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HEPATOPROTECTIVE ACTIVITY OF *ALLIUM CEPA* EXTRACT IN ANTITUBERCULAR DRUGS INDUCED HEPATOTOXICITY IN EXPERIMENTAL ANIMALS

Rajat Sagar[1], Praveen Kumar[2], Dr. Shamim Ahmad[3], Hemant Kumar[4] and Suraj Mandal[5]*

[1,2,3,4] Translam Institute of Pharmaceutical Education and Research, Meerut, 250001, Uttar Pradesh, India.
[5]Pt. Rajendra Prasad Smarak College of Pharmacy, Campus- Kajri Niranjanpur, Khutar Road, Puranpur, Pilibhit, 262122, Uttar Pradesh, India.

***Corresponding Author: Suraj Mandal**

Pt. Rajendra Prasad Smarak College of Pharmacy, Campus- Kajri Niranjanpur, Khutar Road, Puranpur, Pilibhit, 262122, Uttar Pradesh, India.

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ABSTRACT

The current study is an initiative to examine the hepatoprotective activity of *Allium cepa* extract in Anti-tubercular drug induced hepatotoxicity in rats. From the Two group of rats one group was given *Allium Cepa* Extract prior to giving the anti-tubercular drugs for a month time. After the completion of the study the blood of the all animals from both the groups were taken and the liver of all the animals were collected after sacrificing the animal's using euthanasia. The Liver tissues were processed for histology and the blood samples were subjected to estimate biological parameters and hematological parameters. In *in-vivo*, *Allium cepa* extract has no significant decrease in Anti-TB drug induced hepatotoxicity by not reducing the SGPT, SGOT and no significant activity has been observed in other parameters. In histological observations maximum sum score was calculated in disease control group (G2). All the treatment groups showed relatively less damage score when compared to G2 group. Based on the gross and histopathological data, *Allium cepa* extract treated was found to be well tolerated and there is no safety and toxicity concern.

KEYWORDS: *Allium cepa*, hepatoprotective activity, Anti-tubercular drug.

INTRODUCTION

The Greek word for liver is hepar, so therapeutic terms identified with liver regularly begin with hepato or hepatic. Liver is the heaviest gland of the body. Liver assumes a crucial job in digestion, emission and capacity and is a few times alluded as the "extraordinary compound processing plant" of the body in light of the fact that the body relies upon the liver to direct, incorporate, store, and emit numerous vital proteins, supplements, synthetic concoctions and to decontaminate and clear poisons or superfluous substances from the body. Arige et al. [37] Liv sicknesses are essentially brought about by poisonous synthetic compounds, overabundance utilization of liquor, contaminations, and immune system issue. Most of the liver toxic synthetics harm hepatocytes principally by instigating peroxidation of lipid & other oxidative harms. It is a restorative term for harm to the liv brought about by a drug, synthetic or dietary enhancement. Saleem et al.[42]

Structure

The weight of the liver is between 3.17 & 3.66 pounds (lb), or between 1.44 & 1.66 kg & the liver is brownish red with rubber texture. This's approximately triangular & has 2 lobes: anarrower lobe to the right and a lobe to the bottom. Glasson's capsule a fibrous tissuelayer that covers external area of the liver. The peritoneum, a membrane which forms the abdominal lining, also covers this capsule.

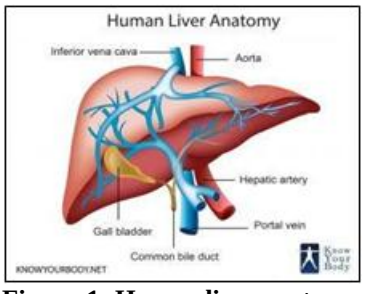


Figure-1: Human liver anatomy.

Function of liver

- The metabolism of Carbohydrates
- Fat metabolism
- The metabolism of proteins
- Drogery and hormone processing
- Bilirubin excretion
- The bile salt synthesis
- Tailoring

- Phagocyticism
- Vit D activation

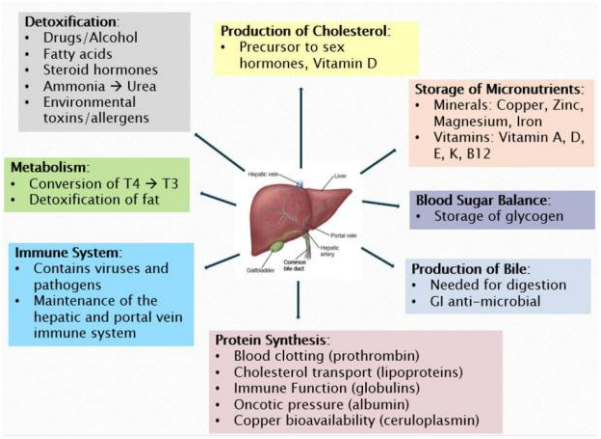


Figure-2: Liver function.

