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Research Article

Nanotechnology for Better Drug Targeting

Nephroprotective Effect of Green Synthesised Gold Nanoparticles Using Bark Extract of *Terminalia arjuna* on Acetaminophen Induced Nephrotoxicity in Male Albino Rat

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Abstract: Green synthesised gold nanoparticles offer a great promise in biomedicine. An overdose of acetaminophen causes severe hepatotoxicity and nephrotoxicity. The development of nephroprotective drugs through eco-friendly production routes is a major challenge for current pharmacology. This study was undertaken to examine the therapeutic effects of green synthesised gold nanoparticles (AuNPs) using aqueous bark extract of *Terminalia arjuna*, on acetaminophen induced nephrotoxicity in male albino rats and also to select the most effective dose of AuNPs to protect from nephrotoxicity . *Terminalia arjuna* , is a herbal plant of high interest in Asian traditional medicine. The bark of this tree has been widely used in the preparation of ayurvedic formulations such as powerful cardiotonic, antioxidative, antiuremic and antimicrobial properties. In this study 36 experimental albino rats were taken and randomly divided into 6 groups. Group 1 served as normal control, Group 2 received acetaminophen intraperitoneally at concentration of 500mg /kg of body weight for 14 days and Groups 3,4,5,6 were co-administered with acetaminophen (500mg/kg/day) along with AuNPs at doses 55, 175, 550, 2000 µg/kg/day intraperitoneally for 14 days. After 14 days all animals were sacrificed for biochemical and histopathological studies. Among different experimental doses of AuNPs (55,175,550, 2000µg/kg/day), 175µg/kg/day showed more potent activity towards biochemical indices and histopathological studies. There was significant ($p<0.05$) increase in Urea, Creatinine, CRP and MDA levels but significant decrease in SOD, CAT and GSH activity in acetaminophen treated group, in comparison to control group but co-administration with AuNPs (175µg/kg/day) restored the activities of these biochemical markers and also of the antioxidant enzymes. Hence,this study confirmed that AuNPs at dose 175µg/kg/day have better nephroprotective efficacy.

Keywords: Acetaminophen, Antioxidant, Biochemical indices, Nephrotoxicity, , *Terminalia arjuna.*

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1. INTRODUCTION

Metallic nanoparticles had become the most active area of research due to their distinct physical, chemical and biological properties.[1] The synthesis of nanoparticles by using various physical and chemical methods is quite expensive and potentially hazardous to the environment.[2] The alternative techniques using natural plant extracts, bacteria, fungi, sugars, biodegradable polymers etc. act as reductants and stabilizing agents for the synthesis of inorganic nanoparticles.[3] The synthesis of nanoparticles using plant extract provides an advancement over other methods because it is simple, cost-effective, environment friendly and relatively reproducible.[4] A metal nanoparticle, especially gold nanoparticles represents huge potential in the field of novel drug delivery and therapeutic applications. In this study, herbal gold nanoparticles were synthesised by reduction of chloroauric acid ($HAuCl_4$) with *Terminalia arjuna* bark extract. *Terminalia arjuna* commonly known as *arjuna*, belongs to the family of Combretaceae. Its bark decoction is being used as an astringent, expectorant, antidysenteric, and demulcent. It has also shown to be useful in the treatment of ulcers, diabetes, anaemia, cardiomyopathy, and cirrhosis [5].Acetaminophen toxicity is caused due to drug metabolism in both the liver and extrahepatic tissues.[6] Through urine only 1% of the drug is excreted without any alteration. At therapeutic doses of acetaminophen, approximately 63% is metabolized via glucuronidation and 34% by sulfation. These phase II reactions primarily occur in the liver producing water soluble metabolites that is eliminated via the kidney. In therapeutic dosing, <5% of N-acetyl p-aminophenol(APAP) is oxidized by the microsomal P-450 enzyme system to a reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI). Glutathione then reduces this electrophilic metabolite and subsequently excrete as mercapturic acid. Traditionally glutathione leads to detoxification of acetaminophen and its metabolites, its conjugates have been involved in the formation of nephrotoxic compounds.[7]In excess of APAP, sulfate and glutathione stores are depleted. This shunts most of the acetaminophen to cytochrome P450 mixed function oxidase system, generating more NAPQI intermediates. There is severe depletion of glutathione due to overdoses of the drug, as well as production of metabolites, which causes toxicity, leaving a large amount of reactive species unbound. These electrophilic intermediates then form adducts with glutathione and sulfhydryl moieties on cellular proteins.[8] This causes disturbance in homeostasis, with subsequent activation of caspases and lysosomal enzymes that initiates apoptosis. This has been revealed in both liver and kidney tissue of animal models resulting in cell death, tissue injury and ultimately organ dysfunction. [9,10] Therefore in this present study, an attempt has been made to evaluate the nephroprotective activity as well as antioxidant property of green synthesised gold nanoparticles (AuNPs) using aqueous bark extract of *Terminalia arjuna* in a dose dependent manner.

