ANTILITHIASIC AND HYPOLIPIDAEMIC EFFECT OF AuNPs OF *SILYBUM MARIANUM* ON LITHOGENIC DIET FED MICE GALLBLADDER

D.Kosalai and *M.Chandran
Research Scholar and Associate Professor,
Department of Zoology,
Thiruvalluvar University, Vellore-632 115, Tamilnadu, India.

Abstract: Cholelithiasis is most prevalent gastrointestinal diseases in Indian sub-continent because of consumption of Westernization diet, sedentary lifestyle and craving for fast food culture. This disease affects all peoples irrespective of gender and ages. However, the prevalence this disease is higher in the youngster age group up to 25 due to soft drink and tea consumption, low physical inactivity, high body mass index (BMI), frequent intake of junk food in the form of sweet foods and high refined sugars, low fibre contents, high fructose intake, fast food, high fat, and low vitamin C intake. Doctors follow surgical treatments such as laparoscopic cholecystectomy and open cholecystectomy, and non-surgical treatments such as endoscopy retrograde cholangiopancreatography (ERCP), oral dissolution therapy and shock wave lithotripsyto cure this gallstone disease. All these treatments are costly and have some post-operative complications. Hence, recent times most of the peoples seek alternative and traditional medication system such as Ayurveda, Yoga, Naturopathy, Unani, Siddha, Homeopathy and Acupuncture to treat various diseases without any side effects and at low cost. Among these, Siddha system of medicine is the foremost than all other medical systems in the world, in which the plants are used in various forms and through various root of delivery system to treat the diseases. Hence, in the present study, the plant *Silybum marianum* plant leaf extract and AuNPs of *Silybum marianum* are used to test the efficacy of gallstone prevention and dissolution in a cholesterol fed mice.

Key words - Gastrointestinal diseases, *Silybum marianum*, AuNPs, Gallstone, Cholesterol

I. Introduction

Gallstones must have been known to humans for many years, since they have been found in the gallbladders of Egyptian mummies dating back to 1000 BC (Berci, 2004 and Gordon-Taylor, 1937). Gallstone disease is a common disease of biliary system and its incidence is increasing every day (Song, et al. 2015 and Teilum, et al. 1986). Each year, roughly 1 million new cases of cholesterol cholelithiasis are discovered (Everhart, 2002, Everhart, et al. 1999 and Sandler, 2002). In India its prevalence was found to be 6.12% in the adult population (Khuroo, et al. 1989). Cholecystectomy and common bile duct exploration were the gold standard treatment for patients with concomitant gallstones and CBDS (Bansal, et al. 2010 and Ye, et al. 2015). Addition to these, non-surgical treatments include dissolutions of gallstones using the potent cholesterol solvent methyl tert-butyl ether (MTBE)(Thistle, et al. 1989), and extracorporeal shock wave lithotripsy (Sauerbruch, et al. 1986). All these treatments are costly and have some post-operative complications. For examples laparoscopic procedures has bile duct injuries (Kaman, et al. 2006), especially in the hands of less experienced surgeons (Hobbs, et al. 2006). A variety of other problems, including vascular injury, retained gallstones, and abscess formation, may also be encountered (Kaman, et al. 2006, Deziel, et al. 2006 and Hjelmqvist, 2000), abdominal wall bleeding, omental bleeding, abdominal vessel injury, netnoperitoneal vessel injury, gastrointestinal perforation, bladder perforation, solid viscenal injury, gallbladder fossa bleeding, bile duct injury, bile leakage, and infection (Ponsky, 1991 and Castro, et al. 1999).). Sonography has fluid in the surgical (McAlister, 2000), concomitant vascular injury (Strasberg and Helton, 2011), bowel and major retroperitoneal vascular injury related morbidity (Giger, et al. 2011, Shamiyeh and Wayand, 2004, Nuzzo, et al. 2005 and Zha, et al. 2010). In the recent days to avoid all these complications, a novel and classical drug therapy for cholesterol gallstones is oral litholysis by the bile acid ursodeoxycholic acid (UDCA) which might be an alternative option arise in the near future. Very recent days, most of the people approach the traditional Siddha practitioners to cure the gall stone without pain and bleeding is wonderful mind change in the people because they believe that the herbal based treatment is 100% safe and cheap when compared to the surgical removal in the hospitals. The WHO has listed 20,000 medicinal plants globally to cure various human diseases in which contribution of India is 15-20% (Gupta and Chadha, 1995 and Pareek, 1996). Among these, a very few herbs have been used in traditional medicines against liver complaints and have been claimed to exert a beneficial action against hepatobiliary diseases and cholestasis, a condition in which the flow of bile from liver to the intestine is reduced or block (Spiridonov, 2012). The *Chamomilla recutita* (chamomile), *Inula helenium* (elecampain), *Taraxacum officinalis* (dandelion), *Hypericum perforatum* (John's wort), *Artemisia* sp, *Achillea millefolium* (yarrow), *Rosmarinus officinalis* (rosemary), *Chelidonium majus* are traditionally used medicinal plants to improve digestion and relieve cholestasis because of having cholagogic (promoting the flow of bile from the liver and gall bladder into the intestines) and choleretic (increasing bile production) properties (Pasechnik, 1966, Popowska, et al. 1975, Baumann, et al. 1975. Okuno, et al. 1984, Benedek, et al. 2006, Hoefler, et al.1987 and Vahlensieck, et al. 1995). The present study plant *Silybum marianum* has gallstone prevention and liver protecting properties which have been documented by many researchers because of presence of phytocompound Silymarin and Silibinin in *Silybum marianum* (Crocenzi, et al. 2000). Hence in the present investigation the goldnano particles synthesised from the leaves of plant *Silybum marianum* were used to evaluate their gallstone prevention and dissolution potentiality in cholesterol diet induced gallstone mice.

II. Materials and Method

2.1. Plant description

Silybum marianum (Milk thistle- silymarin) is a flowering herb related to the daisy and ragweed family. This plant sprouts the leaves in all months throughout the year. It is native to the Mediterranean countries such as southern Europe, southern Russia, Asia Minor, Northern Africa, North and South America and South Australia. It grows in light (sandy), medium (loamy) and heavy (clay) soils and prefers well-drained soil. It can grow in all three types of pH such as acid, neutral and basic (alkaline) soils are highly suitable and can grow in very alkaline soils. It cannot grow in the shade places. It prefers both dry and moist soil. The plant can tolerate strong winds but not maritime exposure. Some people also call it Mary thistle and holy thistle. It prefers sunny, rocky slops, fences in the vicinity of houses, train and village streets and grows up to 60 to 150 cm height. The leaves are simple, alternate (one leaf per node along the stem) and growing at the base of the plant. The edge of the leaf has blades with lobes and teeth. Each leaves end in pointy yellow spines and have large prickly-edged leaves covered with undulating white patches, and stems containing a milky juice. This plant is hermaphrodite, so both male and female reproductive organs are present in the same plant and are pollinated by Bees. Flowering months are June to September. The blossoms have thistle-like, large, reddish-purple flower heads with sharp spines grow in the ends of flower stalks. The fruit is small, hard, shiny, and grey to black colour (an achene) with a silvery pappus or fluff. Each flower can produce up to 190 seeds, averaging 6,350 seeds per plant in its lifetime. The seeds are in three different colours such as yellow, brown and beige. The seeds are wind dispersed therefore heads shatter easily, especially, the over-mature seeds. One half of the total seeds 33% may be lost or have blown away from the centre of the seed head. They may become a weed problem in neighbouring fields due to their wind dispersed nature.

2.2. Plant collection

Plant collection method, time, season, drying and extraction procedures for both the whole plant or plant parts of wild and cultivated varieties are very important because of the quality and quantity of the active constituents of these plants vary greatly with the time of the year, the age of the plant and the method of procuring them. Thus, the therapeutic value and quality of these drugs are also largely dependent on the time of their collection and the method employed in their collection and methods of extraction. Untimely collection and the use of improper extraction methods may result in the production of substandard or even useless drugs. Hence, in the present study, the collection parts, time, season and extraction methods were carefully designed and adopted.

2.3. Plant collection site

The plant *Silybum marianum* were collected from the Ooty (short for Udhagamandalam) is a hill station (resort town) in the Western Ghats mountains in Nilgiris District, is in the southern Indian state of Tamil Nadu. Nilgiri means Blue Mountains in English. This name is given to a range of mountains spread across the borders among the states of Tamil Nadu, Karnataka and Kerala. In Ooty, this species of plants are available mostly in all places including the road side. The plant leaves study were collected from road sides of Nilgiris at altitude 2000 MSL

2.4. Method of plant collection

The wild plants that are growing on the roadsides were identified by their plant, leaf and flower structure and colour. From these plants, the leaves were collected by the hand-picking method. The collection was mostly done in the morning time at the beginning of the flowering to early fruiting. Since the photosynthetic process is most active in the plant during this period and the leaves contain the high amounts of secondary metabolites. All the collected leaves were transferred to perforated polythene bags to minimize the evaporation of moisture content from the plant and bring it to the laboratory.

2.5. Treatment after collection

The leaves of *Silybum marianum* were collected from road side plants usually contains much more amount of dust, dirty, suspended particulate matter aroused from wind and vehicles. So, certain treatments are required prior to drying. The dirty materials adhered to the leaves were completely removed with the help of a soft brush and by soaking the leaves by immersing them inside the water. Finally, the leaves were washed in running tap water followed by double distilled water.

2.6. Shade drying process

Immediately after washing, the leaves were chopped into small pieces to ensure the quick drying and spread on the newspaper under the shaded places, particularly ventilated room. This shade drying is done only to retain the natural colour, stabilize the condition of the drug and to fix their chemical constituents. Drying is also necessary to ensure good keeping quality of the drug, to prevent moulding, to stop enzymatic hydrolysis, to discourage growth of bacteria and insects and to stop chemical and other changes in the drug. The plants drying at higher temperature may destroy the active constituents of the drug, and when dried at a lower temperature over a long period leads to enzymatic hydrolysis and other chemical reactions may take place in its constituents. So, the room temperature is an ideal temperature for the drying process.

2.7. Preparation of leaf powder

The dried leaves were kept in an electrical mixy and made into affine powder. This powder was stored in glass or plastic jar for further use.

