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 those diseases (Umezaki et al., 2015). Thus an attempt has been made for the evaluation of therapeutic use AuNPs and AgNPs in ameliorating cancer and xenobiotic induced liver injury prompted through model hepatotoxicant APAP. In view of that the aim of the current study is to evaluate in vivo protective efficacy towards acute and subchronic exposure of APAP induced hepatorenal toxicity and to investigate the in vitro anticancer potential of gold and silver nanoparticles on different cancer cell lines.

Cancer is one of the most dreaded diseases and spreading with increasing incidence. There is increasing demands for anticancer therapy (Kantarjian et al., 2013) and multidisciplinary researches are making best endeavors to combat the disease, but the sure-shot, perfect cure is yet to be brought into global medicine. Despite many efforts, multi drug resistance is still considered as a major drawback in chemotherapy of cancer which has been the subject of exhaustive experiments recently (Yin et al., 2013). Nanomedicine has added new hope in the therapeutic and pharmaceutical
field. The unique nature of nanoparticles is being exploited by scientists, in hope of developing novel therapeutic agents (Augustin et al., 2016; Meng et al., 2016). AuNPs and AgNPs are widely used in medicine, physics, material sciences and chemistry (Swanner et al., 2015). With this aim, in present study AuNPs and AgNPs were investigated for their anticancer potential.

The in vitro antiproliferative effect of AuNPs and AgNPs were screened against five different cancer cell lines (HepG2, PA1, A549, MCF-7 and HCT-15) and viability of cancer cells were assessed using SRB assay. Both the test drugs were able to inhibit the cell 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. Antiproliferative nature of AuNPs and AgNPs in our study is in consistent with the findings of other authors (Gurunathan et al., 2013; Khan et al., 2016). Concentration, 25ppm 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, 7.070ppm in HepG2, 13.29ppm in A549 and 11.56ppm 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 demonstrated that test drugs were more effective on HepG2 cells, further AuNPs were found more effective as compared to AgNPs in all cell lines.

Effect of the test drugs were compared on all cell lines at a concentration of 25ppm which was assessed by the estimation of LDH leakage in the medium. In the current study AuNPs and AgNPs caused remarkable cell membrane damage to each cell line which was evident from the increased LDH leakage in the medium. LDH is a cytosolic enzyme and is released during the rupture of cell membrane. Our results suggested that LDH activities were found to be significantly elevated in the medium of all cells, treated with test samples. Our results correlate with the previous study of researchers who investigated silver nanoparticles as anticancer agent (Satapathy et al., 2013; Faedmaleki et al., 2014). More LDH was found in the medium of AuNPs treated cells as compared to AgNPs treated cells. Furthermore more LDH leakage was
found in HepG2 cells as compared to other cells which demonstrated that both the test drugs are more effective on HepG2 cells as compared to other cells.

Then the possibility of apoptosis induction on HepG2 cells 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 effect of test drugs is the result of interaction of nanoparticles with the biomolecules (Sellappa et al., 2015). Our results demonstrated that test drugs induced apoptosis, which are in accordance with the observations of other authors (Varun and Sudha, 2015). 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 not clear. Our finding suggests that AuNPs and AgNPs induced antiproliferative effect on HepG2 through induction of apoptosis. Thus these test drugs may be effective alternative in tumor and angiogenesis related diseases.

Thus present study on in vitro anticancer activity of AuNPs and AgNPs strongly suggested that both the test drugs were effective antiproliferative on lung, liver, breast, colon and ovary cancer cells. Both the test drugs were found more effective on HepG2 cell lines as compared to other cells. AuNPs were found to be more effective as compared to AgNPs. Test drugs induced apoptosis, which might be mediated primarily by the activation of elements of the intrinsic pathway but not by death receptors. In the present investigation we revealed that AuNPs and AgNPs appear to be promising anticancerous agents that may be of interest in cancer therapy. However, further studies should be conducted to show the possible mechanism of action.

The liver being largest complex organ in the body plays a vital role in the maintenance of internal environment and preventing the accumulation of noxious compounds by converting them into an appropriate form for elimination (James, 2013). It plays an important role in the metabolism of carbohydrates, lipids and proteins. During the metabolism of xenobiotics, the liver is potentially prone to injury. Impairment in the function of liver is life threatening. Liver disease is a global health problem which can be induced by various factors like viruses, alcohol, toxic bile...
Chapter 5

Discussion

acids, fatty acids, drugs, and immune response (An et al., 2014; Huang et al., 2016). Approximately 18,000 people have died each year in India due to liver diseases (Ilyas et al., 2016).

Various environmental pollutants and clinical drugs, such as acetaminophen and gentamicin, can cause ruthless organ damage through the metabolic activation to highly reactive free radicals including the superoxides and reactive oxygen species (Miettinen and Bjorklund, 2014).

APAP, CCl₄, galactosamine and alcohol are being widely used as model hepatotoxicants which are involved in phase I and phase II of biotransformation of xenobiotics respectively (Dongare et al., 2013; Jaeschke and McGill, 2013; Dixit et al., 2015). In the present study APAP was taken as a model toxicant for the evaluation of therapeutic effectiveness of gold and silver nanoparticles.