Symptoms

- Fatigue
- Tiredness
- Lost weight
- Breakouts
- To vomit
- Yellow skin discoloration (yellowish ness)

Hepatotoxicity

Hepatotoxicity, or liver harm, is brought about by hepatotoxins, which may source from synthetics, dietary supplements, pharmaceutical medications, and therapeutic plants. Eminently, various therapeutic plants are utilized to mitigate disease, especially in customary frameworks of drug, for example, Ayurveda and Traditional Chinese Drug.

These frameworks of drug have been executed for quite a long time for treating different illnesses. A few therapeutic plants fill in as hepatoprotectors against liver harm, while others initiate hepatotoxicity.

Example

These are some medicines which cause hepatotoxicity or give harm to liver

- Anti-tuberculosis drug
- Paracetamol
- Carbon tetrachloride
- Alcohol
- Thioacetamide
- D- galactosamine

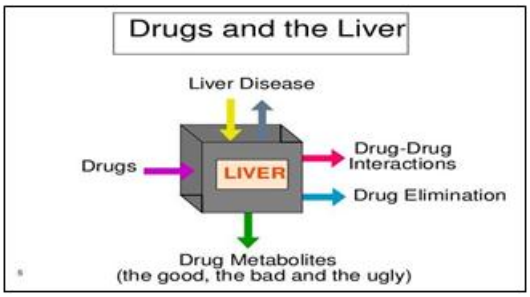


Figure 3: DRUGS & LIVER.

MATERIAL AND METHOD

Authentication of plant- The plant Allium cepa was collected from Agra, Uttar Pradesh, India in the month of May & authenticated the plant by Chaudhary Charan Singh University, under the guidance of Dr. Vijay Malik in India. We air-dried the plant to remove its moisture and this process was done for 3 days & then we grinded it to a fine powder form by using a mixer cum grinder. We then kept the powder sample in an air tight container till we required it. Approximately we extracted 80 grams of plant sample with 500 mL ethanol & water solvent by following a process called soxhlet extraction. Then, we evaporated both the solvents (ethanol and water) under decrease pressure use Rota-Vapor evaporator & we could get viscous semisolid mass. **Zarnowski et al.**[36]

Acute Toxicity Studies- The acute toxicity studies of plant ethanol / water extracts on Wistar strain rat (150-200 g) were determined, & the animals were kept under standard condition as given below. Until experiment, the animals went fasting overnight. Fixed dose methods as per OECD Guideline No. 425 method, administered by CPCSEA, were adopted for toxicity studies. The work was carried out with prior authorization from the Animal Ethics Committee of the Institute (CPCSEA, IAEC / KSOP / E/18/03 approval).The rats had been divided into five classes of six animals each.The rat test groups were provided with the dose of extracts of 200, 400 mg / kg. Carefully observe all rats for any evidence of toxicity within the first four hours, after extract administration, for regularlyafter that for 28 days **Hayes et al.**[19]

Select the animals for caring and handling: - The wistar strain rats are used with 150- 250 g of either sex. Animals were accustomed to normal husbandry condition for a period of 10 days following randomization into various classes. Room temperature: 23 ± 3°c Relative humidity: 50 ± 20% 12 hrs dark and light cycle. All the animals were given the rodent pellet diet, & under strict hygienic conditions ad- libitum was permitted for water. We acquired an ethical clearance for performing animal experiments from the Ethical Committee on Institutional Animals & we have mentioned the IAEC No. (IAEC Registration No. IAEC / xxxx / xxxxx CPCSEA, approval No.). The animals were divided into group control, grouphepatotoxic, low dose of PC, high dose of P.C, group of treatment. In all sets of experiments each consisting of 6 animals

respectively. Once regular the drug treatment was given per oral.