2.8. Preparation of plant extract by Soxhlation method

The base of the thimble was plugged by glass wool. Then, the fine powder of *Silybum marianum* was filled inside the thimble of soxhlation apparatus. Thereafter, the top region of the thimble was filled by glass wool. The bottom region containing boiling flask of the soxhlate was filled with methanol. The electrical heating mantle was switched on. The extracting solvent in boiling flask was heated, and its vapors were condensed by the condenser. After sometime, the methanol extract turned into their original colour ink green indicating the extraction of phytocompounds from the leaf powder. This process was continued up to the green colour of the powder packed inside the thimble turned into pale colour. This method has some advantage when compared to any other method because of large amounts of drug can be extracted with a much smaller quantity of solvent.

2.9. Preparation of 0.5mM stock gold chloride solution

17mg of Auricchloride was taken in a 500 ml of volumetric flask. Along with 200ml of double distilled water was added. The total volume of the solution was made up to 500ml to yield 0.5mM chloride solution.

2.10. Synthesis of AuNPs of *Silybum marianum*

Different concentrations of *Silybum marianum* extracts such as 1.0mL,0.2mL, 0.3mL, 0.4mL, 0.5mL, 0.6mL, 0.7mL,0.8mL,0.9mL and 1.0mL was taken separately in separate container. In each test tube, 10mL of 0.01M of aqueous gold

chloride solution was added and kept at room temperature. In each test tube the colour of the solutions was changed. In this way, different colours such as red, brown, yellow and purple were formed in different test tubes as the particle size changes. The solution containing the size range below 100 nm are considered as green synthesized *Silybum marianum* nanoparticles or nanogold. The bio-reduction of Auric chloride was scrutinized by analysing the coloured mixture by UV-Visible spectroscopy (Hitachi - Inkarp 2300 SICAN, Japan).

2.9. Purification of AuNPs of *Silybum marianum*

The gold nanoparticles of *Silybum marianum* might sometimes contain few unwanted debris from plant materials. For the purification of gold nanoparticles, they were centrifuged by using refrigerated high-speed centrifuge (Kubota 6500, Japan) at 17000 rpm 4ºC for 20 minutes. The AuNPs pellets formed were separated and re-suspended in fresh deionized water and centrifuged thrice to remove the undesirable excess of phytochemicals. Supernatant remaining after centrifuge was lyophilized and stored at 4◦C for further study.

2.10. Characterization study on AuNPs of *Silybum marianum*

The formation of nanoparticles was simply confirmed by absorbance at 720nm using UV–Vis spectrometer (Hitachi Incarp 2300 SICAN, Japan) with distilled water as a reference. The elemental composition of AuNPs of *Silybum maraianum* were tested by Energy dispersive X-ray spectroscopy (EDX) analysis in SEM. High resolution three-dimensional topography AuNPs of *Silybum marianum* were analysed by AFM study. The morphological features of AuNPs of *Silybum marianum* were analysed by using SEM –Scanning Electron Microscope instrument ZeissEVO18 (INCA Oxford Instruments). The shape and morphology of AuNPs of *Silybum marianum* was characterized by TEM- SEAD analysis using an instrument Tecnai G2 20 Twin (FEI).Bio- reduction of chloroauric acid (HAuCl4) by leaf extract *Silybum marianum* confirmed by the analysis of XRD pattern of the green synthesized AuNPs by using the XRDPowder X-ray Diffractometer (D8 Advanced Bruker Axs GmbH, Karlsrute, Germany). The stability of AuNPs were confirmed by analysing Zeta potential of gold nanoparticles synthesized from plant *Silybum marainum* by an instrument nano-partica SZ-100 for Windows (Z Type) (Series nano-particle analyzer) (HORIBA Scientific Copany).

2.11. Experimental design for In-Vivo study

The healthy male mouse *Mus musculus* were obtained from Sunday market, Madurai, Tamilnadu, India. All these mice are wild type; therefore, bring them to rearing in the laboratory. The healthy mice approximate ages 8-12 weeks with body weight 20-25g were housed in ventilated metal cages on a 12 :12 12 hours light/dark cycle (Lights on at 0600 hours) with 4 mice per cage. A conventional rodent diet and water were provided. For this 58 study, totally 30 mice were reared. All 60 animals were divided into five groups. Each group contain 6 animals were housed in separate cage. They are, Group I: Normal control group contain 6 animals were reared for 30 days without any treatment with normal diet. Group II: Negative control group contain 6 animals fed with cholesterol + cholic acids shortly referred as along with normal diet for 60 days (shortly denoted as CD+CA group). Group III: Treated group contain 6 animals fed with normal diet+ cholesterol+ cholic acid + extract of *Silybum marianum* mixture for 60days (CD+CA+SMLE group). Group IV: Treated group contain 6 animals fed with normal diet+ cholesterol+ cholic acid+AuNPs of *Silybum marianum* mixture for 60days (CD+CA+GSAuNPs group). Group V: Treated group contain 6 animals fed with normal diet+ cholesterol+ cholic acid+ ursodeoxycholic acid (A standard gallstone cure drugs) mixture for 60days (CD+CA+UA). After 60 days rearing, all mice were anesthetised by inhalation of 5% isoflurane until their muscular tonus relaxed. Gall bladder along with liver were dissected from one animal in each group and transferred to a standard fixative called bouins solution for histological studies. In order to assess the blood parameters, in the animals of all groups, periodically i.e., 12 days once, the blood samples were collected via cardiac puncture in heparinized screw vial and stored for biochemical analysis. All the experiments were conducted in accordance with guidelines approved by IAEC (Institute Animal Ethical Committee) of Biomedical Research Unit and Lab Animal Center (BEUCAC), Savitha Institute of Medical & Technical Sciences, Chennai-600077.

2.12. Lipid profile

The lipid profile such as Triglyceride, total cholesterol, HDL, LDL and VLDL in the blood serum of control and experimental mice were estimated by using appropriate cholesterol kit and the basis of PEG/CHOD-PAP method.

III. Results and discussion

Gallstone disease is high prevalence among the world populations. Gallstone disease remains as one of the major causes of abdominal morbidity and mortality throughout the world (Johnston and Kaplan, 1993). Currently, it is a frequent problem in developed countries, representing a major health problem (Shaffer, 2005). The frequency rates reported among adult whites in Europe and the United States has varied between 7.5 and 22 % but this disease is uncommon in the Negro (Curb, 1940, Jaffe, 1933 and Ludlow, 1937). Maki (1961) and Kleeberg (1960) found that the disease to be rare among the Chinese and the Japanese. Cholelithiasis represents a significant problem for the health system in both developed and developing societies. It is affecting 10% to 15% of the adult population, corresponding to 20 to 25 million Americans (Pierre, et al. 2019, Schirmer, et al. 2005, Everhart, et al. 1999 and Tazuma, 2006). The prevalence of gall bladder stones varrey widely in different parts of the world. Gallstone disease (GSD) is one of the most common biliary tract disorders worldwide. The prevalence, however, varies from 5.9–21.9% in Western society to 3.1– 112 10.7% in Asia (Chaung, et al. 2013). In India it is estimated to be around 4% whereas in western world it is 10% (Tandon, 2012). Particularly, in India, out of 800 million adult populations, approximately 15% (120 millions) have gallstones. Asymptomatic gallstone disease has a benign natural course; the progression of asymptomatic to symptomatic is relatively low with about 2% become symptomatic each year (Attili, et al. 1995). The highest incidence rates of gall bladder stone in the world are 21.5/100 000 in females in Delhi, 13.8/100 000 in Karachi and 12.9 /100 000 in Quito (Randi, et al. 2006).

Both the surgical and non- surgical treatment followed till date in allopathic medicines to remove gallstone is life threatening in long time because of their lot of post operative complications. Hence, the researcher turned their research towards ancient peoples following plant based traditional treatment to lash out the secrets of curability hidden in the plants. Therefore, traditional, complementary and alternative medicines (referred to hereafter as “traditional medicine”) are commonly used to treat or prevent disease and chronic illness and to improve quality of life. Some evidence points out the presence of promising therapeutic potential in plants (Secretariat- Fifty-Sixth World Health Assembly, 2003). The composition and preparation details for those traditional medicines are not literally documented they are transmitted generation by generation from the ancestors

through oral communication or guru-parambara system (Chandran, et al. 2016). In that way, from the prehistoric period, worldwide many plants were used to cure the gallbladder stone diseases. Turmeric root (Curcuma longa) has a long history as a digestive aid and choleretic in Asian, Ayurvedic, and Western herbal medicines (BDM, 1994 and Blumenthal, et al. 2000). Oregon grape (*Mehonia aquifolium*) is recommended by some herbalists for gallbladder disease (Fetrow and Avila, 2001 and Tierra, 1992). In Chinese folklore, an herb called 'coin grass' was discovered to have gallstone-dissolving properties (Lu, 1991).The black radish is used for treating different problems associated with the gastrointestinal, hepatic and biliary systems in many Asian and African regions (Vargas, et al. 1999). The present research has been carried out to evaluate gold nanoparticles of plant *Silybum marianum* on gallstone dissolution and prevention of gall stone in the high cholesterol diet fed gallstone induced mice. Naturally, the plant *Silybum marianum* has liver protection activities along with gallstone inhibition activities and this was documented by several researchers through many researches on hepatoprotectivity potential of *Silybum marianum*.

The application of nanotechnology for medical purposes has been termed nanomedicine and is defined as the use of nanomaterials for diagnosis, monitoring, control, prevention and treatment of diseases (Tinkle, et al. 2014). The use of nanotechnology in the development of new medicines is now part of our research and in the European Union (EU) it has been recognized as a Key Enabling Technology, capable of providing new and innovative medical solution to address unmet medical needs (Bleeker, et al. 2013, Ossa, 2014, Tinkle, et al. 2014 and Pita, et al. 2016). Hence in the present study, the gold nanoparticles of *Silybum marianum* were synthesized. This green synthesis of AuNPs was confirmed by made a characterization study on it and their medicinal values were evaluated by conducting experimental studies on mice.

