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In-Vitro and *In-Vivo* Hepatoprotective activity of *Abutilon indicum* L., *Alangium salvifolium* and *Broussonetia papyrifera* L. Silver Nanoparticles

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Abstract:

Current study aimed to develop eco-friendly and cost-effective approaches for silver nanoparticle (AgNPs) synthesis using ethanol extract of *Abutilon indicum* L., *Alangium salvifolium* and *Broussonetia papyrifera* L. Silver Nanoparticles and were tested for hepatoprotective activity *In-Vitro* and against paracetamol-induced hepatotoxicity in rats. *Abutilon indicum* L., *Alangium salvifolium* and *Broussonetia papyrifera* L. AgNPs having significant hepatoprotective activity by reducing carbon tetrachloride and paracetamol- induced change in biochemical parameters that was apparent by enzymatic examination and in-vivo study. The plant extracts intrude with free-revolutionary conformation, which may conclude in hepatoprotective action.

Keywords: Silver Nanoparticle, *Abutilon indicum* L., *Alangium salvifolium*, *Broussonetia papyrifera* L. Hepatoprotective.

Introduction:

The metabolism and detoxification of substances that enter the body and may induce hepatic damage, which can result in life-threatening disorders, are critical functions of the liver. Therefore, the liver's effects have been the focus of the main toxicological issues linked to a number of disorders. Hepatotoxic substances often damage liver cells by causing oxidative damage. To treat liver problems, medications of both synthetic and natural origin are available. Liver disorders have traditionally been treated using natural therapies. Due to these reasons, the recent importance of plant-based herbal treatments against drug-induced toxicity has skyrocketed.

The primary toxicological problems accompanying multiple diseases have focused liver for their effects. The liver has played a pivotal role in the detoxification of hepatotoxic compounds that severely affects liver cells and are the reason for hepatic injury and oxidative damage. Therapeutic effects of plant medicines against synthetic drug toxicity have got chief importance recently, and natural treatments have been extensively used against liver diseases.

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Nanotechnology is the term given to those areas of wisdom and engineering where marvels that take place at confines in the nano scale are utilized in the design, characterization, product and operation of accoutrements, structures, bias and systems. Although in the natural world there are numerous exemplifications of structures that live with nano confines (henceforth appertained to as the nanoscale), including essential motes within the mortal body and factors of foods, and although numerous technologies have apropos involved nanoscale structures for numerous times, it has only been in the last quarter of a century that it has been possible to laboriously and designedly modify motes and structures within this size range. The present study was designed to prepare biosynthetic silver nanoparticles (AgNPs) using the extract of *Abutilon indicum* L., *Alangium salvifolium* and *Broussonetia papyrifera* L. and evaluate their *In-vitro* and *In-vivo* Hepatoprotective activity. [1-3].

Characteristics of Nanoparticles:

Experimenters are still challenged by the task of determining the physicochemical properties of nanoparticles and exploring their structure function connections. A crucial limitation is their capability to completely probe the nanoscale realm Different characterization ways are grounded on different physical parcels, thus only furnishing a partial picture of the nanoparticle characteristics. Making matters more grueling yet, the characterization styles themselves can directly affect the measured amounts of nanoparticles [4-6].

Nanoparticles live in colorful chemical compositions ranging from micelles to essence (oxide), from synthetic polymers to large biomolecules. Each of these accoutrements features a fully different chemistry, which can be analyzed by a variety of techniques including optic spectroscopy, X-ray luminescence and absorbance, Raman spectroscopy, and solid- state NMR etc. [7].

Materials and Method:

Plants Used for Phyto-Mediated Synthesis of Nanoparticles:

In the present study the following plants have been used in the Phyto-mediated synthesis of AgNPs.

1. *Abutilon indicum* L. (Malvaceae)
2. *Alangium salvifolium* (Alangiaceae)
3. *Broussonetia papyrifera* (L.) Vent (Moraceae)

***In-vitro* hepatoprotective studies:**

Preparation of solutions:

Toxicants:

1 mg of CCL4 was dissolved in 1 ml DMSO and diluted to 100 ml with MEM 40/50 and 1%

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solutions were prepared.

Sample Solution:

10 mg of test drug was dissolved in 1 ml of DMSO and diluted to 10 ml with minimum essential medium. 50, 100 and 200 µg/ml solutions were prepared by diluting with water.

Standard:

Silymarin at a concentration of 200µg/ml was used as standard.

Cell lines used:

The cell line BRL3A used for screening hepatoprotective activity of the plant extract was obtained from National Centre for Cell Sciences, Pune, India. The description of the cell line is as follows.