2. MATERIAL AND METHODS

2.1 Green synthesis and characterization of gold nanoparticles using aqueous bark extract of Terminalia arjuna

Terminalia arjuna ([Roxb.] Wight & Arn.) bark was collected from the Laterite region of Gope Palace (Raja N.L Khan Women's College) Medinipur, Paschim Midnapore district, West Bengal, India and the plant voucher number is RNLKWC/121/2016. Taxonomic identification was done by botanist Dr. Dulal Chandra Das, Associate Professor, Department of Botany, Raja Narendra Lal Khan Women's College (Autonomous). Green synthesis of gold nanoparticles (AuNPs) were derived from aqueous bark extract of *Terminalia arjuna* and characterized as described by Mitra et al.[11]Hundred millilitre of 1mM chloroauric acid ($HAuCl_4$) solution was reduced by heating for 10minutes at 60-70^{0}C in 10mL of aqueous bark extract of *Terminalia arjuna* to yield a ruby red colored dispersion.

2.2 Experimental animals

Male albino rats (100 - 120gm) procured from authorized Chakraborty Animal suppliers, Kolkata (M/S Chakraborty Enterprise Registration no.: 1443/PO/b/11/CPCSEA), were used for the study. The animals were housed in large, clean polypropylene cages at controlled room temperature (22±4^{0}C) under 12 hrs. light / dark cycles and with relative humidity (50-60%) with proper supplementation of standard food and water *ad libitum*. All animals were acclimatized to the laboratory environment for a week prior to the initiation of treatment. All animal experiments were performed as per the Animal Ethical Committee guidelines of Raja Narendra Lal Khan Women's College(Reference number: 14/ IAEC (05) / RNLKWC /2019) and were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animal(CPCSEA), Government of India(Registration no.:1905/PO/Re/S/2016/CPCSEA).

2.3 Ethics approval and consent to participate

Animal Ethics approval was obtained as per the Animal Ethical Committee guidelines of Raja Narendra Lal Khan Women's College (Reference number: 14/ IAEC (05) / RNLKWC /2019) and were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animal(CPCSEA), Government of India(Registration no.:1905/PO/Re/S/2016/CPCSEA).

2.4 Experimental design

Experimental studies for the protective effect of AuNPs in dose dependent manner against acetaminophen induced nephrotoxicity in rats was conducted as per Organization of Economic Cooperation and Development (OECD) guidelines 425(OECD, 2008).[12]All experimental protocols have been approved by the Constitutional of Institutional Animals Ethics Committee (IAEC) of Raja Narendra Lal Khan Women's College (Autonomous), Midnapore-721102, West Bengal, under registered CPCSEA. A total 36 experimental rats were randomly divided into 6 groups of 6 rats in each cage. Group 1 served as normal control, Group 2 received acetaminophen intraperitoneally at concentration of 500mg /kg of body weight for 14 days and Groups 3,4,5,6 were co-administered with acetaminophen (500mg/kg/day) and AuNPs (55,175,550,2000 µg/kg/day) intraperitoneally for 14 days.

2.5 Sacrifice of animals and blood and tissue collection

The experimental animals were sacrificed after 14 days of treatment for biochemical and histopathological studies. Animals were dissected from the posterior part of the

abdomen, and then blood was collected from the aorta, after that kidney tissue was gently removed. The collected tissue was perfused with PBS and then half of the tissue was stored at-20^{0}C in a sterile container for preparation of tissue homogenates. The remaining other half of the tissue was preserved in 10% of neutral formaldehyde solution until processed for histopathological analysis.

2.6 Separation of serum and homogenization of kidney tissue

Serum was separated from the collected blood sample by centrifugation at 1500 xg for 10minutes and was preserved (-20^{0}C) for carrying out further biochemical investigations. The kidney tissue was homogenized in ice cold phosphate buffer saline (PBS), pH=7.4.The homogenate thus obtained was centrifuged at 1000 xg for 5minutes at 4^{0}C and the resulting supernatant was stored at -20^{0}C for different biochemical estimation.

2.7 Measurement of biochemical markers of nephrotoxicity

The activities of urea[13], creatinine[14] in serum were assessed by using agappe diagnostic -kit.

2.8 Measurement of inflammatory marker

The level of C- reactive protein (CRP)[15] in serum was evaluated by using agappe diagnostic - kit.

2.9 Assessment of lipid peroxidation

Lipid peroxidation of renal tissue homogenate was measured to evaluate the degree of intracellular damage. The renal tissue homogenate was mixed with 20% TCA (1.5mL), 1.34% TBA (1.5mL) then boiled for 30minutes at 100^{0}C and cooled, followed by addition of 2.5mL of butanol. Then the mixture was centrifuged for 5minutes in 2000xg and supernatant was collected. Then optical density of the supernatant was measured at 535nm. TBARS as malondialdehyde (MDA) concentrations were calculated by using the molar extinction coefficient $1.56\times10^5 M^{-1}cm^{-1}$ and expressed as n mol of MDA formed/mg protein.[16]

2.10 Antioxidant Enzyme Profile

Activities of superoxide dismutase (SOD)[17] and catalase (CAT)[18] were assayed from renal tissue homogenate for estimation of intracellular antioxidant enzyme status.

2.11 Assessment of Reduced Glutathione (GSH) Level

The GSH level was estimated from renal tissue homogenate according to Moron et al.[19] The renal tissue homogenate was mixed with 25% of TCA and then centrifuged at 2,000 x g for 15minutes. Then the supernatant was diluted to 1mL with 0.2M sodium phosphate buffer. Later, 2mL of 0.6mM DTNB (Ellman's reagent) was added to it and incubated for10 minutes at room temperature. The optical density of the yellow colored complex was formed by the reaction of GSH and DTNB which was measured at 405nm.