Energy dispersive X-ray spectroscopy (EDX) is a standard method is used to identifying and quantifying elemental compositions in a very small sample of material as small as a few cubic micrometers. EDX analysis of AuNPs of Sylibum marianum leaf extract showed a strong gold peak (Au) confirms the presence of elemental gold. The metallic peak of gold in EDX analysis is present at 2.1KeV. In EDX analysis AuNPs of Sylibum marianum, Au appeared as a mixed compound along with Br Na, Ca, O and C in the reaction medium. SEM-EDX analysis of leaf extract of Ziziphus zizyphus confirmed that the NPs are primarily composed of gold (Aljabali, et al. 2018). Leaf extract of AuNPs of of Adiantum capillus veneris has a strong and clear peak indicates the presence of gold atoms and the weaker signals indicates the presence of oxygen (O), manganese, potassium (K), chloride (Cl), and calcium atoms (Satabdi Rautray and Uusha Rajananthini, 2020). Gold nanoparticles AuNPs of extract of Papaver somniferum possessed spherical shape with average particle size of about 77nm (Wali Muhammad, et al. 2017). The SEM images of AgNPs of *Silybum marianum* showed particles agglomerated, and appear spherical in shape with mean average diameter of 25.26nm (Ayad et al. 2019). Gold nanoparticles (AuNps) of aqueous leaves extract of Acer pentapomicum showed spherical morphology with 18 -25 nm size (Khan, et al. 2018). In the present study, the SEM images of the AuNPs of *Silybum marianum* existed with diameter from 1µm to 2µm with an aggregation. They were in the nano range and with different shapes such as triangular, spherical, oval, and hexagonal and cone. But all these particles are not equally distributed and not have uniformity in their size. The Size and nature of synthesised gold nanoparticles were analysed by X-Ray Diffraction (XRD) and can provide information on unit cell dimensions. The nancrystal of gold showed distinct peaks at 38° ,43° ,65° ,78° and 82°which were indexed to (111), (200), (220), (311) and (222) respectively. These five well-defined peaks indicate the presence of the FCC crystal structure of metallic gold. The set of lattice planes were observed and compared with the reference values of Joint Committee on Powder Diffraction Standards (JCPDS: 89- 3722) These results have a strong agreement with XRD analysis gold nanoparticles of *Ziziphus zizyphus* exhibits four important peaks present in the (20–80) 2θ range. The diffraction peaks of 38.1° relates to (111), 44.5° relates to (200), 64.7° relates to (220), and 77.8° relates to (311) facets of the face center cubic (FCC) crystal lattice (Aljiabali, et al. 2018) and the same values were reported in the analysis of nanostructures of gold nanoparticles (Zuber, et al. 2016) and the planes and face-centered cubic structures of AuNPs *Citrus maxima* peel extract, (Yuan, 2017). EDX studies of AuNPs of aqueous extract of *Fagonia indica* confirmed the presence of face centered cubic structure and elemental composition of the green synthesized gold nanoparticles (Aftab Ahmad, et al. 2015). AFM study of present gold nanoparticles of *Silybum marianum* scan shows non agglomerated, monodispersed distribution with a uniform height distribution between 32.5nm 125 to 36.7nm. The samples are immobilized on the substrate which indicates a uniform coating. The shape of the particles is conical with size ranging from32.5nm to 36.7nm. These results have a strong agreement with AuNPs of leaf extract of Ziziphus zizyphus has uniform distribution and most of the particles were approximately 40–50 nm in diameter with a sphere topology (Aljabali, et al. 2018), aqueous fruit extract of Terminalia arjuna pherical exhibited spherical shape with size in the range 20–50 nm (Gobinath, et al. 2014), gold nanoparticles of mangrove derived cyanobacteria Synechococcus elongates showed cubical shape with the size 84.7 (Asmathunisha, et al. 2018). TEM analysis of AuNPs of *Silybum marianum* showed triangles, pentagons and hexagons and spheres, shape. In all these shape spherical shapes were predominantly present in the AuNPs of *Silybum marianum*. Zeta potential values are often used as a hallmark to prove the stability AuNPs. In the present investigation the zeta potential of AuNPs of *Silybum marianum* is existed as stable colloids and negatively charged because of having -17.6 mV and -22 mV which has an agreement with the results that the gold nanoparticles generally carry a negative charge. The synthesized gold nanoparticles from the plant showed negative charge and were stable at room temperature. Zeta potential showed negative charge (-11.5) which indicated that the sample is moderately stable at room temperature (Koperuncholan, 2015). Generally, the NPs are highly stable when the zeta potential values were more than 25 mV or less than –25 mV) (Hiemenz and Rajagopalan, 1997 and Fourt, 1968). The suspension of AuNPs of *Silybum marianum* is stable with no aggregation up to 8 months. The Cholelithiasis patients normally shows the following histoloigal changes such as gallbladder with cholesterol, distanded columnar cells, hyperplasia and disrupted mucosal epithelial cells (Muna Zuhair, et al. 2011). The hisological changes were observed in cholesterol diet fed mice of the present experiments are as follows, the histological images of gall bladder of cholesterol diet and cholic acid treated group-II mice showed a significant level of increase in their wall thickness and the formation of well-developed out pouching of the mucosal epithelium. The histology of gallbladder showed mucosa with cholesterol stone showing hyperplasia of the epithelial cells and disrupted mucosal epithelium with loss of continuity. All these changes are not observed in the histology of gall bladder of treated group- III, IV and V because, the treated group III and IV contain *Silybum marianum* extract and V has ursodeoxycholic acid along with cholesterol diet and cholic acid. The above findings of the present experimental studies showed a strong agreement with hyperplasia and metaplasia formed in the gallbladder of Cholelithiasis patients (Khanna, et al. 2006 and Elfving, et al. 1999). Administering a parenteral diet to

intensive care patients showed an echogenic appearance after the first 3 days due to the formation of cholesterol crystalsin the bile at the gallbladder level (Treviño-García, et al. 2008). The mucous cells may enhance stone formation by mucous hypersecretion then the stone itself will enhance metaplastic changes along with inflammation and physical injury to the epithelium also he observed (Chang, et al. 1999). The microscopic level observation of gallbladder of all choliolithiasis patients showed cholesterol crystals which is defined as microlithiasis, and it is considered to contribute to the formation of additional stones in the gallbladder (Abeysuria, et al. 2010). Increase in the cholesterol saturation index on the 14th day on a lithogenic diet, which triggers crystal formation (Van Eprecum, et al. 2006). Mice fed a diet supplemented with 1% of cholesterol and 0.5% ofcholic acid during 2 days and following periods of 1, 2, 4, 8,12, 24, and 40 weeks exhibited a prelithiasic phase characterized by a distention of 7.2 mm in average and targeted hyperplasia. (Chang, et al. 1999). In comparison with the Chow gallbladder tissue, the HC group showed an increased epithelial thickness, reachingon average 15.3 Pm (p=0.0004). The wall thickness increased significantly from 108.4 to 296.6 Pm (p = 0.0204), and an increase in nucleus size p = 0.0196 (Lopez-Reyes, 2018). In *Silybum marianum* administered mice, the deleterious effects on histological changes in the gall bladder are very meagre when compared to fat diet fed mice. This is because of the phytocompounds silymarin present in the *Silybum marianum* may protect against gallbladder stone formation by reducing cholesterol output in the bile and by expanding the bile acid pool. The herb does soften gallbladder tissues, increases bile flow, and reduces inflammation and acts as a demulcent, able to promote clearing of any stagnation in the gallbladder. Kercman (1998) reported that silymarin inhibit development of hypercholesterolemia in rats fed cholesterol-rich diet and compared this finding with that produced by probucol, related with an increase in HDL levels and decrease in liver contented of cholesterol (Kercman, et al., 1998). In the present investigation, cholesterol diet plus cholic acid treated mice (CD+CA group) showed a sharp increase of TC TG, HDL-C, LDL-C and VLDL-C were observed in the blood serum. The percentage over control increase was calculated as 138.29% for TC, 315.44% for TG, 29.64%for HDL-C, 107.37% for LDL-C and 81.63% for VLDL-C. The elevation of VLDL-C and LDL-C is closely related to excessive dietary cholesterol (Dietschy et al. 1993). A significant elevation of plasma TC, TG, VLDL-C, LDL-C and lowered HDL-C in 1% cholesterol and 0.5% cholic acid lithogenic (LG) diet fed mice (Papiya Bigoniya et al. 2014). But in CD+CA+SMLE fed groups, all these TC TG, HDL-C, LDL-C and VLDL-Clevel were sharply declined to certain extend. The percent over CD+CA group declined of all lipid profile in CD+CA+SMLE were calculated as -53.32%, 2.21%, 30.76%, 32.&^%, 32.63% and 58.37% respectively. The above findings showed a strong agreement with the findings revealed by Alkuraishy and Alwindy, that ten patients received 600mg of silymarin in the form of oral capsule once daily produce significant reduction in triglyceride, cholesterol, LDL, VLDL but significant elevation in HDL level (Alkuraishy and Alwindy, 2012). Twenty nine healthy persons received 858 mg of (ESM) 128 Extract of *Silybum marianum* seeds (ESM, milk thistle extract) daily for the period of 60 days showed a decrease of serum cholesterol and except the HDL-cholesterol because it increased to certain extant in the ESM group (Vilim Simanex, 2001). The patients having hyperlipidaemia exposed insignificant changes in cholesterol and HDL after treatment with silibinin but triglyceride and VLDL significantly decreased (Lirussi, 2004). The same declined trends were observed in all experimental groups such as CD+CA+GSAuNPs because of the presence of phytocompounds of *Silybum marianum*. The percent over CD+CA decline of TC, TG, HDL-C, LDL-C and VLDL-C were calculated as -56.28%, 13.36, - 41.70%, -36.40 and -68.10 in CD+CA+GSAuNPs received mice. This declined was further increased in CD+CA+UA received mice group when compared to CD+CA group because of the presence of standard gallstone preventing compound ursodeoxycholic acid. In the present investigation, the enzymes such as GGT,ALP, LDH, SGOT and SGPT, biological detergents such as total bilirubin and direct bilirubin, the nitrogen contain waste products such as urea and creatinine contents were analysed in the bood serum of CD+CA+SMLE, CD+CA+GSAuNPs and CD+CA+UA treated group. In these results, the urea and creatinine contents were raised to marked level in cholesterol diet plus cholic acid treated mice (CD+CA group). The American Gastroenterological Association Technical Review on the Evaluation of Liver Chemistry Tests divides test abnormalities into two patterns (Green and Flamm, 2002). Alkaline phosphatase (ALP) is produced mainly in the biliary epithelium, with some isoenzymes being produced in kidney, intestine, leukocytes, placenta, and bone. Elevated ALP gives the “cholestatic” pattern of biliary and biliary tract disease (Tetangco, et al. 2016). The origins of alkaline phosphatase are two, the liver and bone. The biochemical markers, such as serum alkaline phosphatase (ALP) and bilirubin levels, are cheaply and easily sampled and thus commonly used clinically as 129 predictors of CBDS. The serum alkaline phosphatase is persistently elevated for a long period of time, it suggests prolonged cholestasis (Peng, et al. 2009 and Periera, et al. 2000). The serum alkaline phosphatase was significantly elevated in patients with acute calcular cholecystitis with the highest level up to 250 IU/L. These results were correlated with a study done by Thapa etal in 2010; he stated that, the serum level of ALK-P was raised in patients with acute cholecystitis by 1.69+0.118 fold with significant statistical difference. But if serum level of ALK-P was more than 2.5 folds and higher than normal value, the researcher could predict common bile duct stones (Nathwani, et al. 2005 and Padda, et al. 2009). A long-term clinical observation of patients with secondary asymptomatic choledocholithiasis had abnormal GGT serum levels (Yong Mei et al. 2019). A raised GGT level has been suggested to be the most sensitive and specific indicator of CBD stones (Jovanovic, et al. 2011). Inflammation or damage to the biliary tract is signaled by an increase in alkaline phosphatase (ALK-P) which is sometimes confirmed by measurement of gamma-glutamyl-traspeptidase (GGT). Serum bilirubin levels may be elevated in both biliary tract and hepatocellular diseases (Chen, et al. 2009). In the mouse, bile acids, usually cholic acid and cholesterol, are required for dietary induction of cholesterol gallstones (Tepperman, et al. 1964 and Caldwell, et al. 1965). The mean total bilirubin concentration of nb/nb mice with gallstones (2,085±124,M) was higher than that of nb/nb mice without stones (1,727 ± 106, uM, $P < 0.05$). Both groups of nb/nb mice had significantly higher gallbladder bilirubin levels than control mice (221±15 ZM, $P < 0.001$). Total bile acids were similar in control mice (104±5.5 mM) and mice with stones (105±6.7 mM), but significantly lower in mice without stones (80.0+7.0 mM, $P < 0.001$) P (Trotman et al. 1980). SGPT, a cytosolic enzyme, and SGOT, both cytosolic and mitochondrial enzymes (transaminases) catalyze the reversible transfer of α-amine group of aspartic acid, alanine and α -keto group of ketoglutaric acid to form oxaloacetic acid, pyruvic acid and glutamic acid, respectively, via a specific electron transport system (Diehl, et al.1984 and Rej, 1978). The transaminases enzymes such as SGPT and SGOT have been used to indicate the severity in many diseases including liver disease, hepatocyte necrosis and even hepatitis (Braunshtein and Kritzmann, 1937, Zimermann, 1989, Ellis, et al. 1978 and De Ritis et al. 1965). Silymarin (SM) is a C25 containing flavonoid mixture, extracted from the *Silybum marianum* (milk thistle) plant. Today’s standardized (according to its silibinin, often called silybin, content) SM extract contains approximately 65% to 80% flavonolignans (silybin A and silybin B, isosilybin A, isosilybin B, silychristin and silydianin), with small amounts of flavonoids, and approximately 20% to 35% of fatty acids and