Source: Rat

Strain: Buffalo

Tissue/Organ: Normal Liver

Morphology: Epithelial

The monolayer cell culture was trypsinated and the cell count was adjusted to 1.0x 10^5cells/ml using medium containing 10% new born calf serum. To each of the 96-Well Microtiter Microplates, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and different drug concentrations was added to the cells in Microtiter Microplates. The plate was then incubated at 37ºC for 3 days in 5% CO_2 atmosphere and microscopic examination was carried out and the observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50 µl of MTT was added to each well. The plates were gently shaken and incubated for 3 hours at 37ºC in 5% CO_2 atmosphere. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm.

The percentage growth inhibition and percentage cell protection were calculated using the formula;

$$\% \text{ Cell protection} = \left(\frac{\text{Mean O.D of individual test group}}{\text{Mean O.D of control group}} \right) \text{x100}$$

$$\% \text{ Growth inhibition} = 100 - \left(\frac{\text{Mean O.D of individual test group}}{\text{Mean O.D of control group}} \right) \text{x100}$$

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***In-vivo* hepatoprotective studies:**

The present study was undertaken to evaluate the *in-vivo* hepatoprotective activity of the extracts of *Abutilon indicum* L, *Alangium salvifolium* and *Broussonetia papyrifera* (L.) against Paracetamol induced hepatotoxicity.

Selection and maintenance of animals:

Healthy adult male albino rats of *Wistar* strain weighing between 180-220g were obtained from the animal house Accuprec Research Labs Pvt. Ltd. India for the screening of hepatoprotective activity of the plant extracts. The animals were housed in polypropylene cages in adequately, well-ventilated room and maintainedunder standard environmental conditions (22-28°C, 60-70% relative humidity, 12 hr. dark/light cycle). The animals were fed with standard rat feed pellets (Amrut Rat Feed, Nav Maharashtra Chakan Oil Mills Ltd., Pune) and water ad *libitum* (Aquaguard filter water). The study was approved by the institutional animal ethical committee; approval no: 1709/PO/Rc/S/13/ CPCSEA and Protocol reference no. RAL/PRO/PT/23/031-00

Preparation of the drug for the experimental study:

The extracts (Nanoparticle) and the standard drugs were administered in the form of suspension in water with 1% sodium carboxymethyl cellulose as suspending agentand orally received in a dose of 200 & 400 mg/kg b. wt.

Paracetamol (panadol®):

Tablets (500 mg) obtained from Glaxo SmithKline, Dungarvan Ltd. Ireland and orally received in a dose of 640 mg/kg b. wt.

Preparation of the standard:

Silymarin, a known hepatoprotective agent was used as the reference forcomparison at a dose of 50 mg/kg b. wt. in 0.3% CMC.

Experimental design:

Animals were divided into 09 groups comprising of 6 animals in each group. Each group received the following treatment.

Group I	:	Normal control (0.3% CMC)
Group II	:	Animals received paracetamol alone at 640 mg/kg b. wt. (p.o.) dissolved in the vehicle.
Group III	:	*Abutilon indicum* L. at a dose of 200 mg/kg b. wt. and paracetamol 640 mg/kg b. wt. (p. o.) dissolved in the vehicle.

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Group IV : *Abutilon indicum* L. at a dose of 400 mg/kg b. wt. and paracetamol 640 mg/kg b. wt. (p. o.) dissolved in the vehicle.

Group V : *Alangium salvifolium* at a dose of 200 mg/kg b. wt. and paracetamol 640 mg/kg b. wt. (p. o.) dissolved in the vehicle.

Group VI : *Alangium salvifolium* at a dose of 400 mg/kg b. wt. and paracetamol 640 mg/kg b. wt. (p. o.) dissolved in the vehicle.

Group VII : *Broussonetia papyrifera* (L.) at a dose of 200 mg/kg b. wt. and paracetamol 640 mg/kg b. wt. (p. o.) dissolved in the vehicle.

Group VII : *Broussonetia papyrifera* (L.) at a dose of 400 mg/kg b. wt. and paracetamol 640 mg/kg b. wt. (p. o.) dissolved in the vehicle.

Group IX : Animals received silymarin at a dose of 50 mg/kg b. wt. (p. o.) and Paracetamol 640 mg/kg b. wt. (p. o.) dissolved in the vehicle.

All these treatments were given orally for 14 days. On the 14th day, blood was collected from retro-orbital all animals on the same day for biochemical evaluation, then all the animals were sacrificed by cervical decapitation under light ether anesthesia. The major organs (liver, lungs, kidney, brain and heart) were collected from all these animals on the same day for histopathological estimations.