Acute Toxicity Studies

Each time an experimenter uses a chemical or drug or xenobiotic to a physiological system, different types of interactions are anticipated which may be a sequence of dose-related responses or idiosyncratic reactions. In most cases, the reported answers are well expected & needed, although certain outcomes are considered adverse effects which are therapeutically unnecessary. Any of the adverse effects may not be alarming and can be considered a therapeutic effect expansion of the drug but others can be very harmful in nature and even considered dangerous to patients. Testing all substances for the potential for toxicity is thus a routine protocol. The kinds of toxicity tests performed regularly by pharmaceutical manufacturers include acute, sub-acute, & chronic toxicity tests. Acute toxicity is important for calculating & identifying the LD50 dose the dose indicating lethality (causing death) in 50% of the tested animal population. **Hayers et al.**[19]

Acute oral toxicity profiling is usually an original pre-screening procedure for all compounds or product applicants or toxicity evaluation formulations **Hayes, et al.**[19]

Procedure: -For the study, random sampling was performed of wistar strain rats (200- 250 g in weight). OECD- 423 Recommendations were followed for the acute oral toxicity evaluation. The animals fasted with water overnight were administered the extract orally at a dosage rate of 5 mg / kg b.w. and the animals were examined for potential toxic signs such as behavioral changes, locomotion, seizures, and mortality for 14 days. The first 72 hours were regarded most vital, and a close observation was therefore made for the indicated signs during this time span. The sample was discovered to be non-toxic up to 2 g / kg and did not cause the animals tested to die or die.

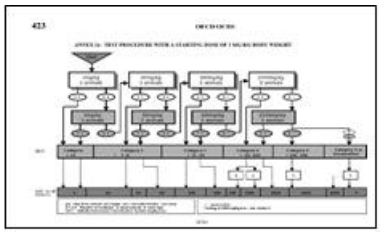


Figure: 4 OECD Guidelines.

Parameters detected

❖ **Biomedical analysis in serum**

- SGOT
- SGPT
- king and kings' method
- Serum bilirubin
- Total protein

❖ **Biochemical estimation in tissue**

- Thiobarbituric active reactive substance
- Catalase
- Superoxide dismutase
- Glutathione

❖ **Histopathology of liver shall be conducted**

Collection of Blood: - Every week of experiment blood sample was collected by retro orbital sinus collection technique in Eppendorf tubes. During the process, the animals were slightly anesthetized by inhalation of ether. The blood samples acquired were centrifuged at 3500 rpm using rime cooling centrifuge for ten minutes after being clotted for half an hour to obtain the serum. Finally, for various parameters, the samples were biochemically estimated. Huma n killing of livestock at the end and collecting their livers Animals were slightly anesthetized by inhalation of ether. The blood samples acquired were centrifuged at 3500 rpm using rime cooling centrifuge for ten minutes after beingclotted for half an hour to obtain the serum. Finally, for various parameters, the samples were biochemically estimated. Human killing of livestock at the end and collecting their livers **Sharma et al.**[6]

Figure 5: Collection the blood by retro orbital method.

Types of Antioxidant test

- Dietary antioxidant
- Natural antioxidants
- Synthetic antioxidants
- Endogenous antioxidants, **Boskou D et al.**[8]

Dietary Antioxidant: -These are secondary metabolites; these plants synthesize to safe themselves aging oxidative stress. Based on chemistry they have four classes

- Carotenoids e.g., a and b carotenes
- Vit C (Ascorbic acid)
- Polyphenolic antioxidant
- Vit E (Tocopherols)

Phenolic acid & flavonoids are the most concentrated antioxidants in all food categories, having the greatest environmental capacity. Flavonoids also have the

greatest in vitro antioxidant power followed by vit C and E, and carotenoids.

Natural Antioxidant

Ayurvedic plants have valuable antioxidant sources. Natural antioxidants are mainly useful for reducing the risk of many diseases such as heart disease, cancer, stroke & increasing the plasma's antioxidant ability. Antioxidant can be found easily in whole plants, such as seed, bark, fruit, root, & leaves Natural antioxidants come predominantly from fruits, grains, vegetables, herbs, and species. According to nature there is a spacious many of naturally occurring antioxidants that differ in composition, mechanism of physical, chemical properties & site of action there **Brown J.E et al.**[9]

Secondary-Antioxidant:- Secondary antioxidants can delay lipid oxidation through a variety of processes, accept transition metal ion chelating, oxygen scavenging, hydrogen replenishment to key antioxidants, UV radiation absorption & detection of reactive species. It should be noted, however, that some types of drugs have more than one antioxidant activity mechanism **Clement et al.** [12]. It is known that different natural phenolic compounds work as principal andsecondary antioxidants through distinct processes. Mega-functional antioxidants have only recently been accessible in one compound, incorporating main & secondary antioxidant properties. Hydroxyl amines, which can scavenge C-radicals, act as both primary and secondary antioxidants.