polyphenolic compounds possessing a range of metabolic regulatory effects (Comelli, et al. 2007). In CD+CA+SMLE and CD+CA+GSAuNPs treated groups, a considerable decline of γGT, ALP, LDH , TB, DB, SGOT and SGPT were observed because of the presence of phytocompounds of silymarin and presence of Ursodeoxycholic acid in UCD+CA+UA experimental group. In silymarin administered rat, the activity of most assayed enzymes and GSH was normalized to the level near the control group, indicating the ability of these substances to restore homeostasis. Similar results were reported with silymarin in a model of cisplatin-induced oxidative stress in liver, and against poisoning from chemicals and environmental toxins (Mansoour, et al. 2006).

Fig: 3.1. Energy dispersive analysis of gold nanoparticles of leaf extract of *Silybum marianum*

Element	Weight%	Atomic%
O K	57.23	86.91
Na K	1.38	1.46
Cl K	8.28	5.68
Ca K	2.61	1.58
Br L	3.37	1.03
Au M	27.13	3.35
Totals	100.00	

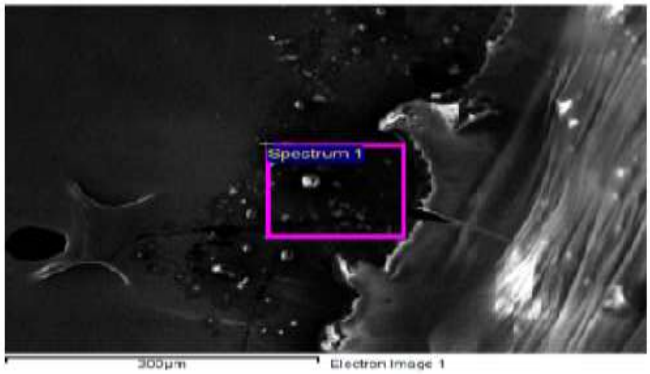


Figure: 3.2. SEM images of AuNPs of *Silybum marianum*

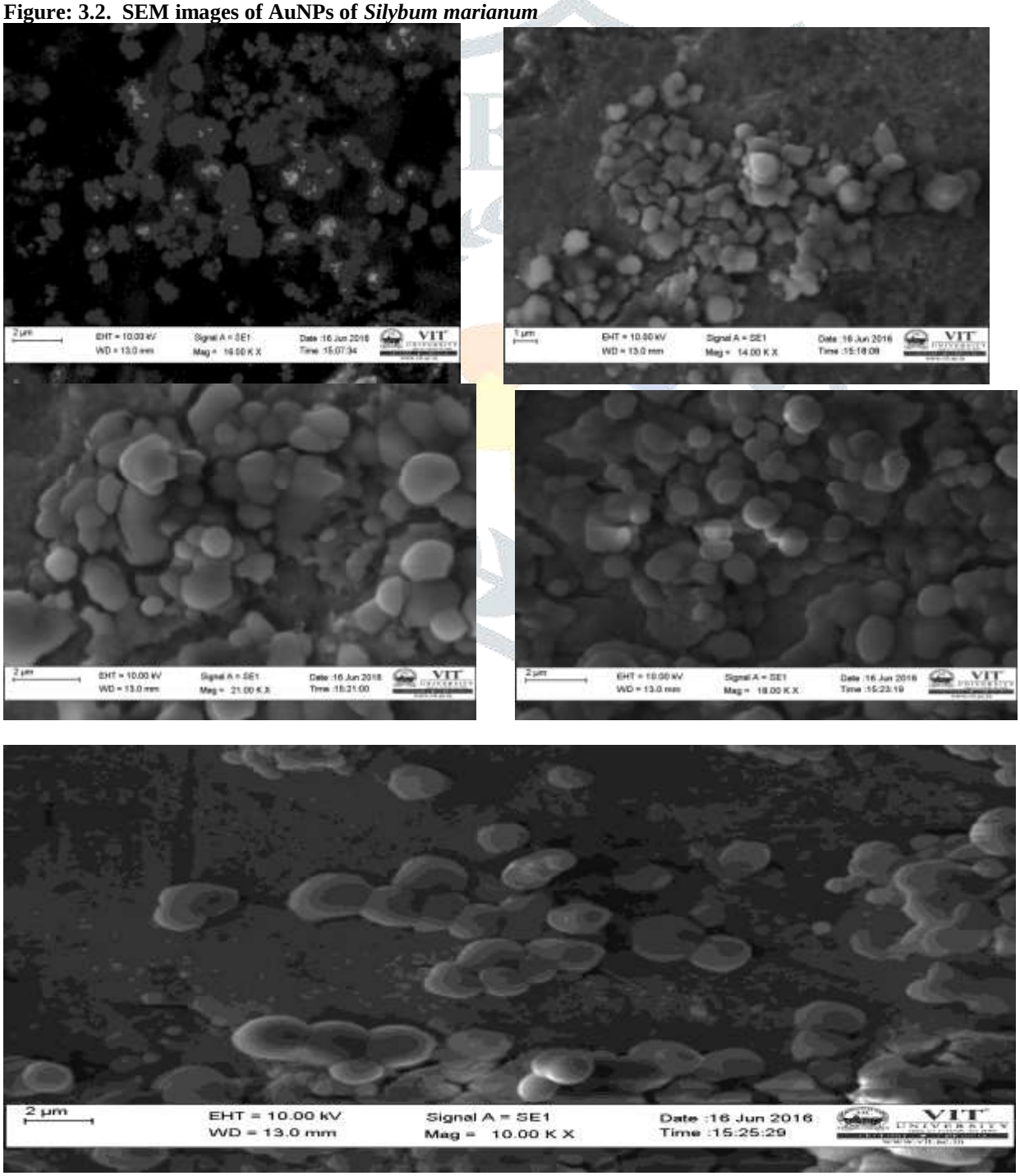


Figure:3.3. Topography of AuNPs of *Silybum marianum*

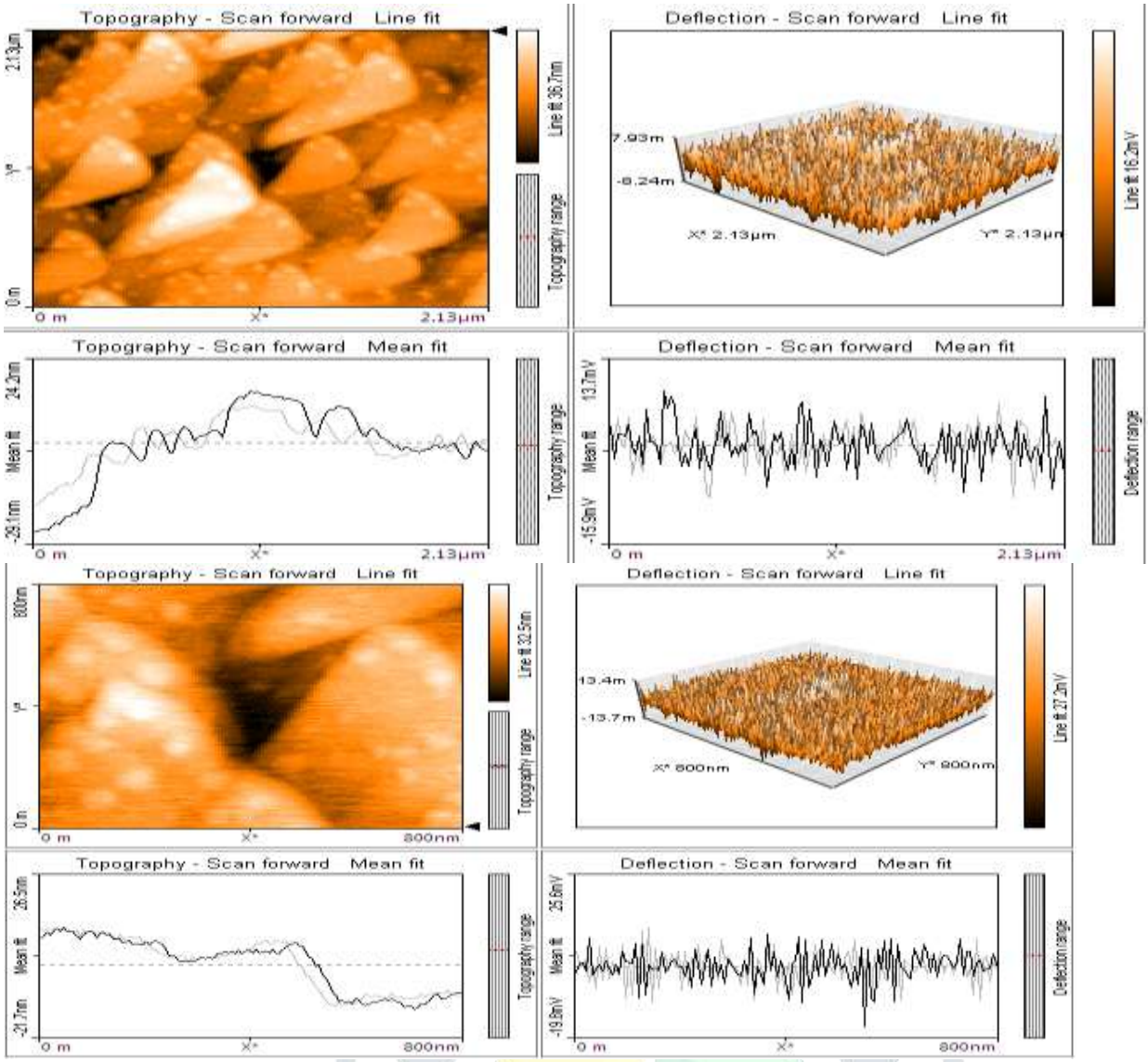
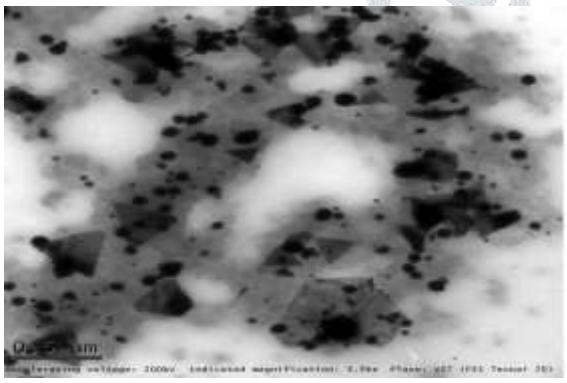
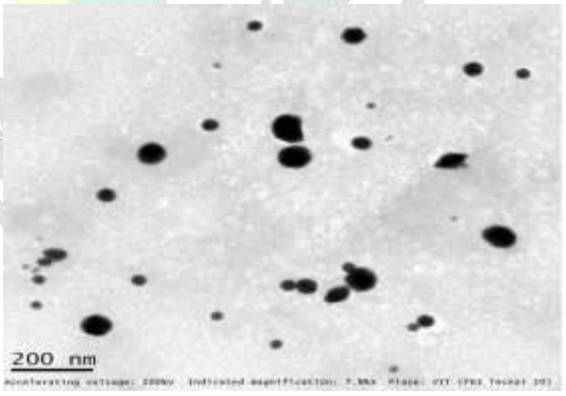


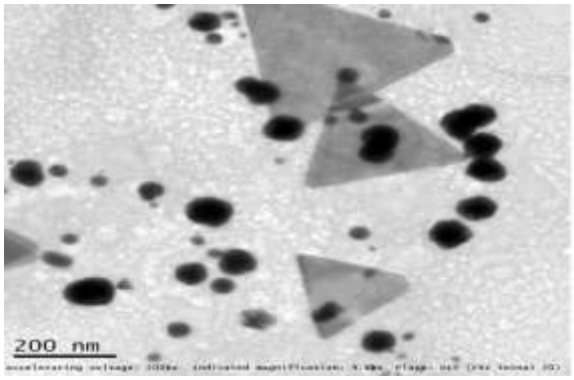
Figure: 3.4. The morphology of AuNPs of Sylibum marianum at different magnifications

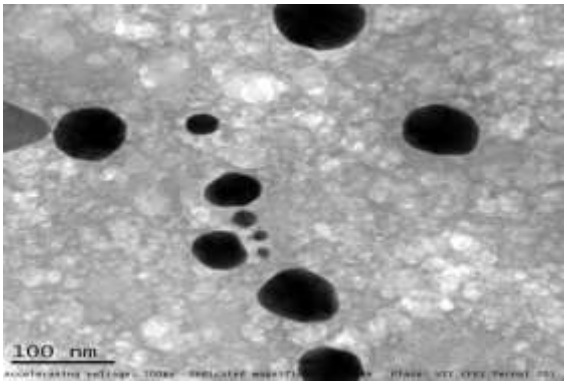
AuNPs of SM at 3.5 KX **AuNPs of SM at 7.8 KX**



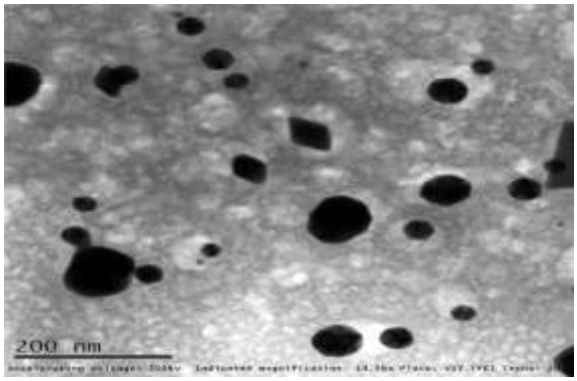


AuNPs of SM at 9.6 KX **AuNPs of SM at 11.5 KX**

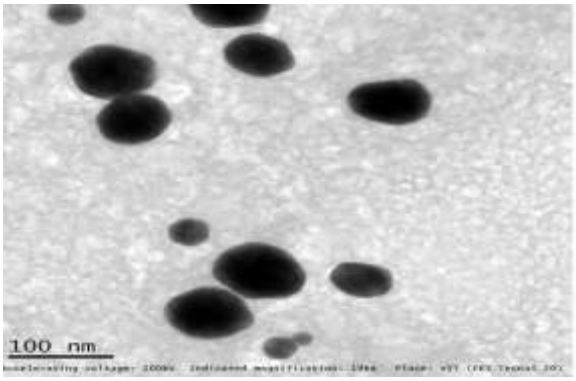




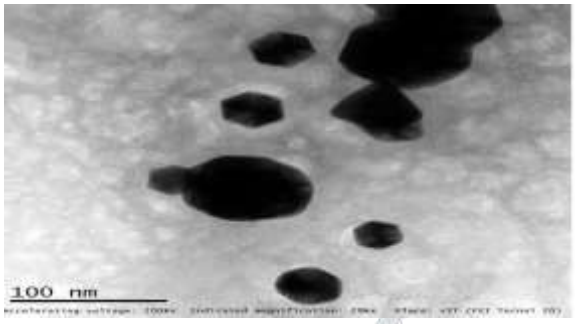
AuNPs of SM at 14.5 KX **AuNPs of SM at 19.5 KX**



