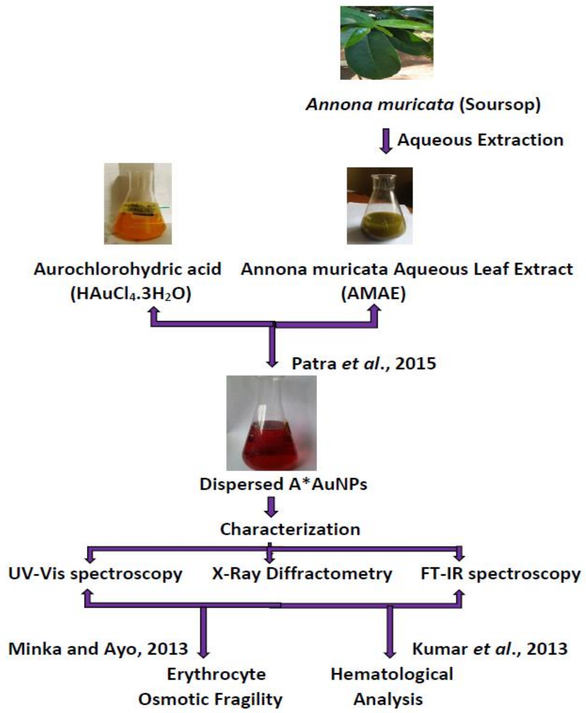
Much attention has been drawn to Plant mediated nanomaterial due to their physico-chemical properties. The role of phytochemicals present in plant extracts in the reduction of metal ion and capping of newly formed particles during their growth processes has been reported (Basavegowda *et al*, 2014).

Hematological investigation can reveal the extent of safety and biocompatibility of metallic nanoparticles in animal models.

The Erythrocyte Osmotic Fragility (EOF) is a measure of the erythrocyte overall response to osmotic pressure, and the tensile strength of the red cell membrane which has been found to be altered in various pathological conditions (Minka and Ayo 2013).

The study presented describes the biofunctionalization of gold nanoparticles by bioreduction of Au^{3+} with aqueous extracts of *Annona muricata* leaf, as reducing and stabilizing agents and their consequent effects on hematological profile and erythrocyte osmotic fragility in male Wister rats.

Materials and Methods



Abstract

Background: Metallic nanoparticles serve as vehicles for delivery of bioactive molecules frompPlants with huge advantages over Crude extract, which includes enhancement of solubility, bioavailability and enhancement of pharmacological activity.
Materials and Methods: *Annona muricata* leaf aqueous extract acted as the reducing and stabilizing agents with aurochlorohydric acid as the substrate. The biofunctioalized gold nanoparticles (A*AuNP) were characterized by UV-Vis Spec, SEM, FT-IR spec and XRD, the effect of administration of this A*AuNPs on hematological profile were determined in the plasma using standard techniques and Erythrocyte osmotic fragility was determined spectrophotometrically.
Results and Conclusion: Bioreduction took place within 45 min of reaction time at 40°C and pH of 9.2. The characterization studies reported that the average crystallite size of the formed nanoparticle was 13nm. The study suggests that A*AuNPs did not exhibit any significant effect (P<0.05) on hematological parameters in rats model but significant reduction (P<0.05, P<0.01) in erythrocyte osmotic fragelity. therefore offers potential to be exploited for various biotechnological use, hence the application of these A*AuNPs for targeted drug delivery in colorectal cancer therapy is already on going in our laboratory.

Keywords: Gold nanoparticles, *Annona muricata* , Hematology, Erythrocyte, Osmotic fragility, A*AuNP.

Results & Discussion

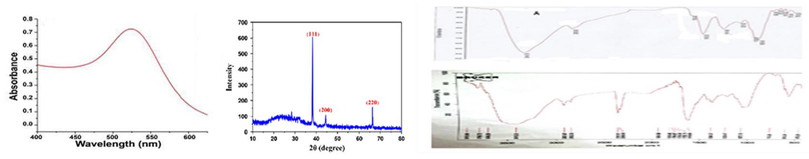


Figure 1. Characterization of annona muricate functionalized gold nanoparticles (A*AuNPs). (a) UV-visible spectra of A*AuNPs); (b) XRD pattern (c) FT-IR of, A*AuNPs (top) and AMAE (bottom)

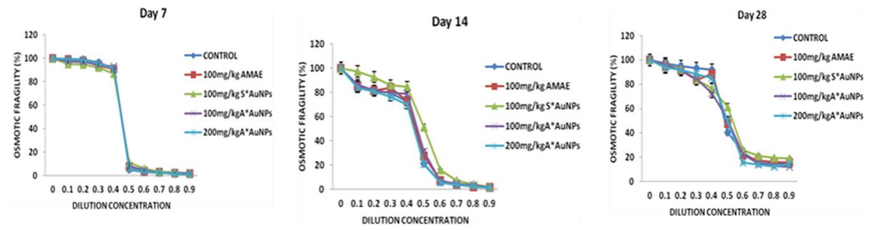


Figure 2. Effect of treatments on Erythrocyte Osmotic Fragiligram at day 7, 14 and 28 respectively. Values are means (n = 6) ± SD.

