Protocol 1: Anticancer potential of nanoparticles (*in vitro*)

**Experiment 1: Determination of antiproliferative effect on five different human cancer cell lines.**

AuNPs and AgNPs were analyzed for their *in vitro* cytotoxicity against five human cancer cell lines (Liver: HepG2, Ovary: PA1, Lung: A549, Breast: MCF-7, Colon: HCT-15) of different tissues at four different concentrations (3.125ppm, 6.25ppm, 12.5ppm and 25 ppm). The viability of cancer cells were confirmed using SRB assay.

*In vitro* anti-proliferative effect of AuNPs

AuNPs demonstrated concentration dependent cytotoxicity against all the cell lines (*Table 1*). The growth inhibition of more than 60% was observed by AuNPs at 25ppm against all the cell lines those were used in the investigation. AuNPs were found more effective on HepG2 cells as compared to other cells and showed 84.3% inhibition at 25ppm as shown in *table 1*.

*In vitro* anti-proliferative effect of AgNPs

AgNPs were able to reduce viability of the cell lines, used in this study in a dose-dependent manner as shown in *table 2*. AgNPs showed more than 50% of cytotoxicity at 25ppm against MCF-7 and showed more than 60% growth inhibition in all other
four cell lines. AgNPs were also found more effective on HepG2 cells as compared to other cells and showed 78.8% inhibition at 25ppm.

**Determination of IC50 of AuNPs and AgNPs**

Based on *in vitro* cytotoxicity study profile, AuNPs were found more effective on all human cancer cell lines as compared to AgNPs. IC50 values of AuNPs and AgNPs were calculated to compare the potential activity of these test drugs against different cell lines. The lower IC50 value corresponds to the maximum cytotoxicity potential of treatment against a particular cell line. **Graph 1** demonstrates that AuNPs revealed extreme activity against HepG2, (IC50 = 7.070 ppm), fair activity against PA1 and A549 (IC50 = 11.56 and 13.29 ppm) and low activity against MCF-7 and HCT-15 (IC50 = 15.83 and 18.63 ppm). **Graph 2** depicts that AgNPs were highly active against HepG2 and HCT-15 (IC50 = 12.27 and 12.89 ppm), moderately active against PA1 and A549 (IC50 = 14.65 and 15.94 ppm) and least active against MCF-7 (IC50 = 24.44 ppm).

**Experiment 2: Evaluation and confirmation of cytotoxicity by LDH assay**

**Graph 3** depicts the percent cytotoxicity of test drugs on selected cell lines which was confirmed by LDH assay. Significantly increased activities of LDH were found after 24h exposure of AuNPs and AgNPs. **Graph 3** showed that both the test drugs were more effective on HepG2 cells as compared to other cells. More over AuNPs showed more effect on all cell lines than AgNPs. AuNPs showed 83.3%, 71.5 %, 71.8%, 60.2% and 61.5% cytotoxicity and AgNPs showed 73.2%, 61.3%, 62.2%, 50.2%, 59.9% cytotoxicity on HepG2, PA1, A549, MCF-7 and HCT-15 cell lines respectively.

**Experiment 3: Induction of apoptosis by test drugs on HepG2 cell line**

DNA fragmentation assay

To detect the mechanism of action of the AuNPs and AgNPs against HepG2 cell line, DNA fragmentation assay was carried out (**Fig. 1**). DNA gel electrophoresis clearly showed that the AuNPs and AgNPs treated cells exhibit DNA fragmentation, which was confirmed by the appearance of a mild DNA smear in lane 3 and lane 4 (AuNPs and AgNPs treated cells) which might be considered as induction of apoptosis, however, no DNA changes were observed in lane 2 (untreated cells).
Outcome

- Both test drugs showed concentration dependent antiproliferative effect on five different cancer cell lines
- Test drugs showed cytotoxicity on different cancer cell lines which was confirmed by LDH leakage assay. AuNPs were found more effective than AgNPs
- Test drugs induced apoptosis in HepG2 cell line which was confirmed by DNA fragmentation assay
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