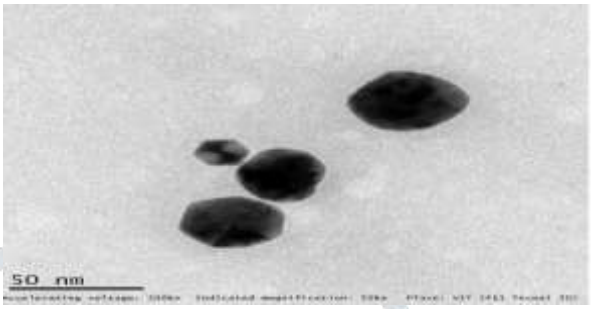
AuNPs of SM at 29 KX



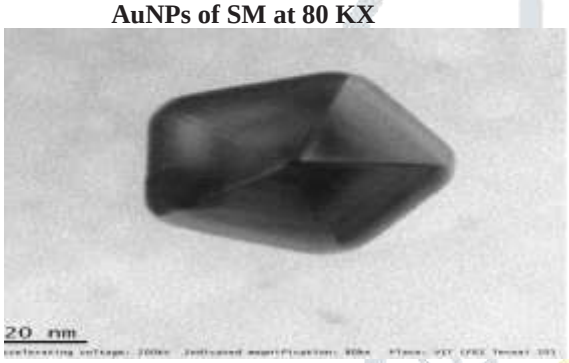
AuNPs of SM at 50 KX



AuNPs of SM at 80 KX



AuNPs of SM at 240 KX



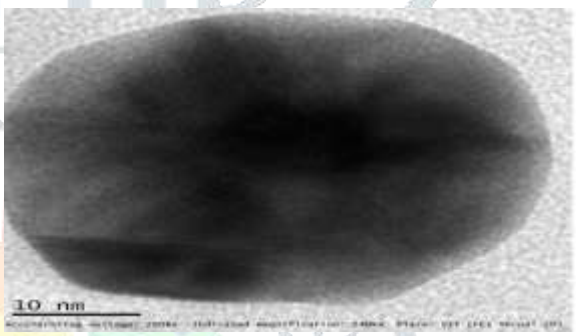


Figure: 3.5. Show the zeta potential of AuNps of *Silybum marianum*

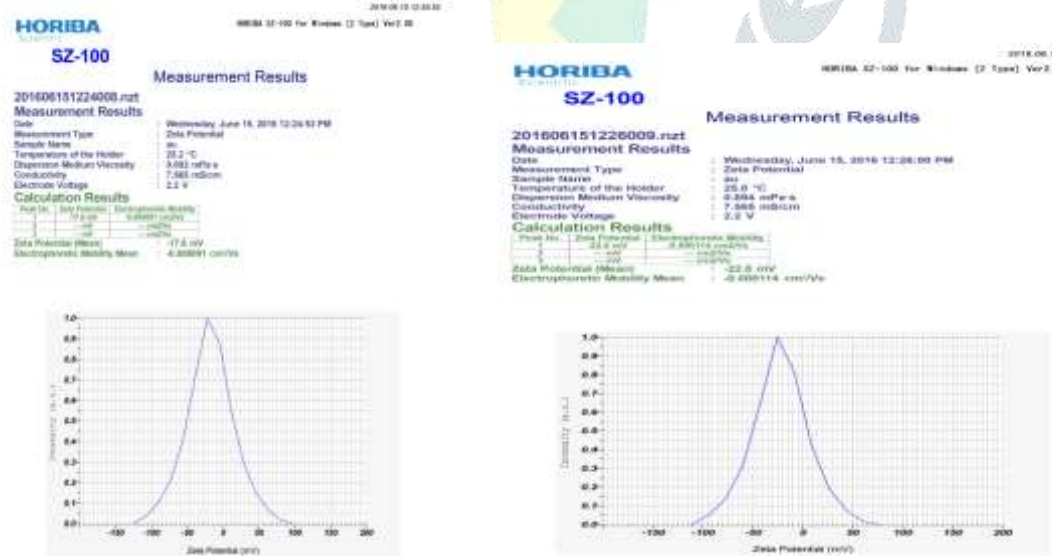


Figure: 3.6. Photomicrograph of histology of normal rodent diet fed mice gallbladder with less cholesterol stone (arrows), (Haematoxylin and eosin stained X10).

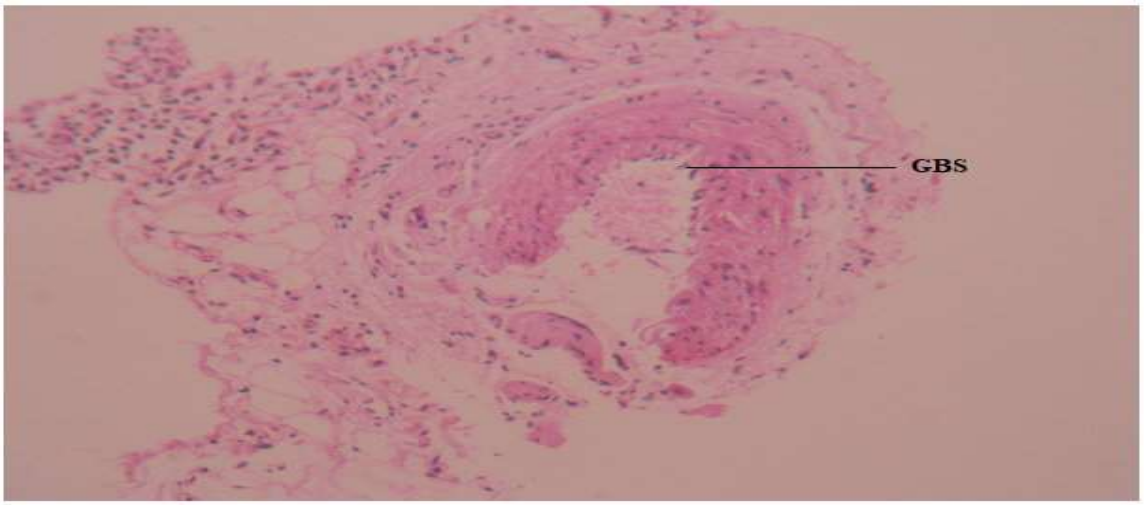


Figure: 3.7. Magnified view of fig 1.4 (Haematoxylin and eosin stained X40).

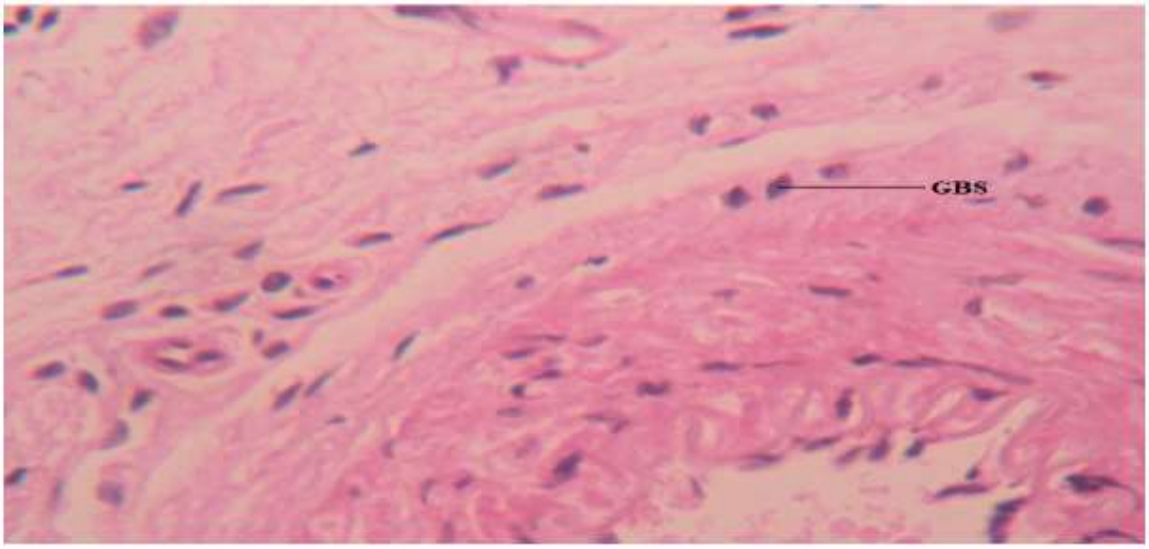
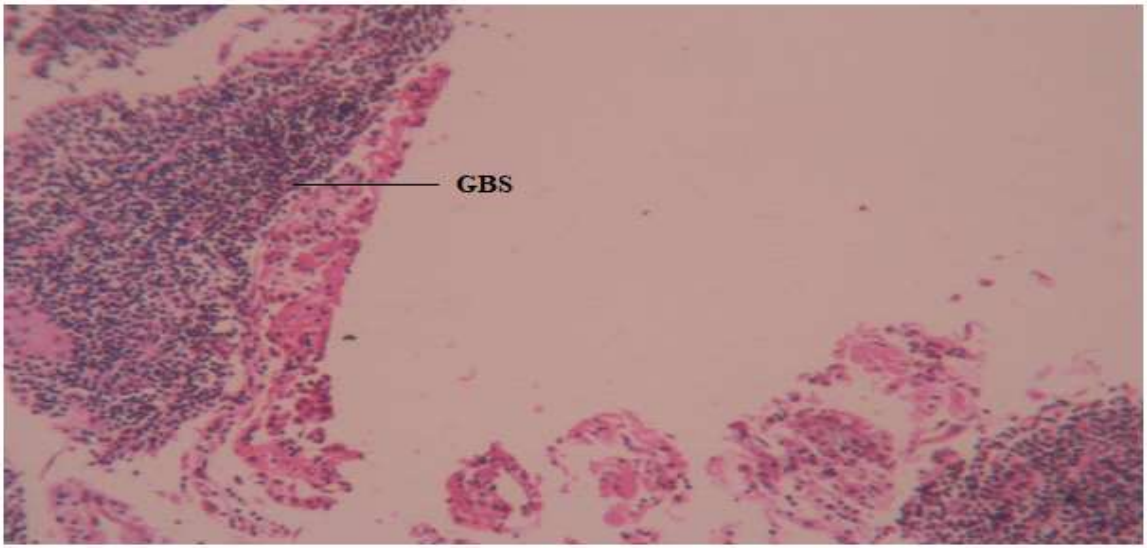
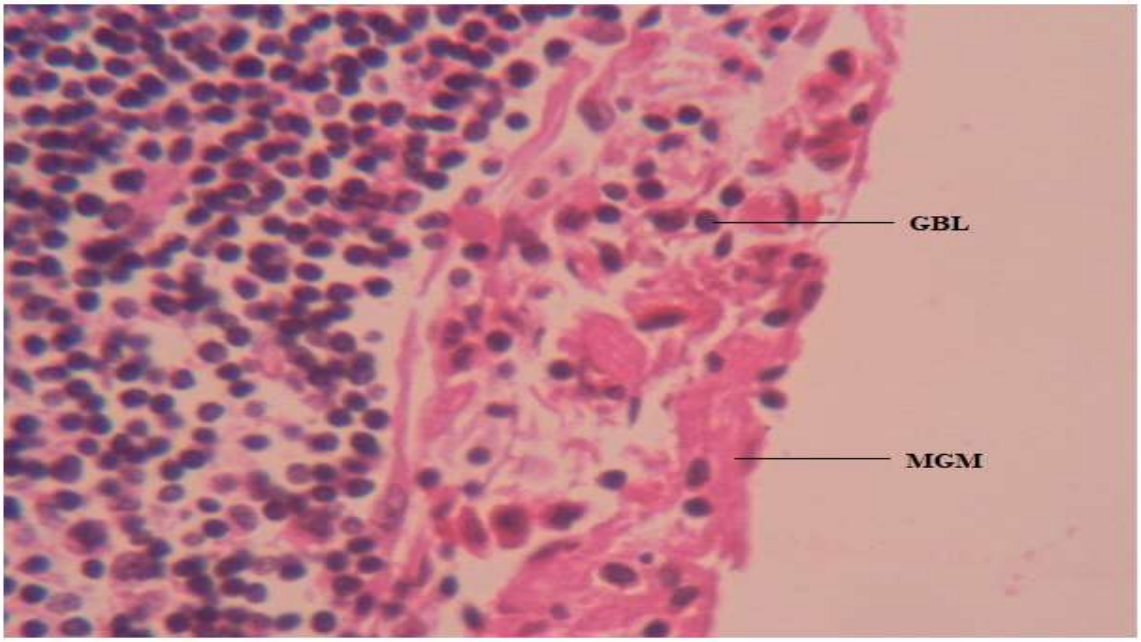


Figure: 3.8. Photomicrograph of histology of cholesterol diet and cholic acid treated mice gallbladder mucosa of with rich cholesterol stone showing a complete disruption of mucosal epithelium (arrows), (Haematoxylin and eosin stained X10).