2.12 Histopathological Assessment

The histopathological analysis of renal tissue was performed by the Iranloye and Bolarinwa[20] method. Kidney tissue was fixed in 10% formalin solution, then dehydrated in graded alcohol (70% - 100%), cleared in xylene. Then paraffin embedding was done at 58^{0}C for 4 to 5 hours followed by paraffin block preparation. Afterwards histological sections were made with thickness of 5µm using a microtome. Then the sectioned tissues were mounted on slides and deparaffinized with xylene, stained with hematoxylin-eosin, followed by mounting with DPX with a coverslip. The prepared slides were observed for histopathological changes under microscope (Olympus model, Japan).

3. STATISTICAL ANALYSIS

The data were entered in Microsoft excel and statistical analysis was done by using the statistical package, Origin 6.1 Northampton, Mass, USA. The collected data was statistically calculated and were expressed as Mean ± SE, n=6. Comparisons were done between the means of control group with all the experimental groups, by one way ANOVA(Analysis of Variance) followed by multiple two tail t- tests. Bars for a specific data differ from each other significantly at the level of ($p<0.05$).

Table 1: Histopathological changes in kidney tissue of different experimental groups. A six-point scale for fibrosis stage was applied for scoring renal toxicity

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Cellular necrosis	0	6	0	0	1	3
Tubular epithelial cell degeneration	0	6	1	0	1	3
Tubular vacuolization	0	5	0	0	2	3
Renal fibrosis	0	4	1	0	1	2

(0) indicates normal kidney, (1) indicates mild toxicity , (2) indicates moderate toxicity, (3) indicates severe toxicity, (4) indicates modest severe toxicity, (5) indicates extremely severe toxicity, (6) indicates extremely severe toxicity and damage.
Group 1: Control, Group 2: Acetaminophen treated, Group 3: Acetaminophen + AuNPs (55 µg/kg/day), Group 4: Acetaminophen + AuNPs (175 µg/kg/day), Group5: Acetaminophen + AuNPs (550 µg/kg/day), Group 6: Acetaminophen + AuNPs (2000 µg/kg/day).

Graphical Representation of Biochemical Indices

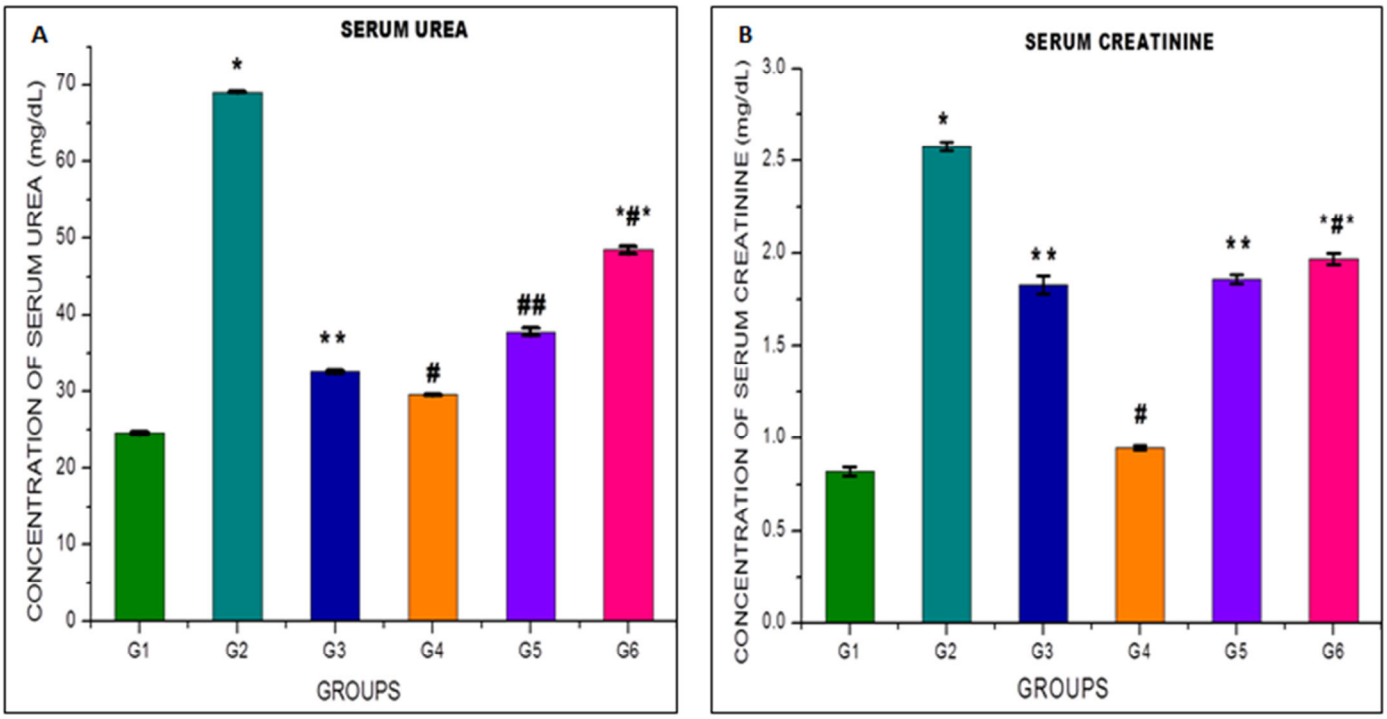


Fig 1A, 1B: Effects of different doses of AuNPs on serum Urea and Creatinine level after intraperitoneal administration of acetaminophen in rats.