Pretreatment of AuNPs and AgNPs did not showed any toxic effect on isolated rat hepatocytes. AuNPs treated cells did not showed any alteration in cell viability when compared with control. AuNPs at 15, 10, 5µg/ml concentration showed 99.0%, 99.0%, 98.2% viability respectively which is almost same as that of control (99.5%). Similar results were shown by AgNPs. AgNPs at 15, 10, 5µg/ml concentration showed 99.0%, 98.0%, 98.5% cell viability which indicates the non toxic effect of test drugs. APAP treatment at 40mM concentration showed significantly reduced cell viability (25%) as compared to control. More over toxicant exposure to AuNPs and AgNPs treated cells showed prevention against APAP induced toxicity as shown in graph 4. AuNPs showed 88.8%, 85.2%, 78.1% cell viability at 15µg/ml, 10µg/ml and 5µg/ml concentration. Similarly AgNPs at three different concentration (15µg/ml, 10µg/ml and 5µg/ml) concentration showed (82.2%, 78.0%, 69.5%) cell viability after toxicant exposure respectively. Similar results were found by silymarin treated cells. Silymarin treatment at 15µg/ml, 10µg/ml and 5µg/ml showed 85.0%, 82.0%, 70.0% viability respectively against APAP exposure.

Outcome

- AuNPs and AgNPs showed non toxic effect towards isolated primary hepatocytes
- Both the test drugs attenuated the toxic effect of APAP in primary hepatocytes
Protocol 3: Effect of test drugs against acute exposure of APAP (in vivo)

Experiment 5: Selection of optimum and effective dose of AuNPs
The present experiment was performed primarily to evaluate the protective potential of AuNPs against APAP induced liver and kidney damage in vivo in rat model. Three different doses of AuNPs (50, 100 & 150µg/kg, p.o.) were selected for this study. Thus, following parameters were assessed to identify the most effective dose.

Serological biochemical observations

Hepatic marker enzymes
Animals administered with APAP demonstrated significant hepatic damage as observed from elevated level of specific enzymes such as serum transaminases, lactate dehydrogenase and serum alkaline phosphatase. The elevated level of these liver function tests were significantly reduced by the three doses of AuNPs when compared with APAP (P ≤0.05) however more recovery was observed at 100µg/kg & 150µg/kg. One-way analysis of variance (ANOVA) was carried out for significant variance between all the groups (P < 0.05) (Table 3).

Triglyceride, cholesterol and bilirubin
Table 4 reveals that APAP caused significant (P ≤ 0.05) increase in TG, cholesterol and bilirubin level which showed alteration in liver function. Treatment of AuNPs at three different doses after APAP exposure showed marked reversal in these indices. Both the higher doses were found more effective, which was confirmed by calculation of percent protection (P ≤ 0.05).

Renal function markers
APAP administration showed significant elevation of urea and creatinine level which indicated decreased glomerular filtration rate. AuNPs and silymarin reduced the elevated levels of renal markers significantly towards normal (P≤0.05) (Table 5). The results were analyzed by ANOVA at 5% level.

Tissue biochemical observations

Lipid peroxidation & reduced glutathione
Acute administration of APAP to rats caused an increase in lipid peroxidation with a concomitant fall in reduced glutathione level in liver and kidney. Data shown in table 6 confirms that APAP accentuate lipid peroxidation which is considered to be an
indicator of tissue damage. Therapeutic agent showed marked reversal in the lipid peroxidation level in both the tissues. All the three doses showed significant diminution ($P \leq 0.05$).

Reduced glutathione is supposed to be an imperative endogenous defense against peroxidative obliteration of cellular membranes. In the present study, a significant decline was observed in the reduced glutathione level after APAP intoxication in liver and kidney (Table 6). Substantially decreased reduced glutathione level was significantly ($P \leq 0.05$) recouped by the treatment of AuNPs.

