Nanomedicine in healthcare contributes to a better understanding of and controlled interactions with biological mechanisms at the molecular level leading to new pathways for diagnosis and treatment of human diseases, in line with the global evolution of medicine. Nanomedicine as a translational science has the goal to provide cost effective novel therapies and diagnostics using the enabling capacity of nanotechnology applied to medicine. This ambition is based on the fact that nanotechnologies provide the tools for analysis and manipulation of biological processes at the nanoscale, where diseases initiate and progress. The result is an increasingly better understanding of the molecular biology of diseases leading to new targets for more specific and earlier diagnostic and therapeutic treatments. These new options cause profound changes in healthcare systems by enabling more personalized, predictive, preventive and regenerative medicine.

Metal nanoparticles have attracted much attention of scientific researchers due to their applications in medicine, biology and material science. Noble metals such as gold and silver have great potential for biomedical applications, not only to deliver pharmaceutics but also to be used as novel diagnostic and therapeutic agents. Gold and silver are among the most studied metal nanoparticles due to their promising properties such as biocompatibility, easy synthesis, and facile surface modification. Thus the interests in these nanoparticles are continuously increased. Extensive ancient
literature reports the presence of significant properties in gold and silver nanoparticles (AuNPs and AgNPs) like antimicrobial, antiangiogenic, antitumor, anti-inflammatory and antioxidant activities.

Mankind is still fighting against many complex illnesses like hepatic disorders, cancer, multiple sclerosis, cardiovascular diseases, Alzheimer’s, Parkinson’s diseases, diabetes as well as some inflammatory or infectious diseases like HIV. Nanomedicine raises hopes and expectations for millions of patients that suffer from these ailments.

Looking in to a variety of biomedical applications of Nanomedicine, we made an extensive literature survey and scientific experimentation and thus an attempt has been made for the evaluation of therapeutic potential of AuNPs and AgNPs in ameliorating cancer and xenobiotic induced hepatorenal injury prompted through model toxicant acetaminophen (APAP).

Therefore in the present study the aim and objectives are as follows:

**Aim:**

**Nanoparticle therapeutics: an emerging treatment modality for cancer and hepatorenal disorders**

**Objectives:**

**Evaluation of test drugs, AuNPs and AgNPs on:**

- Cancer cell lines and primary hepatocytes
- APAP induced toxicity using battery of liver and kidney function tests
- Oxidative stress induced by model toxicant
- Drug metabolizing enzymes
- Regulation of cytokines; TNF-α and Interluekins-6
- Recovery pattern in histological and ultrastructural alterations
- APAP induced DNA damage by comet assay

Thus a thorough scientific study was conducted to arrive at vital assumptions.

The whole study was divided into 5 protocols that include 10 sets of experiments:
Experimental layout

Protocol 1:
Anticancer potential of nanoparticles (in vitro)
Experiment 1: Determination of antiproliferative effect on five different human cancer cell lines.
Experiment 2: Evaluation and confirmation of cytotoxicity by LDH assay
Experiment 3: Induction of apoptosis by test drugs on HepG2 cell line

Protocol 2:
Effect of test drugs on isolated primary hepatocytes (in vitro)
Experiment 4: Confirmation of non toxic and protective effect of test samples against model hepatotoxicant APAP

Protocol 3:
Effect of test drugs against acute exposure of APAP (in vivo)
Experiment 5: Selection of optimum and effective dose of AuNPs
Experiment 6: Selection of optimum and effective dose of AgNPs

Protocol 4:
Assessment of specific hepatocellular markers (in vivo)
Experiment 7: Antipyretic activity
Experiment 8: Choleretic activity