Data are expressed as Mean± SE(n=6).ANOVA followed by multiple two-tail t-test and data with different superscripts (*, **, #, ##,*#*) in a specific vertical column indicates significant difference (p<0.05) compared to the control group.
Group 1: Control, Group 2: Acetaminophen (500mg/kg/day) treated, Group 3: Acetaminophen + AuNPs(55 µg/kg/day), Group 4: Acetaminophen + AuNPs (175 µg/kg/day), Group 5: Acetaminophen + AuNPs (550 µg/kg/day), Group 6: Acetaminophen + AuNPs (2000 µg/kg/day). Data with different superscripts (*, **, #, ##,*#*) in a specific vertical column differ from each other significantly (p < 0.05).

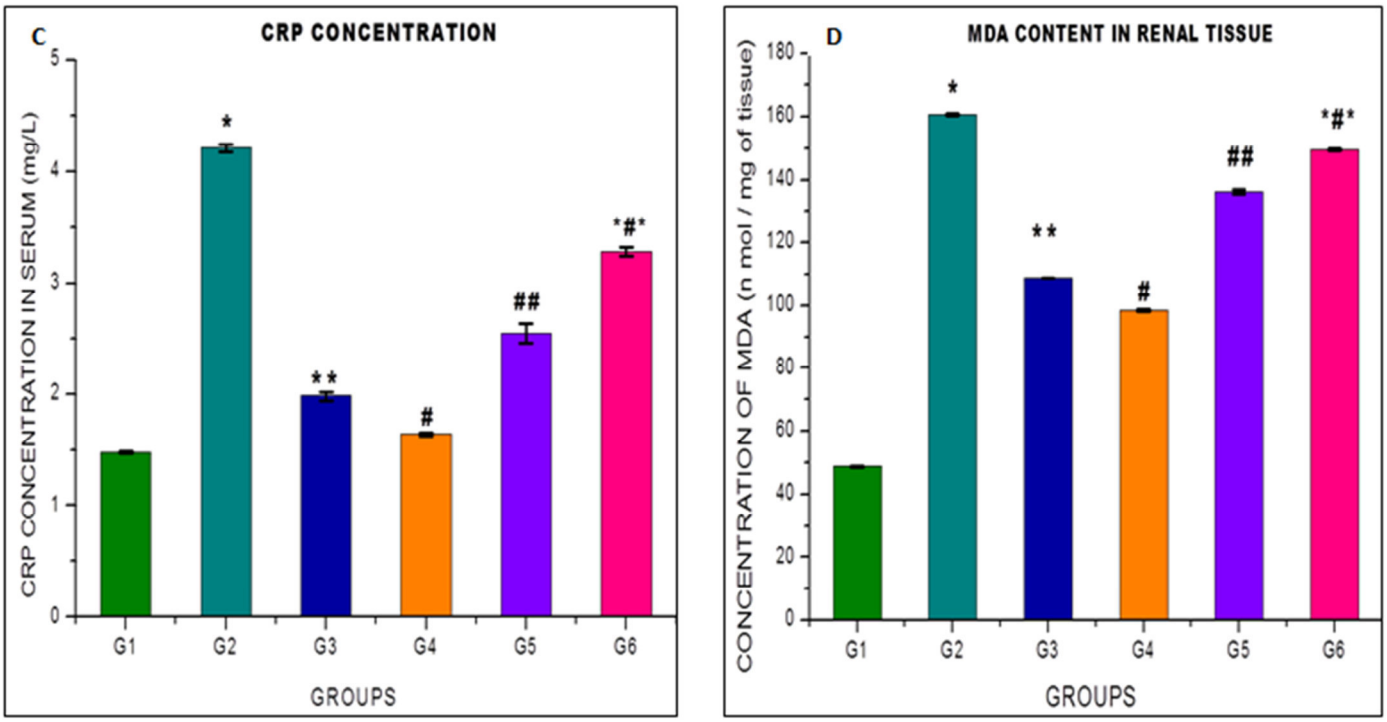
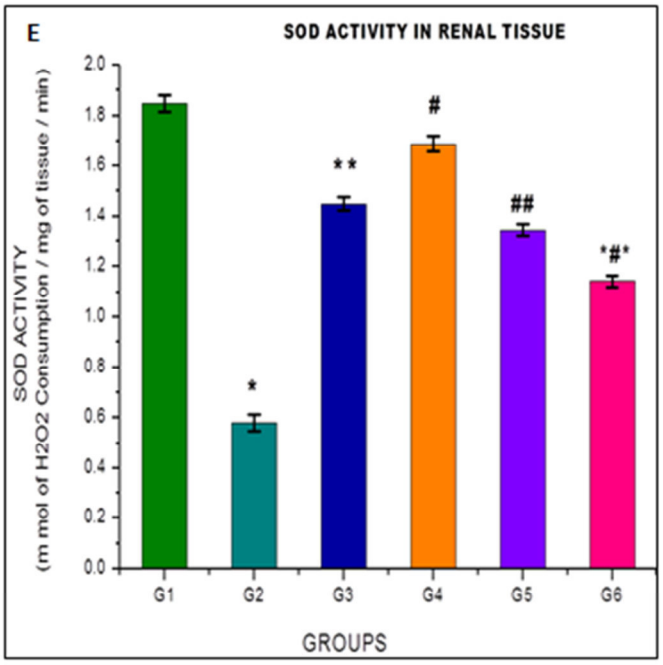
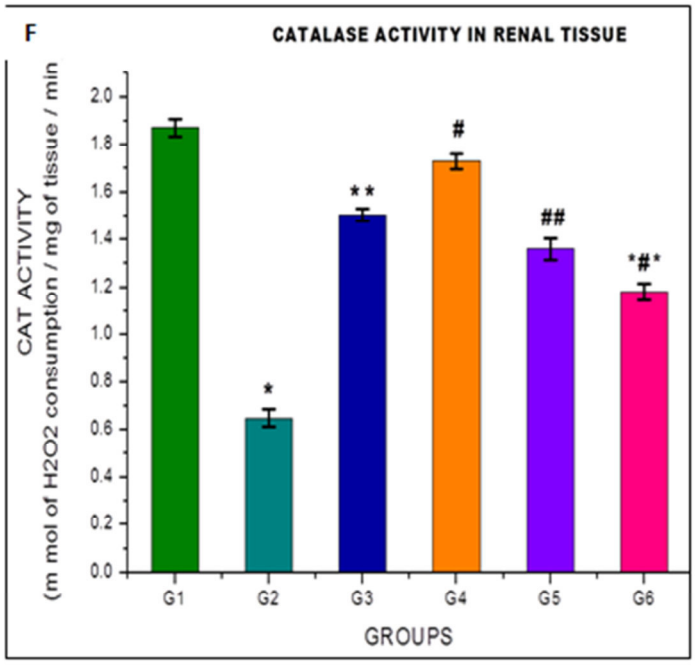