Isolation of blood serum for biochemical studies:

Collected blood was centrifuged (3000 rpm for10 min) to obtain serum. The serum was used for marker enzyme estimation. Assay of aspartate aminotransferase (AST or SGOT) Aspartate amino transferase (AST) catalyzes the following reaction.2-oxoglutarate + L- aspartate glutamate + oxaloacetate + NADH + H^+ malate + NAD^+ The rate of NADH consumption was measured photometrically at 540 nm and is directly proportional to the AST activity in the sample, AST level in serum is expressed as U/L.

Result and Discussions:

In-Vitro Hepatoprotective activity:

Hepatoprotective activity of *Abutilon indicum* L, *Alangium salvifolium* and *Broussonetia papyrifera* (L.) was examined on BRL 3A cell line. BSE exhibited CTC50 of more than 1000 µg/ml. Nontoxic dose of BSE tested for its hepatoprotective activity on BRL 3A cell line intoxicated with CCl_4 showed

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that 57.6%, 53.7% and 61.2% shielding effect by *Abutilon indicum* L, *Alangium salvifolium* and *Broussonetia papyrifera* (L.) AgNPs (200 µg/ml) respectively whereas standard silymarin at 200 µg/ml exhibited 76.3%

Table-1: *in-vitro* hepatoprotective cell %viability

Test drug	Con. µg/ml	% Viability
Normal cells	-	100
DMSO	0.25%	98
CCl_4	1%	20.5
Silymarin+ CCl_4	200	76.3
***Abutilon indicum* L**	50	10.6
	100	38.5
	200	57.6
Alangium salvifolium	50	15.9
	100	36.5
	200	53.7
Broussonetia papyrifera L.	50	16.1
	100	41.2
	200	61.2

Values are expressed as mean ±S.E.M; n=3

Significance level: P <0.05, compared to DMSO cells Significance Level: P<0.05,

In-Vivo Hepatoprotective activity:

paracetamol (640 mg/kg BW, p.o.) dissolved in the vehicle. Based on an earlier study, doses of test drug were administered to rats, whereas the Paracetamol dose was designated according to the findings of Janbaz and Gilani. All of the oral administrations were given in the morning between 09:30 am and 10:30 am and continued for 14 consecutive days. During experimentation, the rats were observed daily for any unusual behavior and death while body weight (BW).

Body Weight:

Normal body weight gain was observed in all animals on Day 1 compared to Day 14

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Clinical Signs:

Table-2: Morbidity and Mortality Check

Animal Group	Sex	Mortality up to
Group-1	Male	00/06
Group-2	Male	00/06
Group-3	Male	00/06
Group-4	Male	00/06
Group-5	Male	00/06
Group-6	Male	00/06
Group-7	Male	00/06
Group-8	Male	00/06
Group-9	Male	00/06

There was no morbidity/mortality throughout the study period.

Gross Pathology:

Table-3: Gross Pathology for animals

Group	Animal I.D	Sex	Observation: -
G-1	G1-M/001	Male	No abnormality detected
	G1-M/002	Male	No abnormality detected
	G1-M/003	Male	No abnormality detected
	G1-M/004	Male	No abnormality detected
	G1-M/005	Male	No abnormality detected
	G1-M/006	Male	No abnormality detected
G-2	G2-M/001	Male	No abnormality detected
	G2-M/002	Male	No abnormality detected
	G2-M/003	Male	No abnormality detected
	G2-M/004	Male	No abnormality detected
	G2-M/005	Male	No abnormality detected
	G2-M/006	Male	No abnormality detected
G-3	G3-M/001	Male	No abnormality detected
	G3-M/002	Male	No abnormality detected
	G3-M/003	Male	No abnormality detected
	G3-M/004	Male	No abnormality detected
	G3-M/005	Male	No abnormality detected
	G3-M/006	Male	No abnormality detected
G-4	G4-M/001	Male	No abnormality detected
	G4-M/002	Male	No abnormality detected
	G4-M/003	Male	No abnormality detected
	G4-M/004	Male	No abnormality detected

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	G4-M/005	Male	No abnormality detected
	G4-M/006	Male	No abnormality detected
G-5	G5-M/001	Male	No abnormality detected
	G5-M/002	Male	No abnormality detected
	G5-M/003	Male	No abnormality detected
	G5-M/004	Male	No abnormality detected
	G5-M/005	Male	No abnormality detected
	G5-M/006	Male	No abnormality detected
G-6	G6-M/001	Male	No abnormality detected
	G6-M/002	Male	No abnormality detected
	G6-M/003	Male	No abnormality detected
	G6-M/004	Male	No abnormality detected
	G6-M/005	Male	No abnormality detected
	G6-M/006	Male	No abnormality detected
G-7	G7-M/001	Male	No abnormality detected
	G7-M/002	Male	No abnormality detected
	G7-M/003	Male	No abnormality detected
	G7-M/004	Male	No abnormality detected
	G7-M/005	Male	No abnormality detected
	G7-M/006	Male	No abnormality detected
G-8	G8-M/001	Male	No abnormality detected
	G8-M/002	Male	No abnormality detected
	G8-M/003	Male	No abnormality detected
	G8-M/004	Male	No abnormality detected
	G8-M/005	Male	No abnormality detected
	G8-M/006	Male	No abnormality detected
G-9	G9-M/001	Male	No abnormality detected
	G9-M/002	Male	No abnormality detected
	G9-M/003	Male	No abnormality detected
	G9-M/004	Male	No abnormality detected
	G9-M/005	Male	No abnormality detected
	G9-M/006	Male	No abnormality detected

No gross lesions were observed in any of the animal of either sex, treated with test item and vehicle.