Acetaminophen/paracetamol (APAP), commonly used analgesic and antipyretic drug is safe at therapeutic dose, but overdose of APAP potentially results in hepatic damage in humans and experimental model animals (Mondal et al., 2016; Huang et al., 2016; Jiang et al., 2015; Werawatganon et al., 2014). During the overdose of APAP, cytochrome P450 (CYP) metabolizes it into highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) (Lee et al., 2013; Sahu et al., 2014) which covalently binds with protein and form acetaminophen-protein adducts (McGill et al., 2013). Formation of more NAPQI results in the depletion of a natural antioxidant, cellular glutathione (GSH), thus induces oxidative stress by the generation of free radicals such as reactive oxygen (ROS) and reactive nitrogen species (NOS) (Mazaleuskaya et al., 2015; Zhang et al., 2015). This results in the depletion of cellular antioxidant defense mechanism in tissues and thus finally causes hepatic damage (Kheradpezhouh et al., 2014) and tubular necrosis in the kidney (Adil et al., 2016) in both humans and experimental animals.

Recently isolated hepatocytes have become important tools to assess the possible hepato-protective effect of test drugs. The techniques for high yield isolation of rat hepatocytes are made it as useful model. Hepatotoxin such as paracetamol, CCl₄, galactosamine etc has been found to cause reduction of cell viability and rise in hepatospecific marker enzymes (Qadrie et al., 2015). In the present investigation,
APAP caused a significant reduction in cell viability which is in accordance with the investigation of other researchers (Bruderer et al., 2015). AuNPs and AgNPs treated cells does not showed any significant alteration in cell viability as compared to control, thus indicated the non toxic effect of test samples on normal hepatocytes. AuNPs and AgNPs significantly attenuated the effect of APAP on hepatocytes and moderately restored their viability. AuNPs and AgNPs showed cytoprotective effect on the isolated hepatocytes thus were selected for in vivo study against APAP induced toxicity.

Hepatocytes plays important role in array of metabolic activities and possess various enzymes. The hepatic damage produced by APAP is associated with the deviation of serum enzyme which may be caused due to structural alterations in the liver. Damage to hepatic cells may result in an oozing out of intracellular enzymes and other cell constituents in to the extra spaces, accordingly leading to a distinct rise of these enzymes in the circulation. Release of intracellular enzymes, like transaminases, serum alkaline phosphatase and lactate dehydrogenase into circulation are the most sensitive and remarkable indicators of hepatocytic injury. In the assessment of hepatocellular damage the estimation of these enzymes in serum is a useful quantitative marker (Hinson et al., 2010; Johnson et al., 2014).

ALT (GPT) is found more abundant in cytoplasm of liver cells and catalyses transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. Whereas AST (GOT) exists in cytoplasm and mitochondria of liver heart and muscle cells and catalyses the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. AST is found in highest concentration in heart compared with other tissues. ALT is more specific to the liver, and is thus a sensitive marker for the determination of hepatic injury (Curtis and Sivilotti, 2015; Sun et al., 2014; Jaeschke and McGill, 2013). If damage is caused in cellular organelles like mitochondria, soluble enzymes like AST normally located there, will also be similarly released. Elevated levels of AST in serum (SGOT) also signify cardiac infarction, muscle injury as well as viral hepatitis. Increased level of AST and ALT in serum indicated cellular membrane damage in liver (Beger et al., 2015). In present investigation, acute and sub-chronic intoxication of APAP results in liver injury which is characterized by leakage of
cellular enzymes in blood stream demonstrating necrosis and fibrosis. Various authors also reported the similar findings (Sharma and Sharma, 2012; Jaeschke, 2015; Mousah et al., 2016).

Alkaline phosphatase (ALP) is a brush border enzyme present in mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone, liver and placenta. It mainly arises from the lining of the canaliculi and also from the sinusoidal surface of hepatocytes which is involved in the transport of metabolites across the cell membrane, synthesis of certain enzymes, secretory activities and glycogen metabolism. The increased level of alkaline phosphatase is sensitive marker of liver damage and the activity of this enzyme rises in many types of liver diseases. Maximum levels are seen with hindrance to the intrahepatic or extrahepatic bile flow (Yamazaki et al., 2013). Acute and subchronic exposure of APAP to animals caused significant elevation in the serum alkaline phosphatase activity (Kocaaslan et al., 2015) which clearly indicated damage in the sinusoidal surface of hepatocytes. The present investigation concur with the findings of various other investigators (El-Sayed et al., 2014; Mousah et al., 2016).

The enzyme lactate dehydrogenase is an intracellular hydrogen transfer enzyme which catalyzes the pH dependent interconversion of lactate into pyruvate. It is present in almost all tissues of the body and is a reliable marker of liver injury. Elevated activity of LDH is found in liver diseases like infective hepatitis, drug induced liver injury and primary or secondary tumors of liver (Iroanya et al., 2014). In the present Investigation APAP intoxication significantly increased the leakage of LDH which clearly indicated hepatocytic injury. Our results correspond with the findings of other authors (McGill and Jaeschke, 2014; Sun et al., 2014).

Therapy of AuNPs and AgNPs significantly stabilize AST, ALT, SALP and LDH levels at all doses, which is an apparent sign of the improvement in the functional status of the hepatocytes. Therapeutic agents significantly neutralize the toxic effect of APAP and attenuated the increased level of these enzymes, thus caused a subsequent recuperation towards normalization, as seen from statistical analysis. Declined levels of AST, ALT and LDH towards the normal value affirm the ability of therapeutic agents to repair hepatocellular membrane which was damaged by APAP intoxication. Therapeutic agents may inactivate the reactive metabolites, which may
inhibit the concentration of intracellular free radicals. Thus AuNPs and AgNPs treatment provided protection against membrane fragility and subsequently leakage of liver marker enzymes into circulation. Our findings corroborates the investigation of other authors (Chen et al., 2012; 2013).