Fig 1C, 1D: Effects of different doses of AuNPs on CRP concentration and MDA content after intraperitoneal administration of acetaminophen in rats.

Data are expressed as Mean± SE(n=6).ANOVA followed by multiple two-tail t-test and data with different superscripts (*, **, #, ##,*#*) in a specific vertical column indicates significant difference (p<0.05) compared to the control group
Group 1: Control, Group 2: Acetaminophen (500mg/kg/day) treated, Group 3: Acetaminophen + AuNPs(55 µg/kg/day), Group 4: Acetaminophen + AuNPs (175 µg/kg/day), Group 5: Acetaminophen + AuNPs (550 µg/kg/day), Group 6: Acetaminophen + AuNPs (2000 µg/kg/day). Data with different superscripts (*, **, #, ##,*#*) in a specific vertical column differ from each other significantly (p < 0.05).





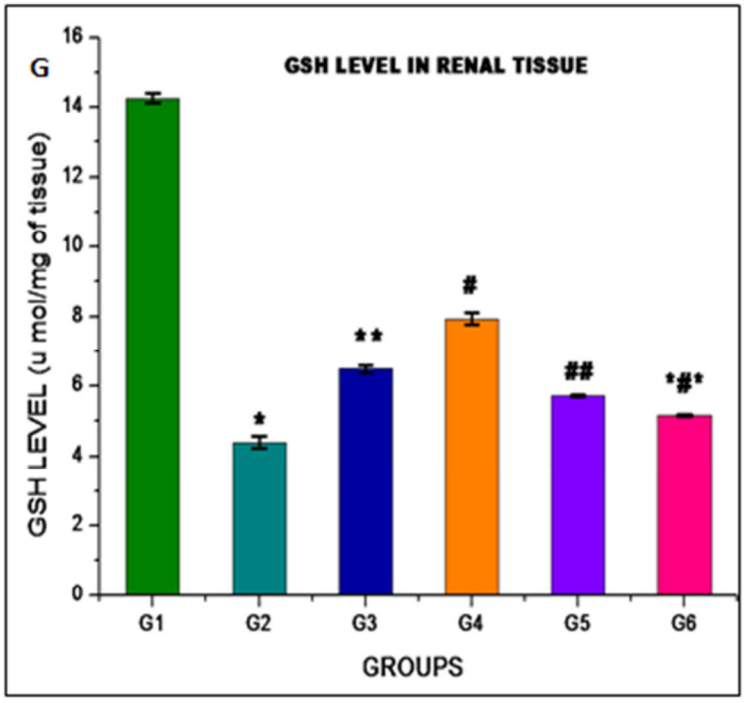


Fig 1E, 1F and 1G: Effects of different doses of AuNPs on SOD, Catalase and GSH activity after intraperitoneal administration of acetaminophen in rats.