Table-4: Biochemistry of serum profile of animals

Species of Animal		**Rat (Wistar)**		**Gender of Animal: Male**			
Group	**Animal ID**	**glucose (mg/dl)**	**ALP (U/L)**	**SGOT (U/L)**	**SGPT (U/L)**	**BNU (mg/dL)**	**Creatinine (mg/dl)**
Group -I	**MEAN**	113.23	130.83	112.22	27.25	7.48	0.80
	SEM	3.04	3.35	2.65	2.33	0.52	0.05
	p-Value	N/A	N/A	N/A	N/A	N/A	N/A

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Group - II	**MEAN**	151.84	137.50	367.63	94.58	10.74	1.73
	SEM	13.29	4.20	45.12	10.29	2.03	0.10
	p-Value	NS	NS	<0.001	<0.001	NS	NS
Group - III	**MEAN**	118.33	131.49	163.86	44.63	9.66	0.80
	SEM	2.33	2.15	12.34	6.42	0.42	0.04
	p-Value	NS	NS	NS	NS	NS	NS
Group - IV	**MEAN**	151.83	178.67	130.14	42.33	11.78	0.95
	SEM	3.21	3.53	4.44	6.66	0.60	0.02
	p-Value	NS	NS	NS	NS	<0.01	NS
Group - V	**MEAN**	119.49	142.31	172.91	74.12	9.55	0.82
	SEM	1.84	1.75	15.25	10.41	0.59	0.04
	p-Value	NS	NS	NS	<0.01	NS	NS
Group-VI	**MEAN**	104.76	128.77	127.29	50.96	9.91	0.83
	SEM	1.89	2.56	8.55	5.73	0.44	0.04
	p-Value	NS	NS	NS	NS	NS	NS
Group-VII	**MEAN**	16.17	151.83	178.67	11.78	11.78	0.95
	SEM	0.19	3.74	4.00	0.58	0.58	0.02
	p-Value	<0.001	NS	NS	<0.01	<0.01	NS
Group-VIII	**MEAN**	16.11	119.49	142.31	10.52	10.52	0.81
	SEM	0.70	1.83	3.11	0.36	0.36	0.03
	p-Value	NS	NS	NS	NS	NS	NS
Group-IX	**MEAN**	14.78	104.76	128.77	9.91	9.91	0.83
	SEM	0.48	3.40	4.01	0.45	0.45	0.03
	p-Value	NS	NS	NS	NS	NS	NS