Protocol 5:
Subchronic study (in vivo)
Experiment 9: Comparison of effective doses of test drugs against APAP toxicity
Experiment 10: Mechanism of restoration by AuNPs towards APAP intoxication
Test Drugs:
Gold and silver nanoparticles (AuNPs and AgNPs) (3-5nm) used in this study were procured from the manufacturer, Gold NanoTech, Inc., Taipei, Taiwan. Nanoparticles were prepared by physical vapor deposition (PVD), suspended in sterilized water which maintains 99.99% purity of gold and silver nanoparticles, and the unique technology was applied to allow our AuNPs and AgNPs to be evenly dispersed in sterilized water. Therefore, unlike nanogold and nanosilver made with chemical reduction which requires the addition of dispersing agent to avoid the aggregation of nanoparticles, the gold nanoparticles used in this study are evenly suspended in water without addition of dispersing agent. This further increases the purity of nanogold. In addition, the 3-5 nm size of AuNPs and AgNPs used in this study can be efficiently excreted and the nanoparticles do not accumulate inside the body.

Protocol 1
Protocol 1 dealt with the \textit{in vitro} anticancer potential of AuNPs and AgNPs.

\textbf{In experiment 1}, \textit{in vitro} anti-proliferative effect of AuNPs and AgNPs were screened against five different human cancer cell lines of various tissues viz. colon (HCT-15), liver (HepG2), ovary (PA1), lung (A549) and breast (MCF-7). Viability of cancer cells was assessed by SRB assay. Both the test drugs were able to inhibit the viability of all the cells in a concentration dependent manner. Our results demonstrated that test drugs mediated a concentration dependent elevation in toxicity on all the cell lines. 25ppm concentrations of both the test drugs were found more effective in each cell line; however both the test drugs exerted more effect on HepG2 cell line as compared to other four cell lines.

Inhibitory concentration 50 (IC$_{50}$) of test drugs were calculated and was found (15.83ppm in MCF-7, 18.63ppm in HCT-15, 7.070 in HepG2, 13.29 in A549 and 11.56 in PA1 cells) for AuNPs and (24.44ppm in MCF-7, 12.89ppm in HCT-15, 12.27 in HepG2, 15.94 in A549 and 14.65 in PA1 cells) for AgNPs which revealed that test drugs were more effective on HepG2 cells, further AuNPs were found more effective as compared to AgNPs in all cell lines.

\textbf{In experiment 2} the comparative effect of AuNPs and AgNPs on all cell lines, at a concentration of 25ppm was assessed by the estimation of LDH leakage in the medium. AuNPs and AgNPs caused remarkable cell membrane damage to each cell
line which was apparent from the increased LDH leakage in the medium. LDH is a cytosolic enzyme and is released during the rupture of cell membrane. LDH activities were found to be significantly elevated in the medium of all cell lines, treated with test samples. LDH was found more in the medium of AuNPs treated cells as compared to AgNPs treated cells. Furthermore, from LDH leakage assay it was confirmed that both the test drugs are more effective on HepG2 cells as compared to other cells.

**In experiment 3** possibility of apoptosis induction on HepG2 by test drugs at 25ppm were investigated and assessed by DNA fragmentation assay. Apoptotic DNA fragmentation is a key characteristic of programmed cell death. DNA ladders of the corresponding AuNPs and AgNPs treated samples confirmed apoptosis and showed that the AuNPs and AgNPs treated HepG2 cells exhibited extensive double strand breaks, thereby yielding a smear appearance. The cytotoxic effects of test drugs are the result of interaction of nanoparticles with the biomolecules. Our results demonstrated that test drugs induced apoptosis. Apoptotic property of gold and silver nanoparticles may be due to their inhibitory activities in several signaling cascades responsible for the development and pathogenesis of the disease which are still unclear.

Our finding suggests that AuNPs and AgNPs induced anti-proliferative effect on HepG2 through induction of apoptosis. Thus these test drugs may be effective alternative in tumor and angiogenesis related diseases.