Bilirubin is the breakdown product of heme and is considered to be the predictable indicator of liver diseases (Afroz et al., 2014; De-Giorgio et al., 2013). Bilirubin is glucuronidated via glucuronosyl transferases in the liver and then the glucuronides (mono or diglucuronides) are actively excreted into bile. Hepatocellular damage leads to a decreased glucuronidation of bilirubin (due to a reduced activity of the glucuronosyltransferases) and to a decreased excretion into bile (due to an impaired function of the transporters). Estimation of bilirubin is the most useful clinical signs to the severity of necrosis and its accumulation determine the binding, conjugation and excretory capacity of hepatocytes. In the present investigation there was a significant rise in bilirubin after APAP administration which may be due to dysfunctional and dystrophic changes in liver (Ekam et al., 2012; Mossanen and Tacke 2015). Elevation in the total serum bilirubin concentration might be due to the failure of normal uptake, conjugation and excretion by the injured liver parenchyma. Hence hyperbilirubinaemia reveals pathophysiology of liver (Seifert et al., 2016). These findings are confirmed by various authors (Ismail and Salem, 2016). Treatment with AuNPs and AgNPs significantly decreased the bilirubin concentration in serum. Same results were obtained by the standard drug silymarin (Kim et al., 2015).

Serum urea, uric acid and creatinine are frequently considered as important markers to estimate the functional status of kidney (Pradhan et al., 2013). It has been suggested that the urea, uric acid and creatinine accumulates in nephrotoxicity and renal diseases, because their rate of production exceeds the rate of clearance due to the imperfection in renal function (de Geus et al., 2012). Urea is a waste product formed from the catabolism of proteins while as creatinine is an amino acid derivative with a molecular mass of 113 Dalton. Creatinine is produced as a waste product of creatine and phosphocreatine and is found almost exclusively (90%) in skeletal muscle tissues. Uric acid is the waste product that results from the breakdown of purine base. Uric acid is made in liver and excreted by kidney. Urea, uric acid and creatinine are usually passed out in the urine. A high blood level of urea (uraemia), uric acid
(hyperuricemia) and creatinine indicates the kidney dysfunction (Roy et al., 2015). Creatinine is usually a more accurate marker of kidney function than urea. In our study acute and subchronic APAP exposure induced nephrotoxicity which was characterized by marked rise of urea, uric acid and creatinine in serum. APAP nephrotoxicity occurs due to its highly reactive metabolite- NAPQI- which arylates proteins in the proximal convoluted tubule, initiating cell death of renal tubular cells (Won et al., 2016). Elevated level of urea, uric acid and creatinine after toxicant administration is also supported by various authors (Ramachandran et al., 2012).

The level of urea, uric acid and creatinine restored near to normal, that state the role of AuNPs and AgNPs in preventing the kidneys from damage (Bektur et al., 2016). The restorative effect of test drugs over the serum urea and creatinine correlate with previous evidence of research made by Brede and Labhasetwar, (2013).

Cholesterol and triglycerides are two forms of fat which plays essential role in synthesis of various hormones and building cell membranes (Toroka et al., 2014). Triglycerides provide much of the energy needed for cells to function. When cells accumulate more free fatty acids (FFAs) than are required for metabolic processes, surplus lipid is esterified and stored as triglyceride in lipid droplets. Elevated level of TG and Cholesterol was observed in APAP induced hepatic damage by various researchers (Aba et al., 2016). These findings are in support of our study, as in present investigation, acute and subchronic intoxication of APAP results in the increased level of cholesterol and TG.

Chloestasis is a condition that causes partial or full obstruction of the bile ducts (Vartak et al., 2016). Bile ducts bring bile from the liver into the gall bladder and the intestines. Bile helps the body to break down fat, process cholesterol and get rid of toxins. Hypercholesterolemia is often caused as a result of chloestasis and may reach a high level as seen in our study after APAP exposure. Various authors have also reported that the alteration in cholesterol takes place due to the imbalance between the normal rates of lipid synthesis, utilization and secretion (Verma et al., 2015).

Free fatty acids (FFA) taken from diet or released from chylomicrons or fat cells into the blood, are rapidly utilized for membrane biosynthesis, energy production through B-oxidation, generation of lipid signaling molecules, post-translational protein
modification, and transcriptional regulation. FFAs are directly taken up by the liver to join the hepatic pool of FFAs and some FFAs are oxidized in the liver for energy, but most are rapidly incorporated into complex lipids (e.g. phospholipids, glycolipids, Triglycerides and cholesterol).

In present study the elevated serum triglyceride level due to APAP intoxication may be due to decreased lipase activity, which involved triglyceride- rich lipoprotein by the extrahepatic tissues. The increase in serum triglycerides are also due to the disturbance of triglycerides secreting mechanism (Wang, 2014). The increased level of TG in serum after APAP exposure is in accordance with the investigation of Khan et al., (2016).

Therapeutic agents significantly reversed the serum level of TG and Cholesterol towards normal. Our findings are in accordance of investigations of Chen et al., (2013), who used gold nanoflakes for the treatment of alcohol induced fatty liver disease.