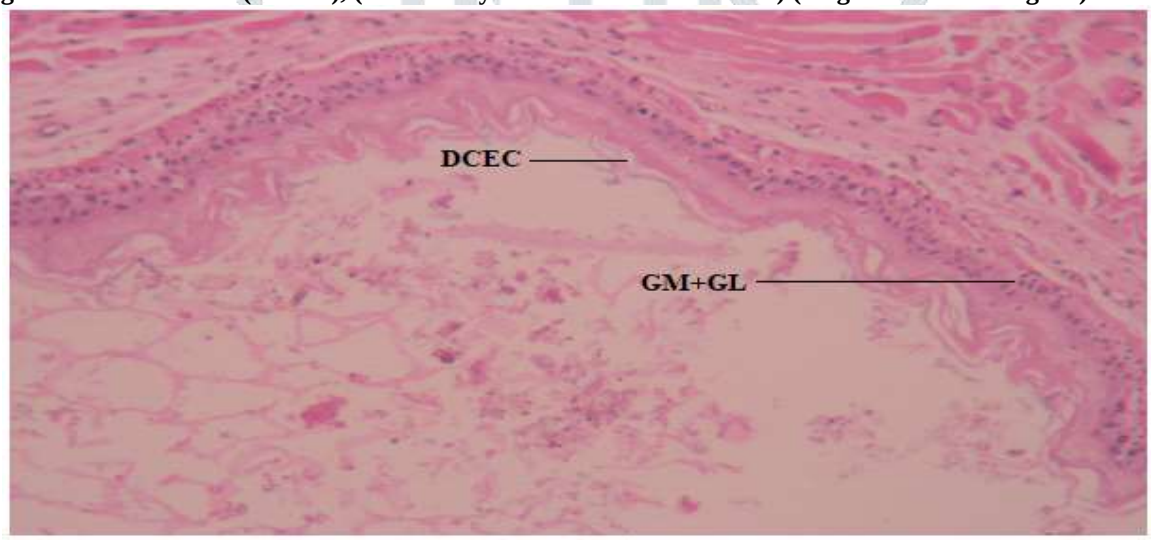
GBS-Gallbladdr Stone

Figure: 3.9. Magnified view of 1.4(X40)



GBS-Gallbladdr Stone, MGMP-Mucous Gland Metaplasia

Figure: 3.10. Photomicrograph of histology of cholesterol diet, cholic acid and *Silibum marianum* leaf extract treated mice gallbladder mucosa of with less cholesterol stone showing a slightly distended columnar epithelial cells with moderate gall stone formation (arrows), (Haematoxylin and eosin stained X40).(magnified view of fig 1.4)



DCEC-Distended Columnar Epithelial Cells

Figure. 3.11. Magnified view of 1.5(X40)

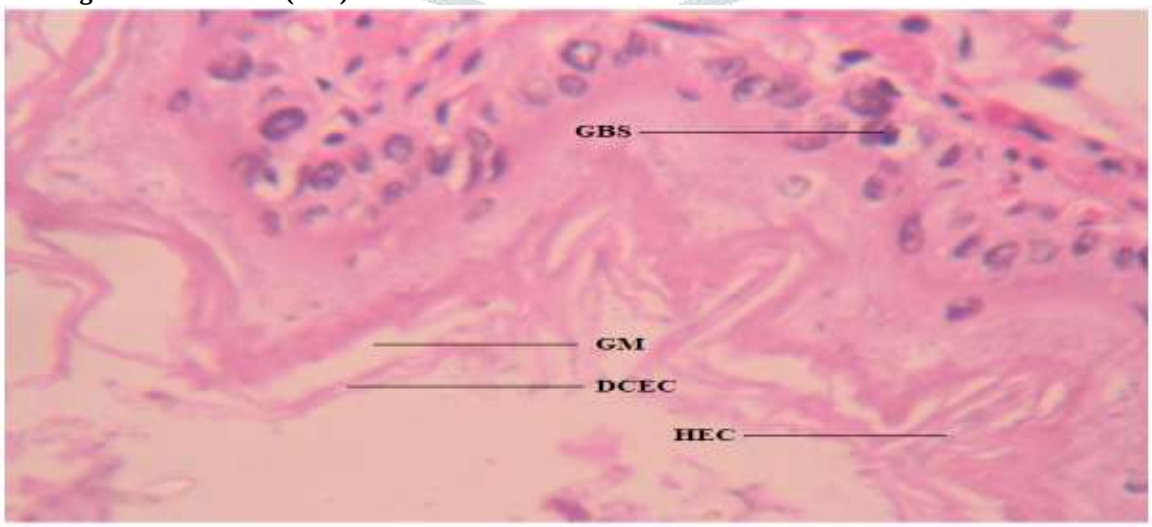
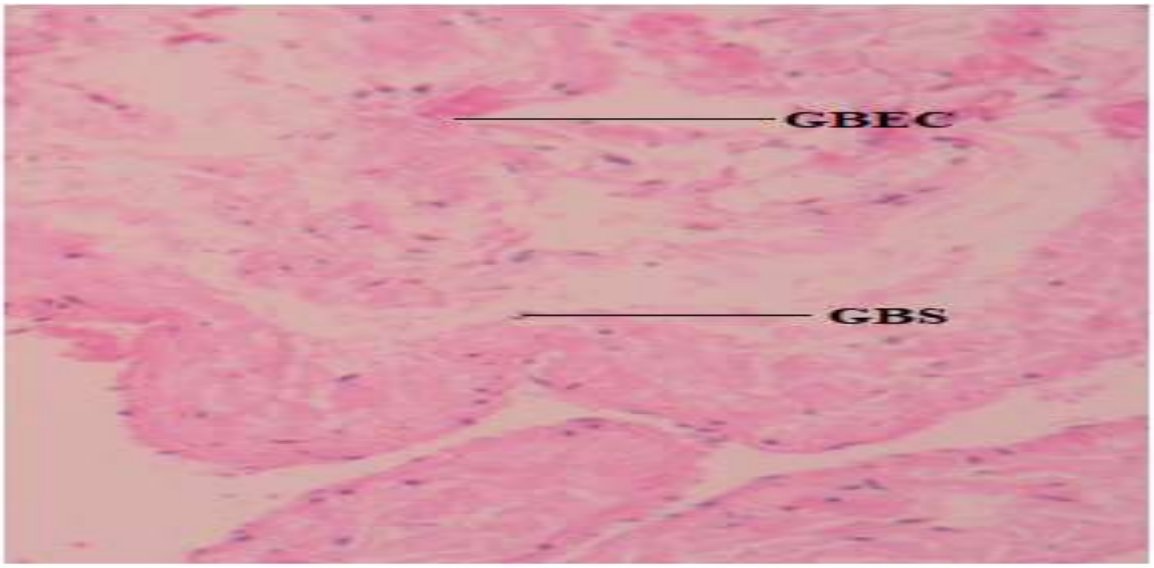
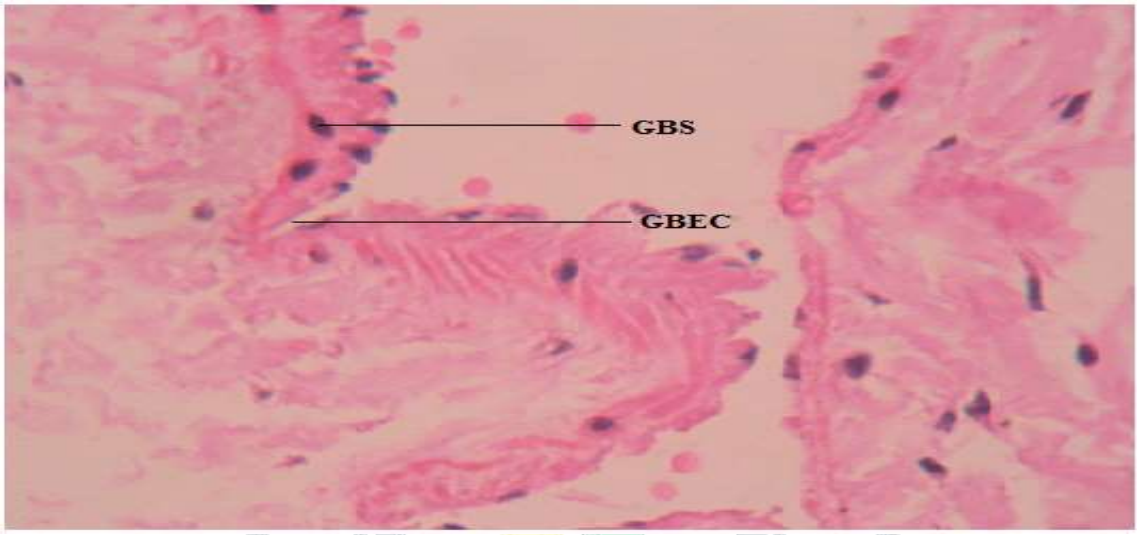


Figure: 3.12. Photomicrograph of histology of cholesterol diet, cholic acid and AuNPs of Silibum marianum leaf extract treated mice gallbladder mucosa of with less cholesterol stone showing a slightly distended columnar epithelial cells with moderate gall stone formation (arrows), (Haematoxylin and eosin stained X10).



GBEC- Gallbladdr Epithelial Cells GBS-Gallbladdr Stone

Figure: 3.13. Photomicrograph of histology of cholesterol diet, cholic acid and ursodeoxycholic acid (A standard gallstone cure drug) treated mice gallbladder with rare gall stone granules on the epithelial layer (arrows), (Haematoxylin and eosin stained X10).