We report for the first time that pure AuNPs and AgNPs exhibited promising *in vitro* cytotoxicity in concentration-dependent manner against a panel of human cancer cell lines of various tissues. Test drugs were found more effective on HepG2 cell line as compared to other cell lines. AuNPs were found to be more effective in all cells as compared to AgNPs. Furthermore test drugs induced apoptosis, which might be mediated primarily by the activation of elements of the intrinsic pathway but not by death receptors. AuNPs and AgNPs appear to be promising anti-cancerous agents that may be of interest in cancer therapy. However, further studies should be conducted to show the possible mechanism of action.
Protocol 2

Fourth experiment of 2nd Protocol dealt with effect of AuNPs and AgNPs on isolated primary hepatocytes. Percentage cell viability of primary hepatocytes was carried out by using trypan blue exclusion test. AuNPs and AgNPs treated cells did not showed any significant alteration in cell viability as compared to control, thus indicated the non toxic effect of test samples on normal hepatocytes. The cell viability rate was significantly decreased (25%) in primary hepatocytes by APAP (40mM) exposure. AuNPs and AgNPs significantly attenuated the effect of APAP on hepatocytes and moderately restored their viability. The result showed that higher dose of AuNPs and AgNPs 15µg/ml showed 88% and 82% cell viability respectively, depicting significant amelioration.

Protocol 3

In protocol 3, experiments 5 and 6 were planned for the selection of optimum and effective doses of AuNPs and AgNPs against in vivo APAP intoxication in rats. In the fifth and sixth experiment, animals were intoxicated with APAP at a dose of 2 g/kg, p.o. once only. In experiment 5 APAP intoxication was followed by the treatment with AuNPs at three different doses (50, 100, 150µg/kg, p.o.) and in experiment 6, after 24 h of APAP intoxication, animals were treated with three different doses of AgNPs (50, 100, 150 µg/kg, p.o.). Results revealed that administration of APAP caused significant increase in the activities of serum transaminases, LDH, SALP, bilirubin, cholesterol, triglyceride, creatinine and urea. Significant rise was also observed in LPO with concomitant decline in GSH, SOD, Catalase, G-6-Pase and ATPase activities in liver and kidney. The altered blood and tissue biochemical variables signify the tissue damage. Therapy with AuNPs and AgNPs showed recovery in all blood and tissue biochemical variables, depicting clear improvement in the functional status of the liver and kidney and thus, showed marked hepatic and renal protective effect. Both test drugs depicted significant effectiveness at all three doses, however 100µg/kg and 150µg/kg doses of AuNPs and AgNPs showed more recoulement.

These findings were further confirmed by the histopathological studies of liver and kidney. Acute exposure of APAP caused degeneration and disintegration of liver cell architecture. Hepatic lesions were characterized by massive hepatic necrosis, distorted
hepatic cords, vacuolization, pyknotic nuclei and obliterated sinusoids in APAP intoxicated group. Therapy with AuNPs and AgNPs at a dose of 50µg/kg showed slight recovery with mild congestion in central vein and distortion of hepatic cords is less. Treatment of AuNPs at 100µg/kg and 150µg/kg doses showed clear central vein, normal lobular pattern with well formed polygonal hepatocytes, having conspicuous nucleus, and clear sinusoidal spaces.

APAP intoxicated group showed distorted renal architecture with degenerated tubular structure, swelling in glomeruli with loss of glomerular space and distorted endothelial lining. AuNPs and AgNPs treatment at all three doses showed restoration of renal histo-architecture with normal glomeruli, uniform glomerular space, maintained endothelial lining and normal tubules. Our results are comparable with standard drug silymarin.

In both the test drugs there was not a significant difference in the percent protection of higher doses, thus 100 µg/kg doses of both AuNPs and AgNPs were further preceded for subchronic study.

**Protocol 4**

Protocol 3 was planned to evaluate and confirm specific hepatocellular markers.

**Experiment seven** was conducted to ascertain the antipyretic activity of AuNPs and AgNPs. AuNPs and AgNPs (100 µg/kg) showed marked antipyretic effects on yeast induced pyrexia in rats at different time interval 1-4 h. Antipyretic effect of test samples was well compared with APAP. Therapy of AuNPs showed better antipyretic effect as compared to AgNPs.