Data are expressed as Mean± SE(n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (*, **, #, ##,*#*) in a specific vertical column indicates significant difference (p<0.05) compared to the control group
Group 1: Control, Group 2: Acetaminophen (500mg/kg/day) treated, Group 3: Acetaminophen + AuNPs (55 µg/kg/day), Group 4: Acetaminophen + AuNPs (175 µg/kg/day), Group 5: Acetaminophen + AuNPs (550 µg/kg/day), Group 6: Acetaminophen + AuNPs (2000 µg/kg/day). Data with different superscripts (*, **, #, ##,*#*) in a specific vertical column differ from each other significantly (p < 0.05).

Representation of histological structure of Kidney Tissue

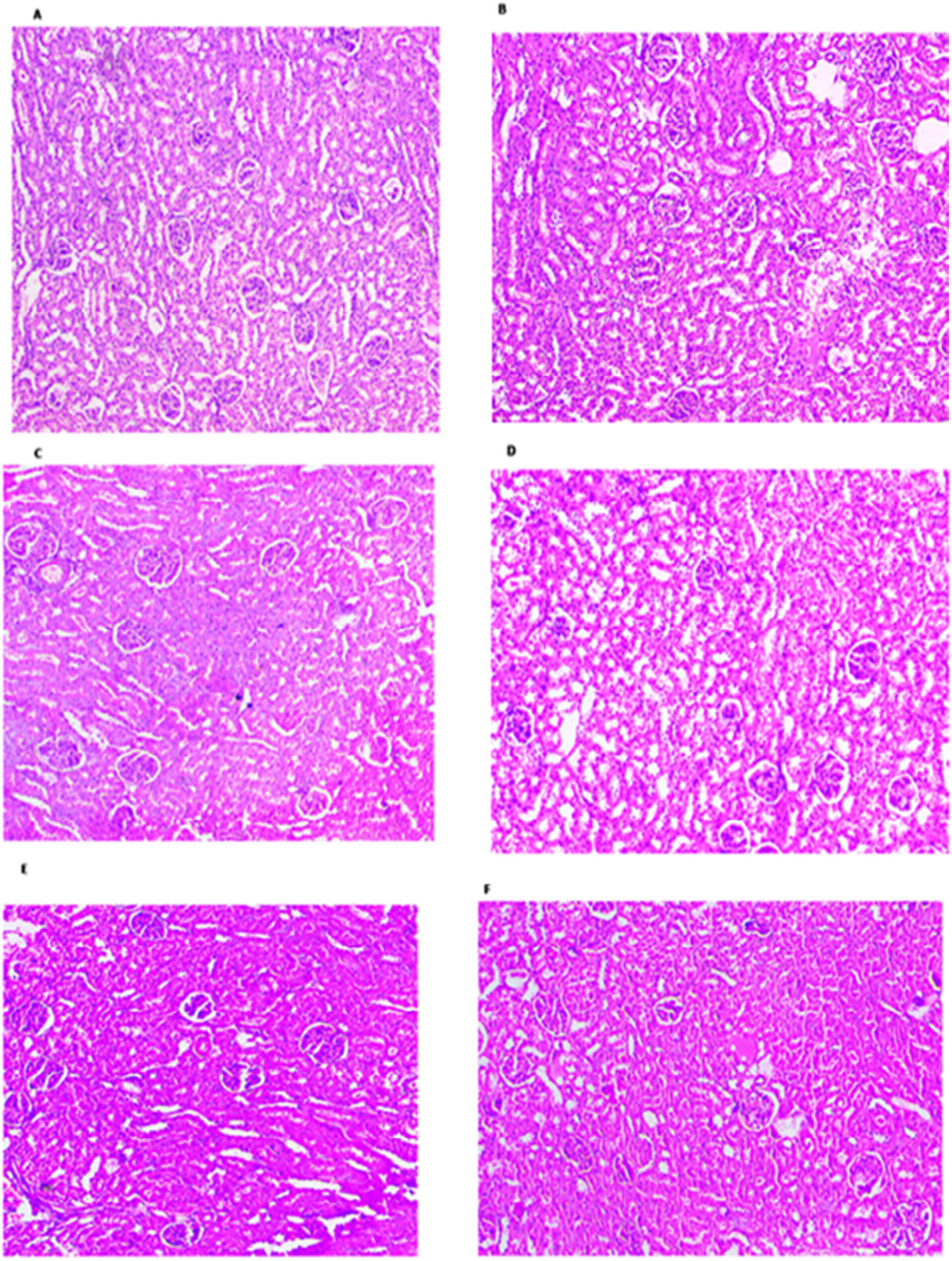


Fig 2: Effects of different doses of AuNPs on histoarchitecture of kidney tissue section on acetaminophen induced nephrotoxicity in male albino rats.

In Figure 2: Section 2A- Group 1: Control, Section 2B-Group 2: Acetaminophen treated, Section 2C-Group 3: Acetaminophen+ AuNPs (55 µg/kg/day),Section D-Group 4: Acetaminophen+ AuNPs (175 µg/kg/day),Section 2E-Group 5: Acetaminophen+ AuNPs (550 µg/kg/day),Section 2F-Group 6: Acetaminophen+ AuNPs (2000 µg/kg/day).