**Enzymatic activities of Superoxide dismutase & Catalase**

SOD & CAT mutually function as imperative enzymes in the eradication of reactive oxygen species (ROS). In the present study, the observed decrease in SOD & CAT activities was associated with the increased oxidative stress caused by acute exposure of APAP to rats. AuNPs at all the three doses recovered SOD & CAT activity in liver and kidney towards normal and protected free radical induced oxidative stress and tissue damage (Table 7). Maximum restoration was found at a dose of 100µg/kg & 150µg/kg.

**Adenosine triphosphatase & Glucose-6-phosphatase**

APAP administration to rats resulted in a significant depletion in the activities of adenosine triphosphatase in liver and kidney and G-6-Pase in liver. Treatment of therapeutic agent showed remarkable ameliorative effect thus maintained enzymatic activities significantly towards normal ($P \leq 0.05$). Significant protection in these parameters was confirmed by Analysis of variance at 5% level (Table 8).

**Histopathological observations (Light microscopical changes)**

**Liver**

The liver sections of control rats showed normal features. Photomicrographs of control rat liver showed normal appearance of hepatocytes with prominent rounded nuclei and well-preserved cytoplasm. Appearance of central vein was clearly visible. Transverse section of liver clearly demonstrates complete hepatic lobules (Fig. 2-3). Acute administration of APAP tempted degeneration in hepatocytes. APAP toxicity was characterized by scattered degeneration, focal necrosis, disturbed cord arrangement and infiltration of lymphocytes (Fig. 4-7). Therapy with AuNPs at a
Chapter 4

Observations

dose of 50µg/kg after APAP exposure illustrated moderate degree of recoupment, however inflammatory cell infiltration still persists (Fig. 8-9). AuNPs treatment at 100µg/kg and 150µg/kg showed significant recovery in liver sections. AuNPs treated animals at a dose of 100µg/kg and 150 µg/kg depicted presence of normal hepatic cords, absence of necrosis and regenerative activity of hepatocytes. Central venous congestion and necrosis that is more severe form of injury was markedly prevented. Cord arrangement was maintained, hepatic sinusoids were seen between the plates of hepatic cells (Fig. 10-13). These histopathological observations basically support the results obtained from biochemical estimations. Silymarin treatment also showed well-preserved hepatic cells (Fig.14-15).

Kidney

Kidney of control rats demonstrated normal characteristics. Proximal and distal convoluted tubules, glomeruli were normal (Fig. 16-17). APAP intoxication for 24 h schedule induced deterioration in the renal histoarchitecture. Ruthless necrotic alterations were observed in the Bowman’s capsules and renal tubules showed hypertrophy in epithelial cells with focal degeneration. Space in the Bowman’s capsules was occupied due to hypertrophied glomeruli (Fig. 18-19). Therapy with AuNPs (50, 100 and 150µg/kg) showed recovery when compared with toxicant however 100µg/kg and 150µg/kg doses showed more recovery. Therapy with AuNPs (50µg/kg) showed improved glomeruli and renal tubules, however lumen of uriniferous tubules were narrowed (Fig. 20-21). AuNPs at 100 and 150µg/kg doses recovered the histological changes with well formed Bowman’s capsules with compact glomeruli and tubules along with a wider lumen (Fig. 22-25). The therapy of silymarin also showed protective effect in Bowman’s capsules, glomeruli and tubules (Fig. 26-27).

Experiment 6: Selection of optimum and effective dose of AgNPs

Blood biochemical observations

Hepatic marker enzymes

Table 9 shows that APAP intoxication caused severe liver injury in various blood biochemical indices. APAP administration caused a significant rise in hepatospecific markers i.e. AST, ALT, LDH, SALP (P≤0.05). Therapy of AgNPs at all doses exhibited significant reduction in these parameters in a dose dependent manner when
compared with APAP treated group. These values were well comparable to silymarin treated group. The results were analysed by ANOVA at 5% level. 100 and 150µg/kg doses of AgNPs showed almost same recovery as calculated by % protection.