In present investigation antipyretic activity of test drugs were carried out. Antipyretics are drugs which decrease body temperature in conditions like fever, which is caused as a secondary impact of malignancy, infection or other diseases (Shi et al., 2012; Mishima-Iwai et al., 2015). Normally the infected or damaged tissue regulates the increased formation of proinflammatory cytokines like interleukin 1β, α, β, and TNF-α, which enhance the synthesis of prostaglandin E2 (PgE2) near hypothalamus and thereby activates the hypothalamus to increase the body temperature (Cavar et al., 2010; Taslipinar et al., 2013).

In the present investigation, rectal temperature was elevated after 18h of yeast injection. It is proposed that the yeast enhanced the production of prostaglandins, which set the thermoregulatory center at a lower temperature (Conti, 2016; Sugita et al., 2016). Treatment with AuNPs and AgNPs allevated the rectal temperature significantly and thus showed antipyretic activity. Inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic activity of test drugs as that of APAP (Safari et al., 2016).

One of the most vital functions of the liver is synthesis and secretion of bile (Yamazaki et al., 2013). Choleretic activity of drug is a good indicator for its safety.
Bile excretion is altered due to hepatic injury by APAP intoxication (Guanabens et al., 2016; Vartak et al., 2016). In the present investigation APAP intoxication reduced the choleretic activity in terms of bile flow and bile solids. DHC (dehydrocholic acid) was used as a standard drug in present investigation as the most important bile acid in mammals is cholic acid. It is a pure biliary salt, which demonstrated stimulatory effect on bile flow.

Injection of AuNPs and AgNPs stimulated choleretic activity which signified no adverse effect of the test drugs on hepatocytes. This is a sign of the healthy status and sturdy stimulating action on the secretary activity of the liver (Kasthuri and Rajendiran, 2009). Thus due to the stimulatory effect on bile output, these drugs may be used in certain biliary disorders.

The biological production of reactive oxygen species primarily superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) is capable of damaging molecules of biochemical classes including nucleic acids and aminoacids (Birben et al., 2012). The most damaging effect is the induction of lipid peroxidation. Lipid peroxidation is a complex process and is considered as the main mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death. It is an extremely oblitative process that induces excess changes in the structure and function of cell membranes and entails oxidation of fatty acids. It is a multi step process and includes initiation, propagation and termination. It is miles widely recognized that polyunsaturated fatty acids (PUFA) are more sensitive to oxidation than the saturated fatty acids. The presence of a double bond adjacent to a methylene group makes the methylene C-H bond weaker and therefore the hydrogen in more susceptible to abstraction. This leaves an unpaired electron on the carbon, forming a carbon-centered radical, which is stabilized by a molecular rearrangement of the double bonds to form a conjugated diene which then combines with oxygen to form a peroxyl radical. The peroxyl radical is itself capable of abstracting a hydrogen atom from another polyunsaturated fatty acid (PUFA) and so of starting a chain reaction to form a lipid hydroperoxide, which may decompose to form peroxy radicals. (Ewertowska et al., 2009). These radicals start a propagation reaction and are maintained until a termination reaction is initiated by other factors e.g. chain breaking.
antioxidants. Lipid peroxidation of cell membranes causes a loss in fluidity and an increase in the permeability of membranes, with resulting loss of cytosolic proteins.

The toxicity of lipid peroxidation products in mammals generally has a role in the pathogenesis of neurodegenerative (Santos et al., 2016; Mitra et al., 2016), inflammatory (Pires et al., 2014), infectious (Ibanez et al., 2010), gastric and nutritional diseases (Repetto et al., 2012), hepatotoxicity (El-Demerdash et al., 2016) and nephrotoxicity (Canayakin et al., 2016). Lipid peroxidation appears to be a major source of endogenous DNA damage which may contribute significantly to cancer and other genetic diseases linked to lifestyle and dietary factors. The products of lipid peroxidation are themselves reactive species and lead to extensive membrane and cellular damage (Fernando et al., 2016). LPO mainly damages kupffer cells as also evident in histological studies.

The thiobarbituric acid (TBA) test is performed to measure the amount of MDA (TBARS) present in the sample. MDA is generated as a degradation product from peroxidised lipids. The level of MDA formation is an indirect measurement of lipid peroxidation. The results of our study demonstrated that acute and subchronic exposure of APAP indicated excessive formation of free radicals and activation of LPO which was confirmed by the significant production of TBARS in liver and kidney, resulting in failure of antioxidant defense system which generated excessive free radicals thus enhancing oxidative stress. The enhancement of lipid peroxidation induced by APAP is also reported in literature showing liver dysfunctions (Adam et al., 2016).

Treatment with AuNPs and AgNPs significantly inhibit LPO in liver and kidney confirming the antioxidant potential of test drugs. Same results were seen by the positive control silymarin. The ability of AuNPs in inhibiting the lipid from peroxidation thereby preventing the ROS generation has restored the imbalances in the antioxidants. This may also be due to the destruction of free radicals that are already formed or by supplying a competitive substrate for unsaturated lipids in the membrane and or by accelerating the repair mechanism of damaged cell membrane. These findings are substantiated by Mohammed and Safwat, (2013).
Reduced glutathione (GSH) is a nonenzymatic tripeptide ($\gamma$-glutamyl cysteinyl glycine) nonenzymatic biological antioxidants (Bilinsky et al., 2015), widely distributed in cells and is recognized as a protective compound within the body which helps to remove toxic free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals and maintains membrane protein thiols, through GPx and GST activities (Gum and Cho, 2013; Jahangir et al., 2014). Determination of total GSH is a key factor to show the antioxidant reserve in the organism (Balouchxadeh et al., 2011). GSH plays a crucial role in protecting biomolecules against xenobiotic induced cytotoxicity by taking part in the removal of reactive intermediates by conjugation reaction, or by free radical quenching mechanism (Zhang et al., 2015). It is extensively known that tissue disorders and injury are the result of GSH deficiency within living organisms (Eugenio-Perez et al., 2016).