**Experiment 8** was conducted to demonstrate the choleric activity of AuNPs and AgNPs which is a good indicator for safety evaluation of test drugs. One of the important functions of the liver is secretion of bile, which plays an important role in digestion and excretion of toxins from the body. The most important bile acid in mammals is cholic acid, thus in present study DHC was used as a standard drug. Choleretic activity was estimated by the rate of bile flow and amount of bile solid contents between 2–5 h periods. Treatment of AuNPs and AgNPs (100 µg/kg) stimulated liver activity which was compared with DHC (standard drug), which indicated no adverse effect of AuNPs and AgNPs on liver cells.
Protocol 5

Protocol 5 dealt with confirmation of protective effect of the selected doses of test drugs against subchronic exposure of APAP.

Experiment nine was conducted to evaluate the comparative effect of selected doses of AuNPs and AgNPs against subchronic exposure of APAP. APAP (20 mg/kg, p.o.) was administered to the animals for 4 weeks (5 days/week) followed by therapy (AuNPs and AgNPs) for 4 weeks (2 days/week). The toxicity was apparent by significant elevation in the activities of transaminases, alkaline phosphates and lactate dehydrogenase in serum. APAP exposure significantly increased the serum cholesterol, triglyceride, bilirubin, urea, uric acid and creatinine level. Tissue biochemical variables viz. LPO, GSH, SOD, CAT, G-6-Pase and ATPase were also altered after intoxication of APAP. AuNPs and AgNPs significantly recouped all blood and tissue biochemical variables towards normal. AuNPs were more effective as compared to AgNPs.

Activities of GSH cycle enzymes such as glutathione reductase (GR), glutathione peroxidase (GPx), Glutathione-S-transferase (GST) and Glucose-6-phosphate dehydrogenase (G-6-PDH) were declined in liver and kidney tissues after APAP intoxication. GR is concerned with the maintenance of cellular level of GSH by affecting fast reduction of oxidized glutathione (GSSG) to reduced form. GPx is a tetrameric selenoprotein which uses reduced glutathione as a cosubstrate and is localized in the cytosol and mitochondria. It is a major enzyme that removes hydrogen peroxides generated by SOD in cytosol and mitochondria by oxidizing reduced glutathione to its oxidized form. GST is a phase II enzyme which plays an important role in cellular detoxification of a variety of electrophilic xenobiotics. It catalyzes the conjugation of GSH with toxic compounds by which the couple of GSH with GST has been reported by a major component of cellular defense against toxic electrophiles. The regeneration of glutathione through GR requires NADPH which inturn is regenerated through glucose-6-phosphate dehydrogenase.

Treatment with AuNPs and AgNPs scavenged and detoxified the endogenous metabolic peroxides generated after injury, thus recovered the alterations in all the GSH cycle enzymes. This might be due to enhance in the level of the GSH substrate
which in turn alleviated the level of peroxides, hence glutathione peroxidase activity in liver was improved. The enhanced level of GSH could cause a proportional detoxification of elevated $H_2O_2$ by GPx. AuNPs were found to be more effective in recovering the activities of GR, GPx, G6PDH and GST when compared to AgNPs.

Liver microsomal enzymes, aniline hydroxylase (AH) and aminopyrine N-demethylase (AND) are cytochrome P450 enzymes. The activity of AH is specific for CYP2E1 and AND is specific for CYP1A2. The damage caused by APAP resulted in a significant loss of drug metabolizing capacity of the liver. The microsomes of liver, originating from the endoplasmic reticulum are most vulnerable to LPO. In the present investigation, results demonstrated that APAP caused significant inhibition in activities of AH and AND due to increased LPO in microsomes which might be due to damage to the smooth endoplasmic reticulum.

Our results specified that AuNPs and AgNPs prevented the event of microsomal LPO significantly hence recovered the activities of AH and AND. Thus AuNPs and AgNPs showed amelioration against APAP intoxication by regulating the levels of drug metabolizing enzymes. Maximum recovery was shown by AuNPs as compared to AgNPs.