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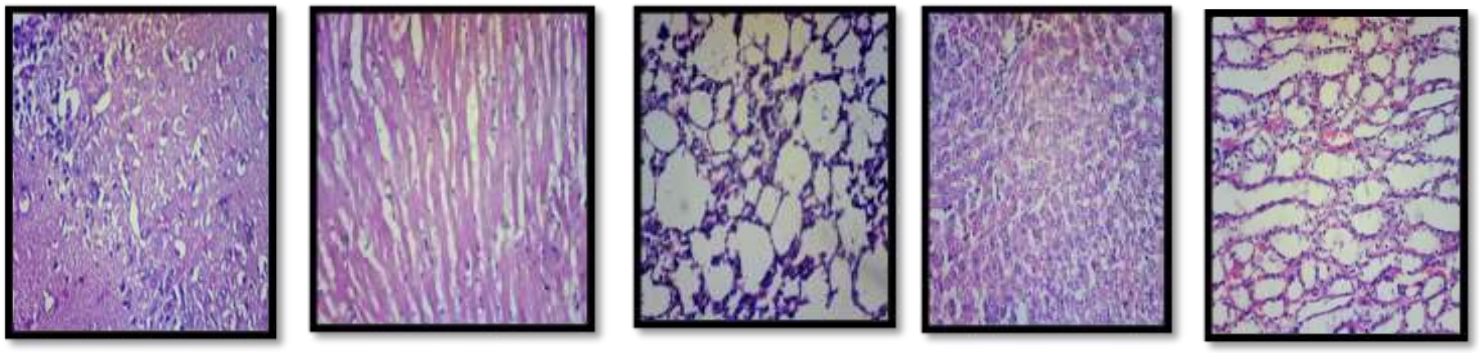
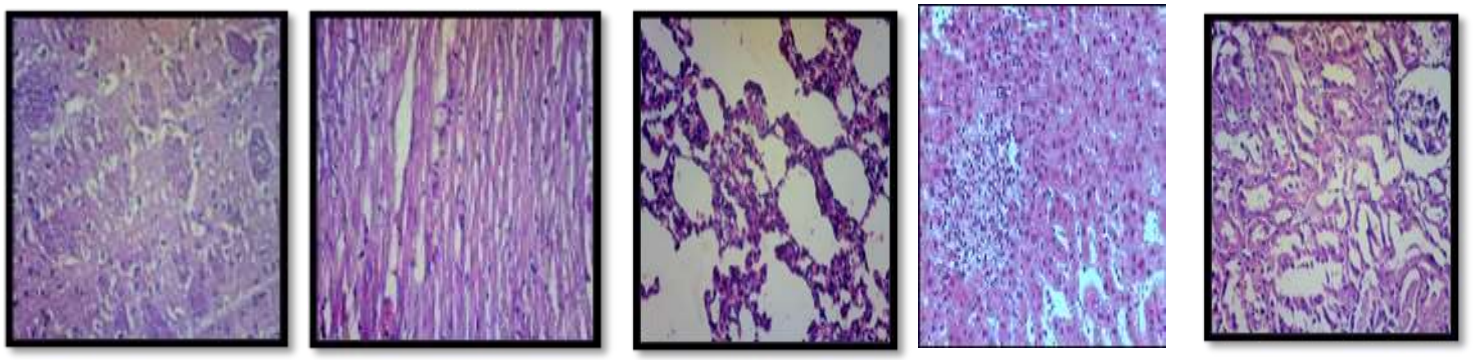
Histopathology:



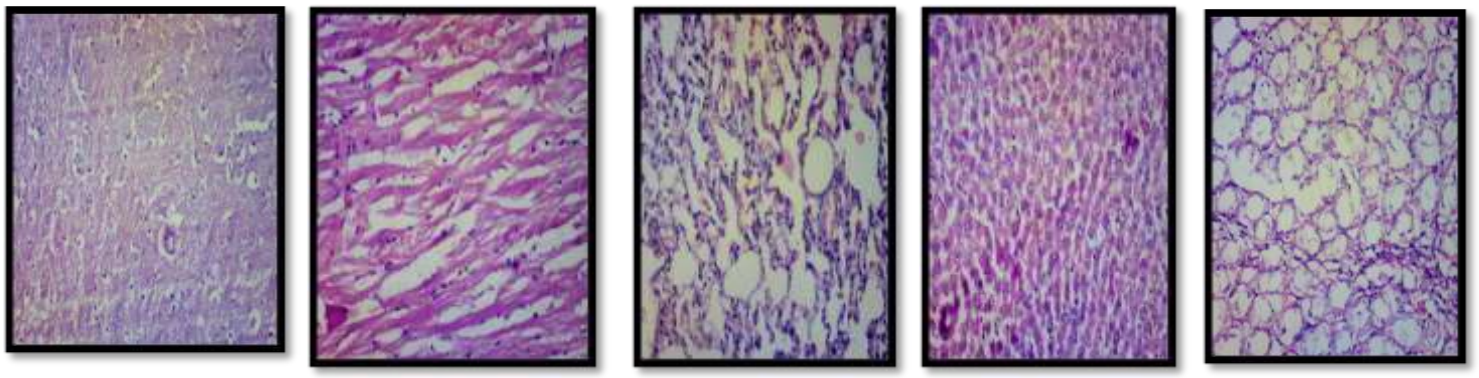
Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-1: Histopathology of Animal I.D. GP1-M1



Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-2: Histopathology of Animal I.D. GP2-M1



Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-3: Histopathology of Animal I.D. GP3-M1