**Triglyceride, cholesterol and bilirubin**

Table 10 summarizes the effect of APAP on lipid profile and bilirubin level followed by subsequent treatment with therapeutic agent. Acute exposure of APAP induced significant elevation of serum triglyceride, cholesterol and bilirubin level (P ≤ 0.05). Treatment of AgNPs at three different doses significantly prevented the APAP induced toxicity. Doses, 100 and 150µg/kg showed more and almost same effect in reducing the elevated levels of these parameters, thus depicting marked protection.

**Renal function markers**

APAP treated animals showed significant elevation of urea and creatinine level which indicated the kidney damage (Table 11). AgNPs and silymarin reduced the elevated levels of renal markers significantly towards normal (P≤0.05). Both the higher doses showed almost same recovery which was confirmed by percent protection.

**Tissue biochemical observations**

**Lipid peroxidation and reduced glutathione**

A significant elevation (P ≤ 0.05) in the level of lipid peroxidation in liver and kidney after 24 h of APAP intoxication was detected in rats. Similarly significant fall in the levels of hepatic and renal GSH (P≤0.05) was also observed. Treatment with different doses of AgNPs reversed these biochemical parameters significantly towards normal in a dose dependent manner (Table 12). 100µg/kg and 150µg/kg doses exhibited protective activity almost equivalent to silymarin as shown by one-way analysis of variance.

**Enzymatic activities of SOD and CAT**

Superoxide dismutase and catalase are antioxidant enzymes which defend against reactive oxygen species. Table 13 represents that enzyme activity of SOD and CAT were significantly decreased after the administration of APAP. Enhancement in the antioxidant enzymatic activities were observed after the treatment with AgNPs at different doses when compared with the APAP treated group (P≤0.05). The results were analysed by ANOVA at 5% level.
Adenosine triphosphatase & Glucose-6-phosphatase
Acute exposure of APAP caused damage to liver and kidney as revealed by alteration in adenosine triphosphatase in liver and kidney & Glucose-6-phosphatase activity in liver. There was significant decline in the activities of these enzymes (Table 14). Therapy with AgNPs recovered the activities of these enzymes significantly towards normal. Silymarin also showed significant elevation in the enzymatic activities when compared with APAP group.

Histopathological observations (Light microscopical changes)
Liver
Liver of control rats showed normal lobular architecture with radiating hepatic cords (Fig. 28-29). After APAP intoxication at a dose of 2g/kg severe necrotic regions were seen. It caused nuclear degeneration and chromatolysis. Sinusoidal dilatation was also seen. Toxicant administration also showed pyknotic nuclei, and perinuclear vacuolation (Fig. 30-32). Administration of AgNPs at 50µg/kg dose improved the structure, cord arrangement was maintained however congested sinusoids were seen (Fig. 33-34). Treatment of AgNPs at 100 and 150µg/kg protected the hepatic lesions more when compared to 50µg/kg. Cord arrangement was maintained along with maintained sinusoidal space and better appearance of nuclei (Fig. 35-38). In silymarin treated group hepatocytes were well formed, cord arrangement was maintained, almost normal portal triad and nuclei also appeared better (Fig. 39-40).

Kidney
Control rats demonstrated normal histoarchitecture of kidney (Fig. 41-42). Kidney of rats after acute administration (2g/kg) of APAP showed severe deterioration in cortical region and hypercellularity in glomeruli, diameters of the tubules were decreased. Apical nuclei were also seen in epithelial cells of tubules. Disrupted endothelial lining was also observed (Fig. 43-44). With the treatment of AgNPs at 50µg/kg dose, significant improvement in proximal and distal convoluted tubules was seen, however tubular obstruction still persists (Fig. 45-46). Better results were observed at the doses of 100 and 150µg/kg treatment of AgNPs, improved structure of glomeruli was noted. The tubules and glomeruli were well organized, the nuclear organization in the epithelium of collecting tubules was normal. Clear and wide
lumens were observed in the renal tubules (Fig. 47-50). Therapy of silymarin showed remarkable improvement in histoarchitecture of kidney (Fig. 51-52).