APAP ingestion showed elevated level of CYP2E1, which plays an important role in production of excess amounts reactive metabolite NAPQI in the liver. NAPQI forms protein and nucleic acid adducts and also generates several reactive oxygen species, leading to increased oxidative stress, mitochondrial and cell membrane damage by increasing lipid peroxidation. In our findings AuNPs and AgNPs showed the ability to restore the level of CYP2E1. Silymarin treated rats showed similar results.

Biochemical studies were substantiated by histopathological studies on both, liver and kidney. Subchronic exposure of APAP to animals showed focal necrosis, nuclear pyknosis, and congestion in central vein and sinusoids in liver. Therapy with AuNPs showed considerable recoupment with better cord arrangement well formed hexagonal hepatocytes with well preserved cytoplasm and clear central vein. Therapy with AgNPs showing recovery in hepatocytes but mild sinusoidal congestion persists.
Kidney of APAP intoxicated rats showed degeneration of tubules, glomerular sclerosis and swelling of glomeruli. Severe necrotic changes were noted in the tubules. Dysplasia was detected in most of the tubules. Treatment with AuNPs and AgNPs provoked recoupment in renal histoarchitecture however AuNPs were much better than AgNPs. After five days treatment of AgNPs histological alterations were less significant. Bowman’s capsule and renal tubules were almost normal in AuNPs treatment depicted improvement in histoarchitecture of kidney. Bowman’s capsule showed comparatively less hypertrophy.

On the basis of observations acquired from 9th experiment of protocol five, it was clearly evident from the percent protection that AuNPs showed better protective effect in comparison with AgNPs. Thus, AuNPs were proceeded further to study their mechanism of action towards APAP toxicity.

**Experiment 10** of protocol five was performed to demonstrate the mechanism of restoration of APAP toxicity by AuNPs. Kupffer cells play a crucial role in normal physiology and homeostasis in liver and participate in the acute and chronic responses to noxious compounds of the liver as well. Direct or indirect activation of Kupffer cells by toxic compounds increases the expression of several cytokine such as transforming growth factor β, tumour necrosis factor-α (TNF-α) and interleukins 1 and 6 (IL-1 & IL-6). They play an important role in monitoring hepatic diseases hence as an indicator of hepatotoxicity.

TNF-α is a pro-inflammatory cytokine secreted by kuffer cells of the liver as an inflammatory response. Increased expression of TNF-α is thus an indicator of immune-pathological response as it induces hepatocyte apoptosis. IL-6 plays a crucial role in liver regeneration and acts as a mediator of cell differentiation and lymphocyte function. It has been found that serum level of IL-6 was related with liver necroinflammatory activity in patients with chronic hepatitis and cirrhosis. Liver damage as seen in subchronic intoxication of APAP appears to be modulated by kupffer cell activation with the release of TNF-α and IL-6 suggesting sizeable inflammation. Treatment of AuNPs markedly restrained the level of cytokines IL-6 and TNF-α may be due to its anti-inflammatory effect. Anti-inflammatory effects of AuNPs may be related to inhibition of the transcription factor nuclear factor-κB (NF-κB), which regulates the expression of various genes involved in inflammation.
The integrity of our DNA is vital to health. However, DNA mainly Guanine base is vulnerable to damage induced by ROS. Xenobiotics also induce single strand breaks in DNA. Formation of lipid peroxides in the liver and kidney tissue after APAP intoxication is one of the sign of membrane damage. APAP also caused severe DNA damage is confirmed from the comet assay. DNA density was measured by using image analysis, determining tail moment, total cellular DNA in the tail and tail length. The current investigation demonstrated a significantly increased DNA damage in liver and kidney of APAP intoxicated rats as compared to the control rats which might be considered as the biochemical indicator of apoptotic cell death. The increase of DNA damage may be due to the enzymes of CYP2E1 subfamily which play an important role in metabolizing APAP and producing toxic metabolite NAPQI, which forms protein adducts and induced oxidative stress and caused DNA damage.

AuNPs treatment effectively suppressed DNA damage induced by APAP showing a significant alleviation in the comet assay parameters (TL, % DNA in tail and TM) as compared to APAP intoxicated group. Decreased DNA damage in animals with AuNPs treatment suggesting that either the system of enzymatic repair was induced and an increase in antioxidant defenses in cell nuclei took place.