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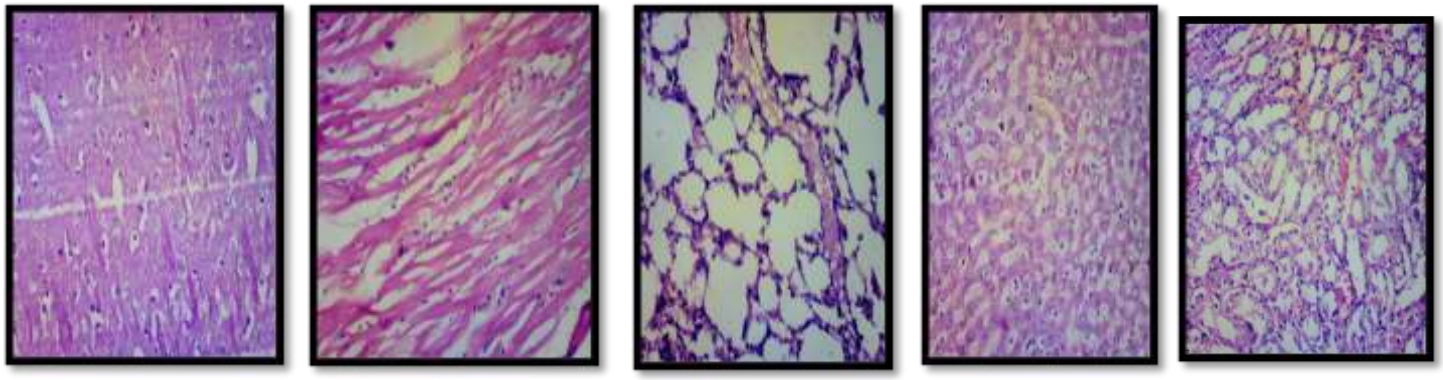
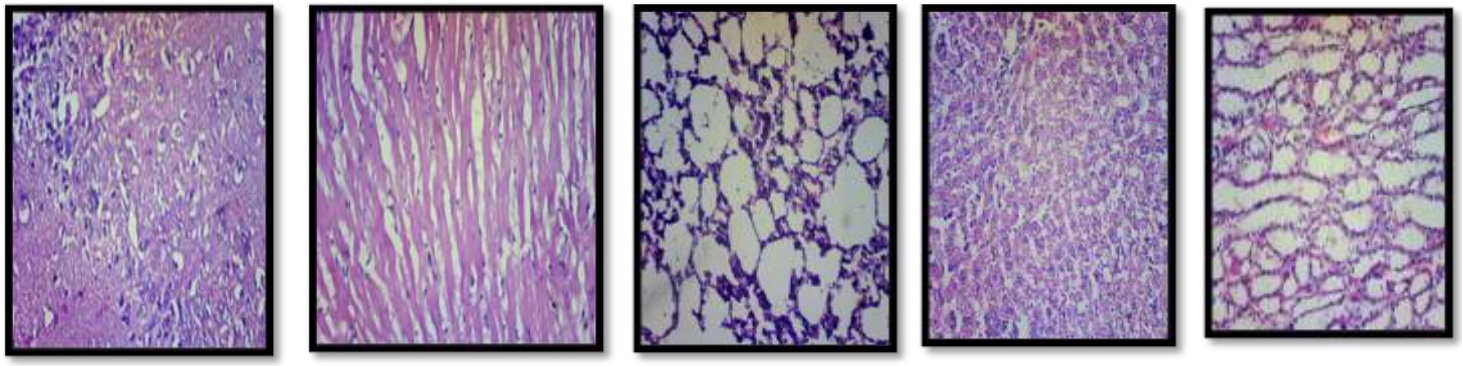


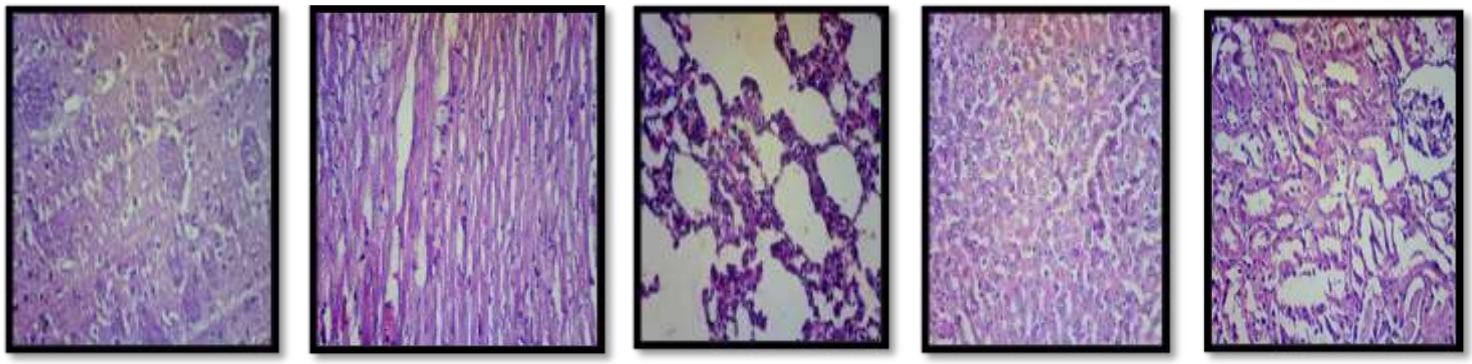
Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-4: Histopathology of Animal I.D. GP4-M1



Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-5: Histopathology of Animal I.D. GP5-M1



Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-6: Histopathology of Animal I.D. GP6-M1

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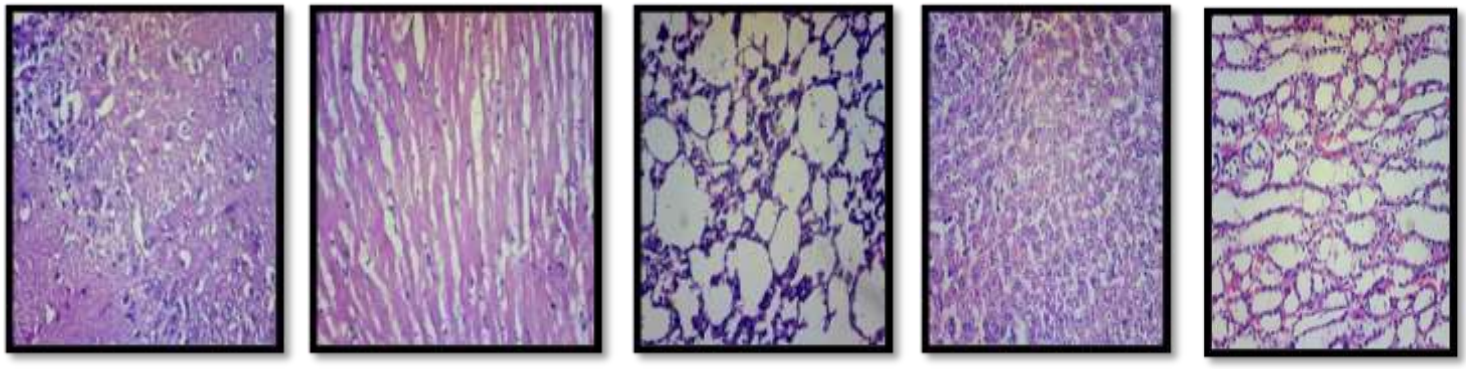
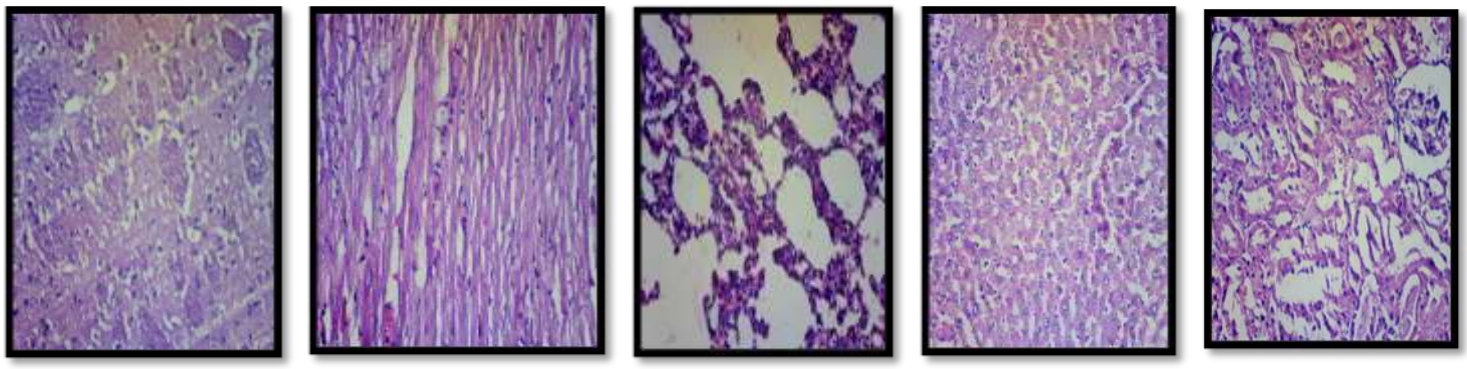