**Outcome**

- Out of three doses of AuNPs and AgNPs, 100µg/kg and 150µg/kg doses showed more recoupment.
- Doses, 100 µg/kg and 150µg/kg of AuNPs & AgNPs showed almost same recovery as calculated by % protection, thus 100 µg/kg dose of AuNPs and AgNPs were further proceeded for subchronic study.
Protocol 4: Assessment of specific hepatocellular markers \((in \ vivo)\)

**Experiment 7: Antipyretic activity**

Table 15 depicts the effect of therapeutic agents on antipyretic activity on yeast induced pyrexia in rats. Treatment of AuNPs (100µg/kg) and AgNPs (100µg/kg) showed significant antipyretic effects at different time interval 1-4 h \((P\leq0.05)\). AuNPs (100µg/kg) showed more antipyretic effect as compared with the therapy of AgNPs (100µg/kg).

**Experiment 8: Choleretic activity**

Choleretic activity of drug is excellent index for safety evaluation. Present investigation on the effects of bile flow (choleretic activity) demonstrated that AuNPs and AgNPs showed discernible choleretic activity in anaesthetized normal rats between 2–5 h periods as shown in table 16. AuNPs (100µg/kg) and AgNPs (100µg/kg) did not show any adverse effect on the choleretic activity of liver. Treatment of therapeutic agents excreted more bile in similar manner as that of DHC (standard drug), when compared with control animals thus showed stimulated activity of liver.

**Outcome**

- AuNPs and AgNPs possessed antipyretic activity
- Choleretic activities of test drugs signified their no adverse effect on physiology of liver
Protocol 5: Subchronic study (in vivo)

Experiments 9: Comparison of effective doses of test drugs against APAP toxicity

Serological biochemical indices

Serum transaminases

The results divulged that array of biochemical alterations produced by APAP were reversed significantly by AuNPs and AgNPs. APAP intoxication for 21 days, elevated activities of AST and ALT were observed when compared to control group. Oral administration of therapeutic agents, AuNPs (100µg/kg) and AgNPs (100µg/kg) significantly attenuated the APAP induced rise in serum transaminases activity. The protective effects of these therapeutic agents were also compared with silymarin treated animals. F values of both parameters were found to be statistically significant at 5% level (Graph 5).

Serum alkaline phosphatase & lactate dehydrogenase

Graph 5 also illustrates that APAP led to severe toxic response by increasing significantly the activities of SALP and LDH. Therapy of AuNPs (100µg/kg) and AgNPs (100µg/kg) for 5 days was found to be significantly effective in alleviating the elevated levels of these enzymes in serum. Silymarin treated rats demonstrated values near to control group (P ≤ 0.05).

Triglyceride, cholesterol and bilirubin

Graph 6 represents the effect of therapeutic agents on APAP on lipid profile and bilirubin in serum of rats. Subchronic exposure of APAP caused significant elevation in serum lipid profile and bilirubin. Treatment of AuNPs (100µg/kg) and AgNPs (100µg/kg) for 5 days significantly (P ≤ 0.05) declined the serum level of TG, cholesterol and bilirubin. Similarly silymarin (50mg/kg, p.o. for 5 days) also significantly decreased the level of these parameters towards normal.