Section 2A: showing normal histological structure of kidney tissue like normal glomerulus, normal Bowman's capsule and normal renal tubules of control group (group 1) rats. Section 2B: Acetaminophen treated group (group 2) showing severe cellular disruptions as damaged glomeruli, loss of Bowman's Capsules, necrosis of tubular epithelial cells or damaged tubules.
Section 2D: Co-administration of AuNPs (175µg/kg/day) along with acetaminophen treatment group (group 4) showing well organized configuration in renal cells with normally repaired glomerulus surrounded by normal Bowman's capsule and normally repaired renal tubules like control group rats (group 1).
Section 2C, 2E and 2F: Co-administration of AuNPs at doses (55,550,2000µg/kg/day) along with acetaminophen treated group (group 3,5 and 6) represented partially organized glomerulus, slightly repaired Bowman's capsule and partially repaired renal tubule structure

4. RESULTS

4.1 Biochemical markers of nephrotoxicity

Evaluation of nephrotoxicity through novel biomarkers includes the measurement of urea concentration and, serum creatinine concentration. Rats treated with acetaminophen (Group 2) showed significant ($p<0.05$) increase in serum urea and creatinine levels compared to control group (Group 1). Co-administration with AuNPs at a dose 175µg/kg/day for 14 days (Group 4) showed significant ($p<0.05$) reduction in the levels of serum urea and creatinine compared to the acetaminophen treated Group 2 rats as well as in other AuNPs co-administered groups in Group 3 treated rats at dose 55µg/kg/day, 550µg/kg/day in Group 5 rats, and 2000µg/kg/day in Group 6 treated rats for 14 days respectively. The nephroprotective effects of AuNPs on serum biochemical markers in acetaminophen intoxicated rats are shown in (Figure 1A,1B).

4.2 Inflammatory marker

CRP is an acute phase protein synthesised by the liver and found in blood plasma, whose circulating concentrations rise in response to inflammation. CRP level was significantly ($p<0.05$) increased in acetaminophen treated (Group 2) rats when compared with the control group (Group 1). After co-administration of AuNPs(175 µg/kg/day) in Group 4 treated rats, CRP level significantly decreased ($p<0.05$) in comparison to Groups 2, 3, 5, and 6 respectively(Figure 1C).

4.3 Lipid peroxidation

Lipid peroxidation was significantly ($p<0.05$) increased in the acetaminophen treated group, as revealed by elevated MDA levels when compared with the normal control group. However, co-administration of AuNPs at a dose (175µg/kg/day) in Group 4 treated animals suppressed MDA level in the renal tissue significantly ($p<0.05$) when compared with acetaminophen treated Group 2 rats as well as in other doses of AuNPs co-administered groups in Group 3, 5, and 6 rats respectively (Figure 1D).

4.4 Antioxidant Enzyme Profile

The level of antioxidant enzymes, SOD and CAT level was significantly ($p<0.05$) decreased in the acetaminophen treated group. However, co-administration of AuNPs at a dose (175µg/kg/day) significantly ($p<0.05$) elevated SOD and CAT activity in the renal tissue when compared with acetaminophen treated group as well as in other doses of AuNPs co-administered groups in Group 3 (55µg/kg/day), Group 5 (550µg/kg/day), and Group 6 (2000µg/kg/day) rats respectively (Figure 1E, 1F).

4.5 Reduced Glutathione (GSH) Level

As shown in (Figure 1G) significant ($p<0.05$) decrease in GSH level in renal tissue was evident in the acetaminophen treated group in comparison to the control group. However, co-administration of AuNPs at a dose (175µg/kg/day) increased the level of GSH ($p<0.05$)significantly when compared with acetaminophen treated Group 2 rats as well as in other doses of AuNPs co-administered groups in Group 3 (55µg/kg/day), Group 5 (550µg/kg/day), and Group 6 (2000µg/kg/day) rats respectively.

4.6 Histopathological analysis

Histopathological findings in the control group (Figure 2A) revealed normal morphology of the renal tissue. Renal corpuscles appeared as dense rounded structures consisting of well-defined glomeruli, surrounded by Bowman's capsule lined by squamous epithelial cells and renal tubules were normally arranged hence, maintaining normal architectural integrity. Administration of acetaminophen showed damaged renal cortex, affected glomeruli with dilated Bowman's capsule and severe tubular necrosis, tubular epithelial cell degeneration, vacuolization and fibrosis in renal tissue (Figure 2B). Co-administration of AuNPs at a dose 175µg/kg/day, in Group 4 treated rats prevented acetaminophen induced changes in glomerular size which almost appeared normal(Figure 2D). In addition, the renal tubules showed normal pattern with minimal vacuolization, least renal cell degeneration, and cellular necrosis in rats treated with AuNPs at a dose (175µg/kg/day) compared to the other treated groups (Table 1, Figure 2C, 2E, 2F).