Synthetic Antioxidant

Synthetic antioxidants are mainly used in sector as phenolic compounds such as Butylated hydroxy--anisole, Butylated hydroxytoluene, Tertiary butyl hydroquinone& Propyl gallate Because of their efficacy and being cheaper, they are mainly used in the food sector. They are toxic carcinogenic, resulting in the need for natural solution. As a healthier and safer alternative to synthetic antioxidants, natural antioxidants have emerged since about 1980 **Yanishlieva, et al.**[50]

Primary antioxidant: -These are also called chain breaking cancer prevention agents, are mixtures equipped to kill lipid FR, mostly by halting their radical condition by exchanging hydrogen and, intermittently, by low reactivity mixtures. These generallystable mixes can't participate in the production of lipid by oxidation, thus breaking the reaction to the chain. In the enlistment time frame, the essential cancer prevention agent is effective; in the proximity, oxidation later starts. Phenol, TPC, Gallic corrosive and its subordinates, flavonoids and various mixtures are essential cancer prevention agents. **Entian KD et al.**[15]

Secondary antioxidant:- Secondary (preventive) anticipates lipid oxidation in an unexpected manner, preventing the beginning by oxidizing themselves rather than oxidizing lipid atoms or turning middle-person or last-person objects created in the midst of responses into non-harmful structures. **Entian KD et al.**[15]

Classification of Antioxidant Methods

1. In vitro antioxidant methods
2 In vivo antioxidant methods

In- vitro antioxidant

Antioxidant capacity assays are broadly classified into two types based on the chemical reaction involved between the antioxidant compounds and the free radicals, **Dontha et al.**[40]

1. Assays focused on the movement of hydrogen atoms (HAT) reaction
2. Assays based on the Electron Transfer (ET) reaction.

In vivo antioxidant

1. Estimation of total Protein
2. Quantitative Estimation of Lipid Peroxides
3. Quantitative Estimation of Glutathione
4. Estimation of Superoxide dismutase (SOD) assay
5. Catalase (CAT) Estimation

In vivo Antioxidant Test

The excised livers were per fused in chilled normal saline to remove all the blood cells. They were then cut into small bits, put in a phosphate buffer (0.1 M, pH 7.4) and homogenized to achieve 20 % homogeneity using Remi homogenizer. The thus obtained homogeneous was subjected to centrifugation for a total of 15 minutes at 1000 g (3000 rpm), and the supernatant taken in a special tube called Eppendorf tube. The final supernatant was used as lipid peroxidation marker and antioxidant enzymes for the determination of malondialdehyde (MDA) **Zadeh et al.,**[31]. **For** SOD, the supernatant was again subjected to centrifugation for 20 min at 10,000 rpm.

Estimation of total Protein[27]

The protein quantity was estimated as per the Lowry's method

Principle:- Proteins structurally contain phenolic groups in the tyrosine & tryptophan residues (amino acid) in it which reacts with copper ions in alkaline environment. Subsequently the reaction product reduces phosphomolybdic phosphotungstic acid (Folin Ciocalteau reagent) to a blue-colored complex which gives a max. absorption in the wavelength region of 660 nanometers. The resulting color intensity depends on the concentration of the aromatic amino acids corresponding to the protein present in the sample & shows variations for various proteins. Most of all methods of protein estimation include BSA as the universally used to ready availability, cost-effectiveness, and high purity. This method is well validated & linear with high sensitivity leading to the possibility of estimation as low as upto 10 µg/ml. It is regarded as the most popular method of protein assay as suggested by literatures. However, no method is without disadvantages & same is true for this method also. Some of the drawbacks of this method include the observed interference from different

components including nonionic & cationic detergents, carbohydrates, lipids, Tris buffer, EDTA & some salts. The incubation time is considered as an important factor in terms of reproducibility & is also crucial in this test. The sensitivity & functionality of the assay relies also upon the pH ofthe system mixture. The working range of pH as optimised for this assay is a pH of 9 to 10.5. One of the major drawbacks of the Lowry method is the precision of the assay within a narrow range of pH. However, the effect of pH can be nullified by taking a very small volume of the sample.