Urea, uric acid and creatinine

Graph 7 assesses the effect of APAP followed by therapy on kidney function tests. APAP induced significant elevation in urea, uric acid and creatinine. These indices were significantly restored with the therapy of AuNPs (100µg/kg) and AgNPs (100µg/kg) (P ≤ 0.05). Results are comparable to silymarin. F values were found to be significant when analyzed by one-way ANOVA.
Tissue biochemical indices

Markers of oxidative stress

In order to assess the effect of therapeutic agents on APAP induced lipid peroxidation in liver and kidney, we observed the levels of TBARS, an index of oxidative damage and one of the principle products of LPO. As revealed in graph (8) the level of TBARS in the APAP intoxicated group was significantly elevated when compared with the control group.

Subchronic administration of APAP depleted significantly the reduced glutathione (GSH) level in liver and kidney. Declined level of cellular GSH below a certain level results in the oxidation of vital protein thiol resulting in cellular toxicity. Treatment of AuNPs (100µg/kg) and AgNPs (100µg/kg) for 5 days significantly prevented the depletion of GSH produced by APAP in a similar manner as that of silymarin. However AuNPs (100µg/kg) were found to be more effective as compared to AgNPs (100µg/kg).

Antioxidant enzymes: Superoxide dismutase & catalase

Graph 9 portrays the activities of superoxide dismutase and catalase. APAP administered group revealed the declined activities of SOD and CAT. 5 days treatment of AuNPs (100µg/kg) and AgNPs (100µg/kg) showed significant (P ≤ 0.05) recovery in the activity of SOD and catalase thus restored the activities of antioxidant defense system in liver and kidney. Similar effect was also shown by the positive control silymarin. Maximum restoration was found by AuNPs (100µg/kg).

Adenosine triphosphatase and glucose-6-phosphatase

Graph 10 demonstrates inhibited activities of adenosine triphosphatase in liver and kidney in APAP intoxicated rats. Treatment of AuNPs and AgNPs for 5 days were significantly effective (P ≤ 0.05) in restoring the activity of this enzymatic variable in both the tissues. Significant improvement was also observed in the enzymatic activity by the standard drug silymarin. Analysis of variance showed significant results.

Glucose-6-phosphatase is a crucial enzyme of glucose homeostasis. In the present research there was a significant fall in the activity of this enzyme in liver (Graph 10). Significant recoupment was found with the AuNPs and AgNPs therapy. However the
protection offered by AuNPs seemed relatively greater in both the above parameters (P ≤ 0.05).

**Evaluation of cellular defense markers (GSH cycle)**
Glutathione redox cycle is a mechanism which scavenges lipid peroxides and hydrogen peroxide.

**Glutathione reductase (GR)**
GR is concerned with the maintenance of cellular level of GSH by affecting fast reduction of oxidized glutathione (GSSG) to reduced form. Graph 11 demonstrates the toxic effect of APAP exposure and protective effect of post treatment with therapeutic agents. Toxicant caused significant inhibition (P≤0.05) in the enzymatic activity of GR in liver and kidney. Therapeutic agents (AuNPs & AgNPs) significantly restored activity of GR (P≤0.05). The effect of therapeutic agents was compared with that of standard reference drug silymarin. AuNPs showed better results when compared with AgNPs treatment.

**Glutathione peroxidase (GPx)**
GPx is a tetrameric selenoprotein which uses reduced glutathione as a co substrate 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. Graph 11 clearly showed a significant inhibition (P≤0.05) in the activity of GPx activity after 21 days of APAP intoxication, when compared with control group. AuNPs & AgNPs independently increased the depleted GPx activity considerably. The recoupment with AuNPs & AgNPs clearly showed significant protection in liver and kidney. AuNPs treated group showed more recoupment as compared to AgNPs treated group.