5. DISCUSSION

Nephrotoxicity mainly occurs when specific detoxification and excretion mechanisms of the kidney become ineffective due to the kidney dysfunction by exogenous or endogenous toxicants. The kidney is a vital organ required by the body to execute several functions including homeostasis maintenance, regulation of the extracellular environment, such as detoxification and excretion of toxic metabolites and drugs.[21]Acetaminophen overdose is associated to many metabolic disorders including increased concentration of serum urea and creatinine due to drug induced nephrotoxicity. The main reason for acetaminophen toxicity is CYP-mediated acetaminophen conversion to highly reactive quinone imine, A-acetyl-p benzoquinone imine. The role of NAPQI in the toxicity of acetaminophen has been reported from the studies.[22] Elevation of serum urea and creatinine is considered as the index of nephrotoxicity. [23]Kidney diseases is associated with reduced urea excretion and consequent rise in the blood concentration because the serum urea production exceeds the rate of clearance.[24] Urea is the principal nitrogenous waste product of metabolism and generated from protein breakdown. On other hand, the kidney also maintains the blood creatinine in normal range. Kidney dysfunction causes rise in creatinine level in the blood due to poor clearance of creatinine by the kidney. Acetaminophen (500mg/kg/day) administration tends to increase serum urea and creatinine level due to deterioration of the kidney function. Co-administration of AuNPs at a dose 175µg/kg/day manifested highest protective activity in Group 4 experimental animals by reducing the elevated levels of serum urea and creatinine in comparison to other doses of AuNPs (55, 175, 550, 2000µg/kg/day)(Figure 1A,1B). CRP is an inflammatory marker and its level rises in the blood due to inflammation in the body. In the present study, administration of nephrotoxic dose of acetaminophen to rats elevated the CRP level due to generation of excess NAPQI intermediate resulting inflammation. On co-administration of AuNPs (175µg/kg/day) significantly decreased the level of CRP when compared to other doses of AuNPs treated rats hence tends to reduce the inflammation in the body (Figure 1C). Several studies have demonstrated that acute overdose

of APAP increases the lipid peroxidation and suppresses antioxidant mechanisms in renal tissue.[25] This is mainly due to free radical- mediated chain reaction that damages cell membranes and MDA is a good indicator of the degree of lipid peroxidation. However in acetaminophen induced rat the MDA levels significantly increased, when compared to the control group. On AuNPs (175µg/kg/day) co-administration, the level of MDA significantly decreased when compared to other doses of AuNPs(Figure 1D). Superoxide radicals are generated during kidney injury at the site of damage and modulate SOD and CAT accumulation, resulting in loss of renal function.[26]Acetaminophen overdose resulted in the reduction of SOD and CAT activities when compared to control group due to failure of antioxidant defense mechanisms to block lipid peroxidation damage. Increase in the SOD and CAT activities was noted in AuNPs (175µg/kg/day) treated Group 4 rats in comparison to other groups due to repairment of antioxidant enzymes, which plays an important role in reno-protection (Figure 1E, 1F). Intracellular GSH plays an important role in detoxification of APAP induced hepatorenal toxicity.[26]Administration of acetaminophen in rats resulted in GSH depletion which is closely related to renal tissue damage. Co-administration of AuNPs (175µg/kg/day) help to uplift the GSH depletion induced by acetaminophen more effectively comparative to other treated groups (Figure 1G). The increase in both enzymatic and non-enzymatic antioxidants might play a significant role in the mechanism of the nephroprotective effect of AuNPs at a dose 175µg/kg/day. From different studies it have been revealed that proximal tubules are the target of APAP. [27]The cellular necrosis may be due to the direct toxic action on the vascular wall or because of insufficient oxygen delivery caused by injury of the capillary wall. [28] The toxic effect of APAP and its metabolites leads to accumulation of denatured cellular material in the cell lumen.[29] In the current study, results from histopathological examination showed clear evidence of nephrotoxicity after administration of acetaminophen. Tubular necrosis was the most relevant histopathological change. The other important finding of this study was that co-administration of AuNPs (175µg/kg/day) showed fewer microscopic changes than acetaminophen treated groups. The biochemical and histopathological results obtained from the study confirmed that AuNPs (175µg/kg/day) might be potential and most effective in protecting acetaminophen induced nephrotoxicity in experimental rat models.

6. CONCLUSIONS

The findings emphasized on the potential use of green synthesised gold nanoparticles (AuNPs) using aqueous bark extract of *Terminalia arjuna* at a dose 175µg/kg/day showed more potent nephro-protective activity as well as antioxidant property in the experimental animals. Ultimately, green synthesised gold nanoparticles (AuNPs) would be effective in the protection of renal dysfunction due to its improve drug efficacy and effective drug delivery. Overall, this study demonstrates multipotent activity of AuNPs, and its biocompatibility and non-toxicity make it suitable for the protection of nephrotoxicity.

7. ABBREVIATIONS

Green synthesised gold nanoparticles (AuNPs), chloroauric acid ($HAuCl_4$), N-acetyl-p-benzoquinone imine (NAPQI), Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Organization of Economic Cooperation and Development (OECD), Institutional Animals Ethics Committee (IAEC), C- reactive protein (CRP), malondialdehyde (MDA)**,** superoxide dismutase (SOD) and catalase (CAT), Phosphate buffer saline (PBS).

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10. AUTHORS CONTRIBUTION STATEMENT

Mousumi Mitra had contributed in content, literature search, data acquisition, data analysis, manuscript preparation and manuscript editing. Dr. Amit Bandyopadhyay had contributed to manuscript editing. Gouriprasad Datta had contributed to manuscript editing. Dr. Dilip Kumar Nandi had contributed in concept, design, manuscript editing and statistical analysis. All authors have read and approved the manuscript for submission.

11. CONFLICT OF INTEREST

Conflict of interest declared none.

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