Ultrastructural study also showed severe degenerative changes in various cell organelles after APAP intoxication in liver and kidney. APAP exposure caused deterioration in nuclear envelop, degeneration in mitochondria, degranulation of endoplasmic reticulum and cytoplasmic vacuolation in liver. Significant recovery was observed in transmission electron microscopic study after treatment of AuNPs. Outer membrane, inner membrane, mitochondria and nucleus were normal in appearance.

Electron micrographs of kidney of APAP intoxicated rats showed significant decline in number of mitochondria in proximal convoluted tubules (PCT), margination and clumping of nuclear chromatin, vacuolation of brush bordered microvilli, damaged basement membrane of glomerular capillary and damaged foot processes of podocytes. AuNPs treatment demonstrated well formed glomeruli with intact basement membrane, well maintained foot processes and podocyte pedicles, increased number of mitochondria having cristae and well formed lateral processes of tubules, normal size of proximal tubular lumen.
Hepatorenal damage induced by APAP was associated with a variety of biochemical abnormalities and these could usually be attributed to the release of intracellular constituents into the circulation, following loss of integrity of the cell membrane or interference with normal metabolism and function of hepatocytes. ROS triggers host of disorders in body resulting in tissue damage and necrosis in many instances.

Acetaminophen is comprehensively metabolized by conjugation with sulphate and glucuronic acid. A small fraction of APAP is oxidized by CYP$_{450}$ enzymes in the liver which results in generation of highly reactive species N-acetyl-p-benzo-quinoneimine (NAPQI), which is normally detoxified by conjugation with GSH which triggers tissue damage. Exposure to high doses of APAP, the capacity of its removal by hepatic conjugate with glucuronide and sulfate is exceeded and more reactive NAPQI is formed. Elevated NAPQI level limit the ability of GSH to detoxify it which resulted in the consumption of liver GSH stores and covalently bind with cellular macromolecules and results in cellular damage.

APAP was associated with a variety of biochemical abnormalities and these could usually be attributed to the discharge of intracellular ingredients into the circulation, following loss of integrity of the cell membrane. In addition, APAP directly reduced cellular proliferation to induce oxidative stress, declined adenosine triphosphatase level and alter Ca$^{2+}$ homeostasis. Mitochondrial damage is an important feature which is associated with early depression of mitochondrial function.

AuNPs and AgNPs showed strong hepatic as well as renal protective potential, however, AuNPs were found to be more effective in ameliorating hepatorenal abnormalities caused by APAP. This data provides a scientific validation for the traditional uses of AuNPs and AgNPs in the treatment of hepatorenal disorders.
It is concluded that AuNPs and AgNPs showed potential anti-cancerous effect and ameliorated the hepatorenal damage as well. The possible mechanism responsible for the therapeutic efficacy include the following:

- AuNPs and AgNPs inhibited the growth of different cancer cells by the induction of apoptosis
- Test drugs showed non toxic effect and attenuated the APAP induced toxicity on primary hepatocytes
- Antioxidant activity of AuNPs and AgNPs as indicated by decrease in lipid peroxidation and increase in reduced GSH level
- Test drugs may possess the ability to block the bioactivation of APAP by inhibiting CYP450 2E1 activity and its expression
- AuNPs and AgNPs may be protecting the liver by preventing lipid peroxidation of the endoplasmic reticulum because it scavenges the superoxide anion and hydroxyl radicals
- Drugs, AuNPs and AgNPs lead to prevent the damage induced by reactive metabolite NAPQI as it has remarkable effects on tissue thiol status by increasing intracellular GSH level
- AuNPs diminished the release of proinflammatory cytokines, IL-6 and TNF-α
- AuNPs prevented the DNA damage

Thus these nanoparticles can be used for the development of drugs, however more studies with molecular considerations are needed in order to strengthen the mechanism of action of AuNPs and AgNPs, which may raise a hope for the patients with these ill effects.