GSH is a scavenger of toxic metabolite NAPQI in APAP intoxication (Huang et al., 2016). Declined level of GSH is implicated in the development of lipid peroxidation in APAP treated rats (Lorincz et al., 2015). In the present investigation, acute and subchronic intoxication of APAP caused a significant depletion in hepatic and renal GSH.

Treatment with AuNPs and AgNPs after APAP intoxication resulted in the accelerated recovery of the GSH content in liver and kidney. The enhanced levels of GSH effectively scavenged toxic metabolites and reactive oxygen species, which reduced APAP-induced liver and kidney injury and promoted regeneration in tissues (Roy et al., 2013). These findings correspond with the investigation of Boonruamkaew et al., (2016).

Superoxide dismutase and catalase are the major enzymes, which play an important role in the eradication of ROS produced from the redox process of xenobiotic in tissues (Bhattacharyya et al., 2014).

The ubiquitous superoxide dismutases (SODs) are the most important intracellular antioxidant enzymes present in all aerobic cells that catalyze the disproportionation of superoxides to molecular oxygen and peroxide thus are critical for protecting the cell against the toxic products of aerobic respiration (Tai et al., 2015). Superoxide and superoxide-dependent formation of hydroxyl radicals are important in oxygen toxicity.
If unchecked, reactive oxygen species (ROS) including the superoxide radical can result in inflammation and inflict cell injury that includes DNA damage mediated by Fenton chemistry (Thanan et al., 2013). This ROS-mediated cellular damage is implicated in various human pathologies, including cardiovascular disease, cancer, hepatic injury, kidney damage, aging and neurodegenerative disease (Ohnishi et al., 2013).

Catalase is a haemoprotein acting as an oxidant in which it works as peroxides and defends cells from the accumulation of H$_2$O$_2$ by catalyzing it to form H$_2$O and O$_2$ (Campomanes et al., 2015). Therefore, diminished activity of CAT may result in a number of harmful effects due to the failure to scavenge superoxide radical and hydrogen peroxide (Wang et al., 2016).

It has been reported that APAP intoxication can lead to alteration in gene expression and declined the activity of SOD and catalase in liver and kidney (Palani et al., 2011). Our study also showed the same results with declined activity of catalase and SOD in liver and kidney. It may be due to the elevated production of reactive free radicals, reduction of intracellular GSH, and may be due to increased lipid peroxidation (Hamza and Al-Harbi, 2015a).

Both the therapies significantly recovered the activity of CAT and SOD in a similar manner as that of silymarin, the standard drug, revealing their potentials to prevent the accumulation of excessive free radicals, thus defending the liver and kidney from APAP intoxication. Our results are in consistent with the investigation of Chen et al., (2013).

Adenosine triphosphatase (ATPase) is a mitochondrial lipid based membrane bound enzyme and precept donor of free energy within the living organisms (Santacatterina et al., 2016). Any change in membrane lipids ends in alterations in membrane fluidity, which in turn changes the ATPase activity and cellular function. Using ATPase activity measurement is taken into consideration as the appropriate index of membrane damage. Pathological methods that intrude with the production of ATP might also intrude with sodium pump activity, which in turn decreased cellular function. The cellular organelles for free radical generation encompass mitochondria, endoplasmic reticulum and plasma membrane. It has been suggested that oxidative
damage of ATPase activity is critical for mitochondrial membrane damage. Free radicals produced in mitochondria are regularly released into the cytosol. The production of ATP within the mitochondria includes the shipping of protons across the internal mitochondrial membrane through the electron transport chain. Uncoupling of oxidative phosphorylation leads to decline in ATPase activity (Kheradpezhouh et al., 2014; Badr et al., 2016). Many investigations have revealed that activities of Na⁺/K⁺ and Ca²⁺/Mg²⁺ ATPase had been inhibited by way of the induction of oxidative stress. It’s found that exaggerated calcium influx into cells is a crucial signal which could result in death of cell. Some of toxic consequences of medication are related to an increase in intracellular calcium. Role of mitochondria in the maintenance of cell energy metabolism has long been recognized. Some of toxic results of drugs are associated with a growth in intracellular calcium and lack of adenosine triphosphatase activity in liver and kidney (Woodhead et al., 2012; Abdelmegeed et al., 2013). It might be due to dysfunctional and dystrophic modifications inside the mitochondria and cell membrane permeability after toxicant exposure.

We have observed that activity of membrane enzyme ATPase reduced drastically in liver and kidney after APAP exposure. This inhibition of adenosine triphosphatase by APAP exposure has also been confirmed with the aid of other investigators (Bhattacharya et al., 2012). Sizable recuperation by the treatment of AuNPs and AgNPs in ATPase activities indicated membrane stabilizing effect of therapeutic agents which can be associated with better shaped mitochondrial assembly and thereby stopping the intense depletion in the activity of ATPase.

Glucose-6-phosphatase is a key enzyme involved in regulation of homeostasis of glucose level in body by catalyzing the terminal enzymatic step of both gluconeogenesis and glycogenolysis by converting glucose-6-phosphate into glucose and inorganic phosphate. G-6-Pase is associated with the luminar side of endoplasmic reticulum and its declined activity specifically reveals injury to this organelle. So this enzyme can be used as a diagnostic tool to quantify the hepatotoxic effect of various toxicants (Shakya and Shukla, 2011).