GBS-Gallbladdr Stone, GBEC- Gallbladdr Epithelial Cells

Figure: 3.14. Changes in glucose and selected serum lipid profile in different CD+CA+SMLE, CD+CA+GSAuNPs and CD+CA+UAtreated mice

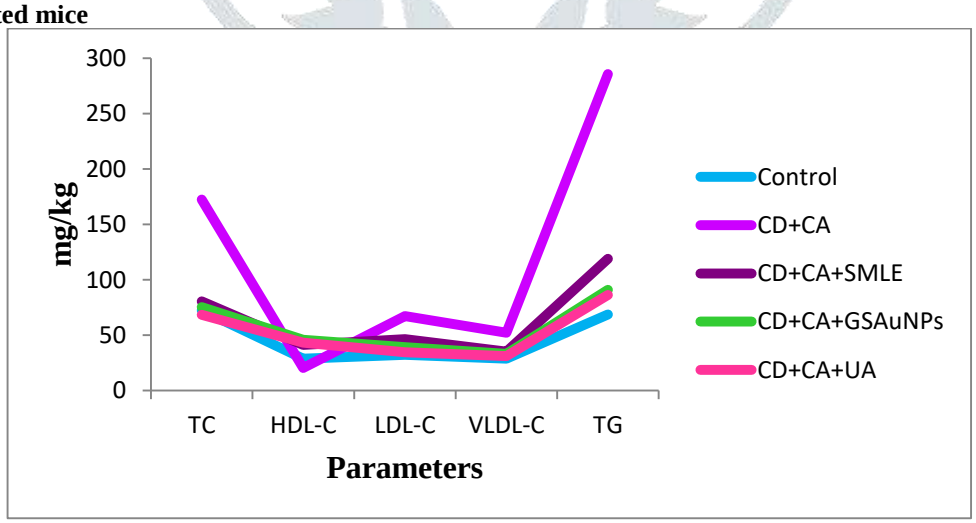
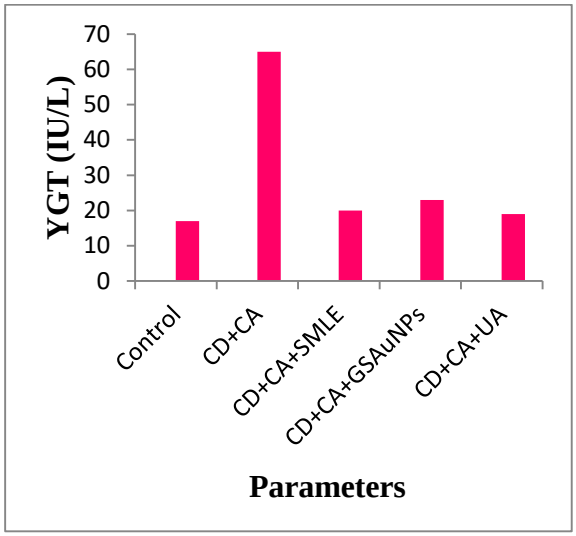
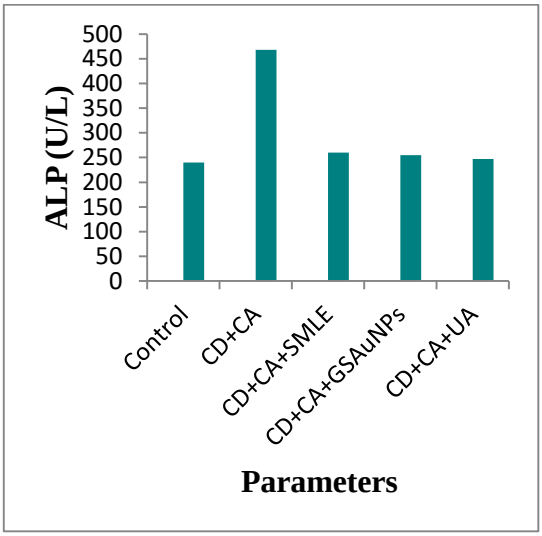
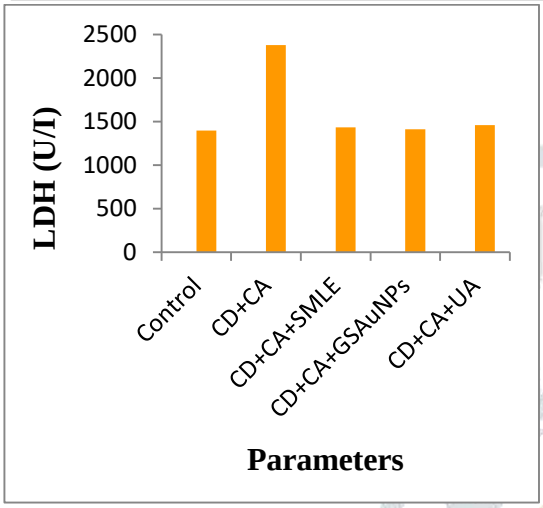
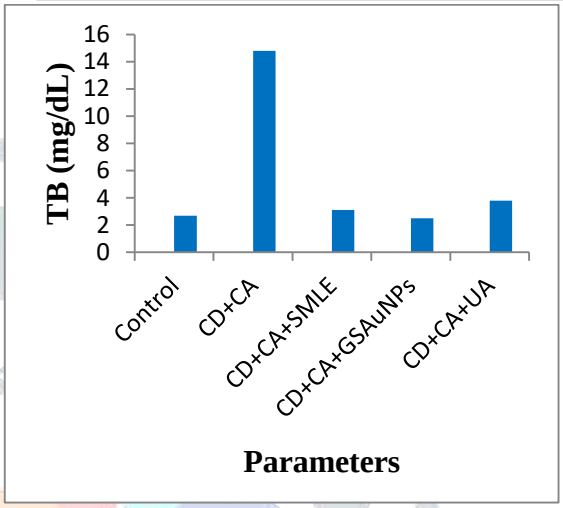


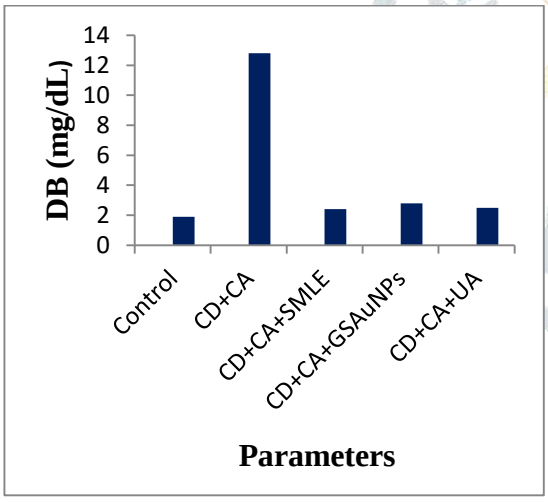
Figure: 3.15. Changes of serum γGT, ALP, LDH , TB, DB, SGOT and SGPT enzymes and TB and DB in CD+CA , CD+CA+SMLE, CD+CA+GSAuNPs and CD+CA+UA treated group of mice

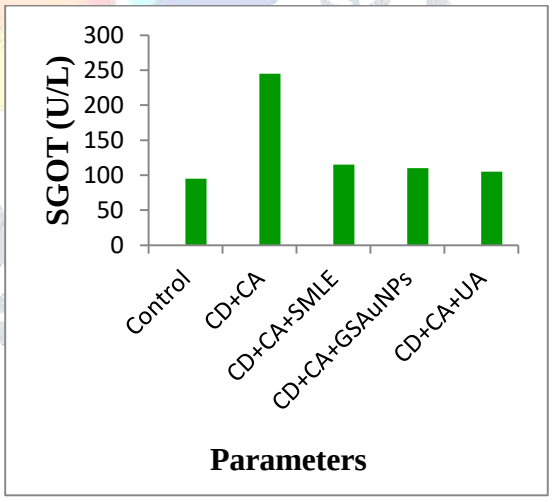

YGT (IU/L)
70
60
50
40
30
20
10
0
Control
CD+CA
CD+CA+SMLE
CD+CA+GSAuNPs
CD+CA+UA
Parameters

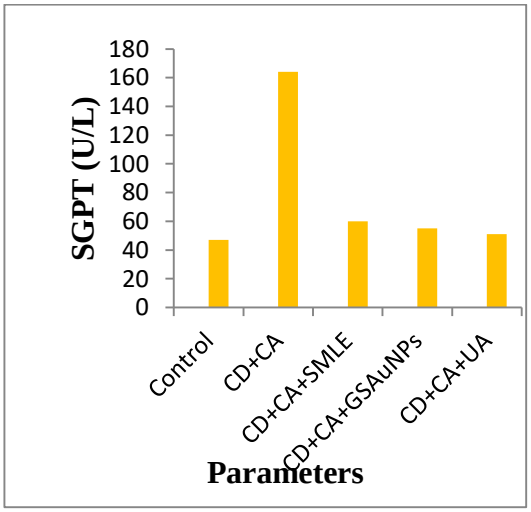

ALP (U/L)
500
450
400
350
300
250
200
150
100
50
0
Control
CD+CA
CD+CA+SMLE
CD+CA+GSAuNPs
CD+CA+UA
Parameters


LDH (U/l)
2500
2000
1500
1000
500
0
Control
CD+CA
CD+CA+SMLE
CD+CA+GSAuNPs
CD+CA+UA
Parameters


TB (mg/dL)
16
14
12
10
8
6
4
2
0
Control
CD+CA
CD+CA+SMLE
CD+CA+GSAuNPs
CD+CA+UA
Parameters


DB (mg/dL)
14
12
10
8
6
4
2
0
Control
CD+CA
CD+CA+SMLE
CD+CA+GSAuNPs
CD+CA+UA
Parameters


SGOT (U/L)
300
250
200
150
100
50
0
Control
CD+CA
CD+CA+SMLE
CD+CA+GSAuNPs
CD+CA+UA
Parameters


SGPT (U/L)
180
160
140
120
100
80
60
40
20
0
Control
CD+CA
CD+CA+SMLE
CD+CA+GSAuNPs
CD+CA+UA
Parameters

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