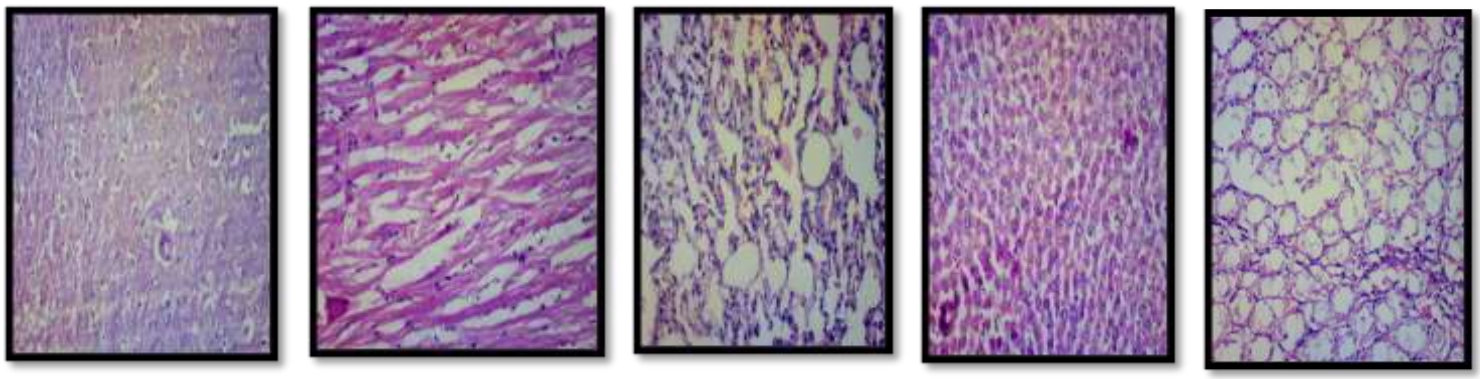
Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-7: Histopathology of Animal I.D. GP7-M1



Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-8: Histopathology of Animal I.D. GP8-M1



Brain **Heart** **Lungs** **Liver** **Kidney**

Figure-9: Histopathology of Animal I.D. GP9-M1

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Conclusion:

In the present study *Abutilon indicum* L., *Alangium salvifolium*,*Broussonetia papyrifera* (L.), were selected. As free radicals and lipid peroxidation play an important role in hepatotoxicity and depleted reduced glutathione (GSH) increases the risk of oxidative stress induced liver damage, the selected extracts AgNPs were evaluated for their *in-vitro* and *in-vivo* Hepatoprotective activity.

In the *In-Vitro* the extracts AgNPs express good hepatoprotective activity. In this study CCL_4 Induced hepatotoxicity compared with standard drug Silymarin 200 µg/ml. test nanoparticle is the exhibits 57.6%, 53.7% and 61.2% Viability.

In the present study the hepatoprotective activity of AgNPs of extracts of *Abutilon indicum* L., *Alangium salvifolium*, and *Broussonetia papyrifera* (L.), were evaluatedin PCM induced hepatic damage. The elevated levels of serum biochemical parameters like SGPT, SGOT, ALP, cholesterol, total bilirubin, and direct bilirubin were used as markers of liver damage. Treatment with lower dose and higher dose of selected AgNPS produced dose dependent reduction in PCM induced rise in biochemical parameters. Silymarin at 50 mg/kg b. wt. dose significantly prevented such rise inboth prophylactic and curative treatments. The histopathological study also supported the biochemical findings. However, the prophylactic treatment was found to be more effective than the curative treatment.

Two doses of Nanoparticle were taken for further study on PCM model. As risk of PCM induced liver toxicity, the Nanoparticle formulation was evaluated against PCM induced hepatotoxicity in rats. Nanoparticle formulation at 200 mg/kg, 400 mg/kg dose showed comparable result with standard silymarin in studies.

In *in vivo* studies Paracetamol (drug) were selected as toxicants, since they are most widely used substances and are responsible for about 30-50% of hepatic disorders. Paracetamol (PCM) produces hepatotoxicity mainly by enhancing lipid peroxidation, produced hepatic damage by free radical generation.

The hepatotoxicity of toxicants/hepatoprotective activity of the extracts (Nanoparticle) were evaluated by estimating serum SGPT, SGOT, ALP, cholesterol, total bilirubin and direct bilirubin along with histopathological examination of liver tissue.

Histopathological studies:

- In normal control group hepatic globular structure was found to be normal. No abnormality detected.
- In paracetamol treated group (toxic control) up to 80% derangement of cord, fatty and

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vacuolar degeneration, necrosis, and cellular infiltration have been observed without any regeneration.

- Groups treated with 200 and 400 mg/kg of *Abutilon indicum* L., Nanoparticleshowed less than 40% derangement of hepatic cord, fatty and vacuolar degeneration, necrosis and less than 25% cellular infiltration have been observed. Moderate to good regeneration is also observed.
- Groups treated with 200 and 400 mg/kg of *Alangium salvifolium*, Nanoparticle showed less than 40% derangement of hepatic cord, fatty and vacuolar degeneration, necrosis and less than 25% cellular infiltration have been observed. Moderate to good regeneration is also observed.
- Groups treated with 200 and 400 mg/kg of *Broussonetia papyrifera* (L.) Nanoparticle showed less than 40% derangement of hepatic cord, fatty and vacuolar degeneration, necrosis and less than 25% cellular infiltration have been observed. Moderate to good regeneration is also observed.
- Group treated with 50 mg/kg of silymarin showed less than 25% derangement of cords. Very good regeneration also observed with normal hepatic features.

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