Table 1 Red Blood cell differentials of rats after 28 days of treatment

Group	RBCs (x10³/µl)	Hb (g/dl)	PCV(%)	MCHC(g/dl)	MCH (pg)	MCV (fl)
I - 9% NaCl Baseline	3.61±0.30^{abcde}	11.20±0.20^{a}	40.16±0.20^{ab}	42.20±2.00^{abce}	23.97±2.00^{ace}	62.47±1.00^{ace}
II - 100mg AMAE /Kg b.w	3.47±0.40^{bacde}	11.57±0.10^{bc}	41.53±0.10^{be}	42.70±1.00^{ba}	24.77±1.00^{b}	63.17±1.00^{b}
III – 100mg S*AuNPs /Kg b.w	3.75±0.10^{cabde}	11.31 ±0.30^{cb}	43.01±0.20^{c}	41.82±3.00^{cad}	23.89±1.00^{cae}	62.42±2.00^{ca}
IV – 100mg A*AuNPs /Kg b.w	3.33±0.40^{db}	12..03 ±0.10^{d}	38.98±0.30^{da}	41.37±2.00^{dc}	24.20±1.00^{d}	61.83±3.00^{de}
V – 200mg A*AuNPs /Kg b.w	3.64±0.20^{eabc}	11.22 ±0.20^{ae}	41.57±0.10^{eba}	42.10±1.00^{ae}	23.83±2.00^{eca}	62.00±2.00^{eca}

Data are presented as mean (n = 6) ± SEM. Values in the same column and category not sharing a common letter (a–e) differs significantly at p < 0.05. Hb, hemoglobin; cells; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; PCV, pack cell volume; MCHC, mean corpuscular hemoglobin content

Table 2 Platelets, white blood cells and differentials of rats after 28days of treatment

Group	Platelet (µl)	WBC (x103/µl)	Lymphocyte (%)	Monocytes (%)	Neutrophils (%)
I - 9% NaCl Baseline	302.67±2.08^{ac}	3.32 ±0.08^{a}	64.53±0.30^{ade}	13.63±0.40^{abcde}	28.89±0.60^{ae}
II - 100mg AMAE /Kg b.w	306.17±0.08^{bde}	3.87 ±0.10^{b}	72.16±0.20^{b}	13.51±0.50^{bacde}	33.15±0.30^{bc}
III – 100mg S*AuNPs /Kg b.w	302.35±3.01^{ca}	4.12 ±0.20^{ce}	79.01±0.50^{c}	13.97±0.10^{cabe}	13.83±0.50^{cb}
IV – 100mg A*AuNPs /Kg b.w	305.33±4.73^{dea}	3.53 ±0.10^{d}	67.88±0.30^{dae}	13.51±0.50^{dab}	31.76±0.30^{d}
V – 200mg A*AuNPs /Kg b.w	305.00±3.46^{ed}	4.15 ±1.00^{ec}	97.47±0.60^{ea}	14.01±0.20^{ecab}	28.43±0.40^{ea}

Data are presented as mean (n = 6) ± SEM. Values in the same column and category not sharing a common letter (a–d) differs significantly at p < 0.05. WBC, white blood count

Conclusions

The aqueous leaf extract of *Annona muricata* initiated nucleation and growth of the nanoparticles.

Alkaloids, flavonoids and tannins present in the plant extracts have been considered to be responsible for the bioreduction process.

The result obtained in this study clearly revealed the role of *Annonna muricata* biofunctionalized gold nanoparticles in maintaining erythrocyte membrane integrity and, consequently, decreasing the degree of hemolysis in male Wister rats.

The non- significant effects of *Annonna muricata* biofunctionalized gold nanoparticles on various hematological indices compared to control, an indication that they are safe, provides useful suggestions of their biocompatibility in living systems.

Hence, their use for target drug delivery in colorectal cancer therapy in already on going in our laboratory.

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Acknowledgments

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