**Glucose-6-phosphate dehydrogenase (G-6-PDH)**
The regeneration of glutathione through GR requires NADPH which inturn is regenerated through glucose-6-phosphate dehydrogenase. APAP intoxicated rats showed significant decrease in the activity of G-6-PDH in liver and kidney (Graph 12; P≤0.05). Treatment with AuNPs and AgNPs significantly (P≤0.05) improved the level of G-6-PDH in liver and kidney. AuNPs treatment was found more effective.
**Glutathione-S-Transferase (GST)**

GST is a phase II enzyme which plays an important role in cellular detoxification of a variety of electrophillic 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. Graph 12 shows the effect of APAP intoxication on GST. Significant (P≤0.05) inhibition in the activity of GST was noted after APAP exposure. Therapy with AuNPs and AgNPs significantly (P≤0.05) recovered the activity of GST in liver and kidney. AuNPs showed better recoupment than AgNPs.

**Microsomal lipid peroxidation, Aniline hydroxylase and Amidopyrine-N-Demethylase**

Graph 13 demonstrated that subchronic exposure to APAP caused significant elevation in microsomal LPO and dramatic alterations in the activities of drug metabolizing enzymes i.e., aniline hydroxylase and Amidopyrine-N-demethylase. In liver microsomes, AH and AND activities were significantly inhibited whereas significantly increased TBARS was seen in APAP intoxicated animals (P≤0.05). Treatment for 5 days with AuNPs and AgNPs recovered the AH, AND activity and reduced the microsomal LPO significantly, (P ≤ 0.05) which was confirmed statistically. The maximum recovery in these parameters was noted by the treatment of AuNPs as compared to AgNPs.

**Cytochrome P450 2E1**

CYP2E1 is also involved in the production of toxic metabolite NAPQI during APAP intoxication. In the present study, APAP intoxication significantly increased CYP2E1 activity as compared with control group. Treatment with AuNPs and AgNPs for 5 days, significantly (P ≤ 0.05) reduced the activity of CYP2E1 (Graph 14). These effects were comparable to standard drug silymarin. Percent protection revealed that AuNPs were more effective than AgNPs.

**Histopathological observations (Light microscopy)**

**Liver**

Histopathological observations of control liver showed polygonal hepatocytes cordially arranged around central vein (Fig. 53-54). Subchronic exposure of APAP induced intense focal necrosis in the liver, which is evidenced by increased
cytoplasmic eosinophilia and nuclear disintegration, Kupffer cell hyperplasia and portal inflammation. Liver sections of APAP intoxicated rats also illustrated congestion of the central vein and distraction of the surrounding sinusoids, necrosis of centrilobular cells, nuclear disintegration, pyknosis, karyorrhexis and cytoplasmic eosinophilia (Fig. 55-58). Therapy with AuNPs depicted considerable recoupment with better cord arrangement, hepatocytes were well formed, granulation and vacuolation was not seen and nuclei were normal, thus reestablishing cord arrangement (Fig. 59-61). After 5 days therapy of AgNPs, improved histoarchitecture of the liver with better formed hepatocytes, reduced necrosis, and congestion in sinusoids were observed (Fig. 62-64). Silymarin treated rats showed hexagonal hepatocytes cordially arranged around central vein with conspicuous nuclei (Fig. 65-66).

Kidney
Kidney of control rat liver showed normal histoarchitecture (Fig. 67-69). Histological alterations in the kidney after exposure of APAP revealed degeneration of proximal tubules, glomerular sclerosis, and swelling of glomeruli. Severe necrotic changes were noted in the Bowman’s capsule. Dysplasia was detected in most of the tubules. Karyopyknosis was also seen (Fig. 70-72). Treatment with AuNPs and AgNPs provoked recoupment in renal histoarchitecture, however AuNPs were much better than AgNPs. AuNPs treatment depicted improvement in histoarchitecture of kidney. Glomeruli, nuclei of both cortical and medullary region and Bowman’s capsules were normal (Fig. 73-74). After five days treatment of AgNPs, histological alterations were less significant. Bowman’s capsule and renal tubules were almost normal (Fig. 75-76). Silymarin therapy recovered the histoarchitecture of kidney almost towards normal (Fig. 77-78).