Liu et al. (2016) suggested that the G-6-Pase activity might serve as an indicator of initial hepatic damage and might occur in advance of histologically evident organ
damage. Reduction of G-6-Pase activity was used to document the destructive property of degradation product of LPO process. Various authors reported that APAP caused significant depletion in the glucose-6-phosphatase activity (Yamaki et al., 2016). Exposure of APAP caused the significant loss in the activity of G-6-Pase. Oral administration of both therapeutic drugs restored the enzyme activity in a similar manner as that of standard drug silymarin. This might be due to the inhibition of ROS generation by the therapeutic agents which resulted in reduced LPO. The therapeutic nature of test drugs can also be seen in the ultrastructural study, where they enhanced the regeneration of cell organelles.

GSH cycle enzymes and reduced glutathione regulates gene expression, apoptosis, transmembrane transport of organic solutes and detoxifies toxic metabolites of drugs. It is very important to maintain the reduced glutathione in cell/tissue (Yang et al., 2012; McGill et al., 2013), and its severe depletion is reported to cause tissue injury (Khayyat et al., 2016; Tobwala et al., 2015).

Glutathione peroxidase (GPx) (a selenium containing enzyme) is concert with catalase and superoxide dismutase (SOD) function to protect the cell from damage due to ROS (Aycan et al., 2015). Two third of GPx is present in the cytosol and one third in the mitochondria. It detoxifies peroxides with GSH and acts as an electron donor in the reduction reaction, producing GSSG as an end product. GPx plays a crucial role in \( \text{H}_2\text{O}_2 \) catabolism and the detoxification of endogenous metabolic peroxides and hydroperoxides, which catalyzes GSH and protects the organism from oxidative damage GSH as a cofactor of the glutathione peroxidase is the only mitochondrial defense to cope with \( \text{H}_2\text{O}_2 \) produced endogenously in aerobic cells (Liu et al., 2015; Jadeja et al., 2015).

Glutathione reductase (GR) is a member of the flavoprotein disulfide oxidoreductase family and exists as a dimer (Ahmed et al., 2016). It is regulated at the level of transcription as well as by posttranslational modifications. Alterations in GR expression and activity have been implicated in cancer and aging. It is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidized glutathione to reduced form in a process that requires NADPH. It is an important source of reducing equivalents during oxidative stress generated by ROS (Takemoto et al., 2014; Li et al., 2015). GSH and its linked
enzymes especially GR, plays a vital role in scavenging ROS, maintaining GSH pool and providing clinical recoveries. It is reported that, the decreased GPx activity leads to H₂O₂ accumulation in the liver which in turns inactivates SOD (Sharoud, 2015).

GSSG under the influence of GR and reduced nicotinamide adenine dinucleotide phosphate (NADPH) is reduced to GSH (Nylova et al., 2014). G-6-PDH activity is the main source of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) that is an essential supplier of reducing equivalents. Reduced activity of this enzyme may lead to a decrease in NADPH concentration, which is necessary for proper functioning of GR (Ignacio et al., 2016).

GST is a group of Phase II detoxification enzymes that catalyzes the conjugation of GSH to a wide variety of endogenous and exogenous electrophilic compounds. Thus it plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione (Grelier et al., 2010). GST binds to lipophilic compounds and acts as an enzyme for GSH conjugation reactions (Zhang et al., 2012). The ability of GST to alter the level of intracellular GSH in the liver in response to generation of ROS has been implicated in protection of cells against free radicals inducting agents (Mannery et al., 2010).

A significant decrease in hepatic and renal GR, GPx, GST and G6PDH activities after APAP intoxication as seen in the present study are also supported by the findings of various investigators (Ucar et al., 2013; Chen et al., 2014; Hamza and Al-Harbi, 2015a; Mazaleuskaya et al., 2015).

The activities of GR, GPx, GST and G6PDH were recovered after 5 days treatment with test drugs which is the sign of enhanced detoxification of H₂O₂. Our findings are in consistent with the results of other investigators (Chen et al., 2013; Kalyanaraman, 2013; Shine et al., 2014; Abirami et al., 2015; Hamza and Al-Harbi, 2015b).

The cytochrome P₄₅₀ is a super family of haemoproteins, which play a key role in the metabolism of xenobiotics. The majorities of P450s are located in the membrane of endoplasmic reticulum which catalyzes a wide range of chemical transformations including redox reactions (Hur et al., 2012). Numerous forms of cytochrome P₄₅₀ have been identified in various tissues including the kidney and the nomenclature is based on amino acid sequence homology (Du et al., 2015). Cytochrome P₄₅₀ are
mainly liable to detoxification of both endogenous and exogenous compounds. During the detoxification reactions, involving the CYP\textsubscript{450} in microsomal system, electron flows from NADPH or NADH through a flavoprotein CYP\textsubscript{450} to different isomorphic factors of CYP\textsubscript{450}. Hepatic microsomal cytochrome P\textsubscript{450} enzymes cause the oxidative metabolism of steroids, other endogenous compounds and fatty acids (Johnson et al., 2014; Szilagyi et al., 2016).