Experiment 10: Mechanism of restoration by AuNPs towards APAP intoxication
Proinflammatory Cytokines: TNF-α and IL-6
Graph 15 depicts the inflammatory response induced by APAP, which was expressed by TNF-α and IL-6. Subchronic exposure of APAP significantly elevated the levels of TNF-α and IL-6 in serum (P≤0.05). AuNPs treatment showed significant suppression of the inflammatory response against APAP toxicity (P≤0.05) hence recovered the
ability of damaged liver to perform its normal function. AuNPs showed 70% and 75% restoration in TNF-α and IL-6 respectively.

**Assessment of DNA damage by comet assay**

In the present investigation comet assay was carried out to calculate the single-strand DNA breaks in liver and kidney cells. Subchronic exposure of APAP was found genotoxic. APAP administered group showed significantly (P≤0.05) elevated tail length along with tail moment and DNA damage as compared to control group. Therapy with AuNPs showed genoprotective effect significantly (P≤0.05) by reducing tail length, tail moment and DNA damage which was confirmed by percent protection (Graph 16 and 17).

**Histopathology (Transmission Electron Microscopy)**

**Liver**

EM of control rat liver showed well formed nucleus with distinct nuclear membrane containing nucleolus, mitochondria were distributed uniformly and had normal appearance with well formed crests, endoplasmic reticulum were present between mitochondria with normal appearance. Glycogen rosettes were normally distributed (Fig 79-81). APAP caused deformed nuclear envelope, condensation of nuclear chromatin, disorganization and degranulation of endoplasmic reticulum, vacuolation in cytoplasm due to steatosis was observed (Fig.82-85). Treatment with AuNPs improved the process of regeneration. Liver sections of AuNPs treated rats demonstrated sharply defined boundaries of the hepatocytes. The nucleus was normal in appearance, nuclear envelopes were intact, majority of the mitochondria were normal with better formed cristae, endoplasmic reticulum was significantly improved, abundant glycogen rosettes were clearly seen (Fig. 86-89). Therapy of silymarin depicted improved structure of mitochondria, endoplasmic reticulum and prominent nucleus (Fig. 90-92).

**Kidney**

Ultrastructure of kidney of control rats showed prominent nucleus with regular nuclear membrane, well formed endoplasmic reticulum and well arranged mitochondria with cristae. Proximal convoluted tubule (PCT) showed the presence of thin profuse microvilli projections from apical surface of cells. Distal convoluted tubule (DCT) depicted abundant mitochondria. In glomeruli, well formed foot
processes were clearly seen (Fig. 93-95). Kidneys of APAP exposed rat revealed declined number of mitochondria in PCT as compared to control. In renal epithelium of PCT numerous fragmented rough endoplasmic reticulum (RER) and increased number of lysosomal related structures within the epithelial lining cells in PCT were revealed. Podocyte processes were broadened with increased fusion of foot processes. Brush border microvilli were greatly deformed with vacuolation (Fig. 96-99).

EM of kidney treated with AuNPs demonstrated well formed glomeruli with intact basement membrane, well maintained foot processes and capillary endothelial lining. Increased numbers of mitochondria having cristae and well formed lateral processes were clearly seen. Proximal tubular lumen was normal in size. Distal tubules also demonstrated basement membrane with an intact nucleus and numerous mitochondria (Fig. 100-103). EM of kidney treated with silymarin showed well formed microvilli, regular foot processes and well maintained basement membrane with regular capillary endothelial lining (Fig. 104-106).

**Outcome**

- Subchronic studies demonstrated that test drugs protected against CYP2E1 mediated oxidative stress, markers of cellular injury and antioxidant defence system. AuNPs showed better recovery, thus were processed for further study to know their mechanism of action
- AuNPs diminished release of pro-inflammatory cytokines, TNF-α and IL-6 which suggested the deactivation of Kupffer cells. AuNPs prevented the DNA damage and restored ultrastructural alterations