Liver microsomal enzymes such as amidopyrine-N-demethylase (AND) and aniline hydroxylase (AH) are cytochrome P450 enzymes. Usually the activities of these enzymes are estimated in liver microsomes to evaluate the metabolic potential of drug metabolizing enzymes (DMEs). The main function of phase I metabolism is to prepare a compound for phase II metabolism. Phase II metabolism leads to true detoxification of drugs and xenobiotics which results in water soluble products which can be easily excreted. It is reported that APAP administration in experimental animals caused the reduction in aniline hydroxylase and aminopyrine -N-demethylase activities in liver microsomes (Mach et al., 2013; Simeonova et al., 2016). The microsomes of liver derived from the endoplasmic reticulum are prone site of lipid peroxidation. In the present study results demonstrate that APAP caused decline in aniline hydroxylase and amidopyrine-N-demethylase activities, which are also confirmed by the earlier findings. This may be due to the damage to the microsomal oxidation system. We assessed the effect of APAP and therapeutic agents on hepatic protein levels of CYP2E1. APAP intoxication significantly elevated expression of CYP2E1 when compared with control rats, as also investigated by other authors (Martin-Murphy et al., 2013; Jing et al., 2015).

APAP ingestion induces the levels of CYP2E1, which plays an important role in production of excess amounts reactive metabolite NAPQI in the liver (Zanger and Schwab, 2013). 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 as confirmed in our study.

In our findings AuNPs and AgNPs showed the ability to restore structural and functional integrity of hepatocytes and hence recouped drug metabolizing enzymes significantly towards normal. Silymarin treated rats showed similar results. This
restoration activity of therapeutic agents may be due to their antioxidant nature (Woolbright et al., 2012).

Kupffer cells are resident macrophages of the liver and 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 cause increase in the expression of several cytokines such as transforming growth factor β, tumor 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 liver injury (Liu et al., 2015; Yang et al., 2012).

The pro-inflammatory cytokines (TNF-α, IL-6, IL-8, etc.) are thought to be responsible for the pathogenesis of advanced liver diseases associated with CCl₄, APAP and alcohol induced toxicity (Polat et al., 2015; Ilyas et al., 2016).

TNF-α is a pro-inflammatory cytokine secreted by Kupffer 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 (Ilyas et al., 2016).

IL-6 plays a crucial role in liver regeneration and acts as a mediator of cell differentiation and lymphocyte function (Tan et al., 2016). It has been found that serum level of IL-6 was related with liver necroinflammatory activity in patients with chronic hepatitis and cirrhosis (Lee et al., 2013). It has been proposed that IL-6 might be significantly involved in fibrotic changes, partly by altering intrahepatic expression of other cytokines.

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 signifying sizeable inflammation (Polat et al., 2015).

Treatment of AuNPs markedly restrained the level of cytokines IL-6 and TNF-α may be due to anti-inflammatory role of test drug. Anti-inflammatory effects of test drugs may be related to inhibition of the transcription factor nuclear factor-κB (NF-κB), which regulates the expression of various genes involved in inflammation. These
findings are in consistent with the investigations of Chen et al. (2013) who used gold nanoflakes for the reversal of alcohol induced inflammation and liver injury.

Increased ROS would cause the oxidative DNA damage (Li et al., 2015). Various investigators have proposed that xenobiotics cause single strand breaks in DNA due to increased ROS and inhibition of DNA repair enzymes. The comet assay is a rapid, sensitive and versatile method for the quantification of DNA damage in the individual cells both in vitro and in vivo (Gunasekarana et al., 2015). Damaged DNA strands were broken into fragments and migrated towards anode during electrophoresis. In this assay cells with damaged DNA showed increased migration of DNA fragments (comet tail) from the nucleoid (comet head) which may also be a characteristic of DNA fragmentation associated with the necrotic/apoptotic process of death (El-Kott and Bin-Meferij, 2015). It has been reported that long time exposure to CCl₄, APAP and alcohol caused oxidative DNA damage in the target organs, which could be blocked by antioxidants (Lorenzo et al., 2013; Lee et al., 2013; Speit et al., 2014).

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 significant 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 increment 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. APAP induced DNA damage was also proposed by various investigators (Yang et al., 2013).

AuNPs treatment in our study was effective in preventing DNA damage induced by APAP showing a significant decrease in the comet assay parameters (Tail length, % DNA in tail and Tail moment) compared to APAP intoxicated group. Decreased DNA damage in animals with AuNPs treatment, suggesting that either the system of enzymatic repair was induced and/or an increase in antioxidant defenses in cell nuclei took place.

The histopathological observations support the biochemical analysis. Light microscopical observations demonstrated that liver sections of control rats showed
normal architecture of hepatocytes with maintained cord arrangement and sinusoidal space. Central veins and portal veins do not show any alterations. Liver sections from APAP intoxicated animals demonstrated the destruction of architectural pattern, focal necrosis, sinusoidal congestion, focal damage around the central vein, feathery degeneration, loss of lobular architecture with damaged cellular outlines and congestion are seen, nuclei have become condensed and pyknotic. Many other investigators have also demonstrated the histological changes in the liver following administration of APAP and our results correspond with their studies (Kyriakides et al., 2016; Ilavenil et al., 2016; Yang et al., 2013). The liver sections of the animals treated with the gold, silver nanoparticles and standard drug silymarin demonstrate recovery of toxic effects and showed almost normal lobular architecture, central and portal veins appear normal. In these groups no necrosis or fatty changes or any inflammatory signs were seen.

Degenerative nephrotoxic effects of APAP have also been estimated through remarkable alteration in histopathological features of kidney. Light microscopy of the kidney sections of control rats showed normal architecture apparently with normal renal cortex and well formed nuclei. In the rats administered with APAP, kidney sections were characterized by glomerular degeneration, tubules showed marked alterations, interstitial mononuclear cell infiltration and fibrosis and vascular congestion in tubular blood vessels were observed in renal cortex, Bowman’s capsules were deformed. The animals in the groups treated with AuNPs and AgNPs showed almost complete recovery from toxic effects with well formed Bowman’s capsule, normal glomeruli and tubules.

Ultrastructural alterations after APAP intoxication in the liver included disruption of the cytoplasmic and endoplasmic reticulum, severe degeneration of smooth and rough endoplasmic reticulum and disappearance of mitochondrial cristae have also observed (Badr, 2013). The nucleus becomes lopsided with condensation of chromatin and the endoplasmic reticulum was dilated, angular mitochondria along with vacuolation. Vacuolation due to steatosis is also seen after administration of APAP. Significant improvement was observed in electron microscopic study after AuNPs treatment. AuNPs treatment showed normal nucleus with intact nuclear membrane, mitochondria
were normal in appearance, endoplasmic reticulum, mitochondrial crests and matrices were clear.

Subchronic intoxication of APAP caused significant decline in number of mitochondria in proximal convoluted tubule, margination and clumping of nuclear chromatin, vacuolation of brush bordered microvilli, degeneration in podocyte pedicles, foot processes and severe damage in capillary basement membrane. 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.

APAP is biotransformed and eliminated as non toxic glucuonic acid and sulfate conjugates. CYP2E1 participates in metabolizing small portion of APAP to NAPQI which is normally detoxified by conjugation with GSH. After high dose of APAP the capacity of its removal by hepatic conjugate with glucuronide and sulfate is exceeded hence more reactive NAPQI is formed. More NAPQI is conjugated with GSH which caused depletion in hepatic GSH thus more NAPQI covalently bind with cellular macromolecules which results in cellular damage. NAPQI induced the oxidative stress which results in DNA damage.

The hepatic and renal protection by AuNPs and AgNPs as studied by biochemical parameters clearly indicated that different treatments with AuNPs and AgNPs showed different degrees of therapeutic activity; however AuNPs showed best result as compared to AgNPs.

Therapeutic potential of AuNPs and AgNPs may be attributed due to their antioxidant property as strongly showed by reduced LPO and maintained GSH contents by enhancing the synthesis of GSH and by recovering the activities of GR, GPx, GST and G-6-PDH. Activities of SOD and CAT are also restored by the therapeutic agents. The activities of microsomal drug metabolizing enzymes (MDME) are restored, thus demonstrated the ameliorative effect of AuNPs and AgNPs at cellular level. Histopathological and ultrastructural studies also indicate the improvement in the structural and functional integrity of the cells which grant additional support to proposed mechanism of action. Activities of major cellular enzymes such as adenosine triphosphatase and glucose-6- phosphatase were also recovered after
therapy of AuNPs and AgNPs. Present investigation strongly recommends that AuNPs and AgNPs significantly recouped the vital liver and renal function tests (AST, ALT, SALP, LDH, bilirubin, urea, creatinine uric acid, triglycerides and cholesterol). Choleretic activity of test drugs was investigated in which liver showed slightly enhanced release of bile flow/bile solids after AuNPs and AgNPs administration that indicated no side effect of the drug on secretary activity of the liver. AuNPs prevented the DNA damage, maintained the regulation of proinflammatory cytokines (IL-6, TNF-α) which clearly indicated its therapeutic potential at molecular level. AuNPs and AgNPs per se groups did not showed any significant alteration in any of the biochemical parameters which signified the non toxic property of test drugs.

Thus, the test drugs ameliorated the hepatorenal damage and showed potential anticancerous activity as well. The possible mechanism responsible for the therapeutic efficacy includes the following:

- Inhibited the growth of different cancer cells by the induction of apoptosis which may protect the integrity of multicellular organisms and thus allowing for the selective removal of unwanted or damaged cells
- Showed non toxic effect to primary hepatocytes
- Attenuated the toxic effect of APAP on primary hepatocytes
- Acted as a free radical scavenger intercepting those radicals involved in toxicant metabolism induced by microsomal enzymes
- Inhibited LPO
- Maintained non-enzymatic antioxidant status in terms of GSH contents thus recycling the status of antioxidant defense system
- Increased enzymatic activities (GR, GPx, GST, G-6-PDH, ATPase, G-6-Pase, SOD and CAT)
- Recovered the activity of drug metabolizing enzymes, AH and AND
- Inhibited the activity expression of CYP4502E1
- Diminished proinflammatory cytokines, TNF-α and IL-6
- Recovered DNA damage
Therapeutic potential of AuNPs and AgNPs against hepatorenal toxicity by APAP can be attributed due to their antioxidant activity. The test drugs might be enhancing the synthesis of GSH which helps in the elimination of more NAPQI hence prevented the covalent binding of this toxic metabolite with the biomolecules hence averted the generation of ROS and preventing cellular damage. Test drugs may also possess the ability to block the bioactivation of APAP by inhibiting CYP2E1 activity or it may directly combine with free radicals and obstruct the formation of these radicals.

AuNPs and AgNPs also showed the excellent anticancer potential against different cancer cell lines. Thus it is concluded that AuNPs and AgNPs can be used for the development of hepatoprotective, nephroprotective and anticancerous drug after further preclinical and clinical studies, which may raise a hope for the patients with these ill effects.