Reagents

1. Alkaline solution

A. 2% (w/v) Sodium carbonate in 0.1 M NaOH.
B. 1% (w/v) Copper sulphate
C. 2% Sodium potassium tartrate

Working alkaline solution: A (48 ml) + B (1 ml) + C (1 ml)

A. Tock standard bovine serum albumin (BSA) – 1 mg/ml
B. Working standard BSA (100 µg/ml) diluted the stock ten times
C. Folin-ciocalteau reagent (ice-cold) diluted with equal amount of water at the time ofuse.

A 0.1 ml supernatant of the homogenate was correctly integrated with 0.9 ml of DDW followed by 5ml of working alkaline solution work applied. At room temperature it was stored for ten minutes then combined with 0.5 folin- ciocalteau 50 % reagent. The solution was again incubated at room temperature, & the absorbance measured at 750 nm against. The protein content was found interpreted in the sample from the normal BSA curve.

Quantitative Estimation of Lipid Peroxides

Lipid Peroxides (LPO) Estimation.[27]

Principle

The results of lipid peroxidation are due mostly to oxidative damage caused by free radicals. During this process, peroxides as a complex mixture were produced as primary products which subsequently produced carbonyl compounds as a breakdown product. One of such hallmark biomarkers is popularly known as malondialdehyde (MDA)which is a carbonyl compound of such origin. MDA is known to form a featured chromogenic compound pink in color by reacting with 2 thiobarbituric acid molecules which give 540 nm of wavelength absorbance. The characteristic color was weighed against a blank, photometrically. The concentration of malondialdehyde was estimated by the chromophore value (1.56 x l05 M−1cm−1) and explain in terms of percentage relative to the control. This method in terms of measuring lipid peroxidation is very popular, widely accepted and considered universal. It is also known for estimating TBARS (the reactive substance of thiobarbiuric acid).

Reagents

1. KCL (0.15M)
2. 0.8% TBA solution
3. 30% TCA solution

Preparation of Reagents

A. Potassium chloride solution (0.15 M): 2.3 g of potassium chloride in 200 ml of DDW was dissolved.
B. TBA (0.8%): 0.8 g TBA in 99ml of DDW and 1ml of glacial acetic acid wasdissolved.
C. TCA (30 %): 30 g TCA in 100ml of DDW was dissolved.

Procedure

One ml aliquot was extracted from the tissue homogenate followed by an additional 0.5 ml TCA aliquot (30%) followed by a TBA reagent (0.5 ml 0.8%). The tubes were covered in aluminum foil and placed for 30 mints in a water bath at a temperature of 800C. The tubes were then deposited in cold ice water for 30mints. Following this stage, tubes underwent 15 mints of centrifugation at 1000 g (3000 rpm). The supernatant was read at wavelength of 540 nm for absorbance at room temperature against predefined blank. In their respective amounts, the blank is nothing, but the same mixture as above prepared for testing mixing the same quantities of distilled water,TCA and TBA.

Calculations

The amount of MDA in a tissue sample was calculated according to the followingequation, and the result was showed as millimoles of MDA / mg protein.

$$\text{Mmoles of MDA} = \frac{\text{Volume of assay X O.D (540)}}{0.156}$$

Where = final volume of test solution
O.D = optical density at 540nm

Quantitative Estimation of Glutathione

Reduced Glutathione (GSH) estimation[14]

The liver content of reduced glutathione was measured using the well-known Ellman's method in 1959, This methodogy of photometric evaluation requires the use of DTNB, as 5, 5-dithiobis- (2-nitrobenzoic acid), which is reduced by -SH groups and produces 1mole of 2-nitro-5-mercaptobenzoic acid (per SH mole)

Procedure

A supernatant aliquot (1 ml) with 4 % sulfosalicylic acid (1 ml) has been treated for precipitation followed by a cold digestion cycle at 4 ° C for 1 h. Then the sample was subjected to centrifugation for 15 min. at 1200 revolution per min while keeping in temperature of 4° C throughout. The phosphate buffer volumes (2,7 ml, 0,1 M, pH 8) and 5, 5 dithiobis 2-nitrobenzoic acid (DTNB) (0,2 ml) were then added to the phosphate buffer; the mixture had a yellow color production that can be read at

a wavelength of 412 nm immediately against a suitable blank. Using the normal curve,the results were measured, & expressed in words.

Estimation of Superoxide dismutase (SOD) assay[28]

Principle

In presence of vapor, pyrogallol (1, 2, 3-benzenetriol) undergoes autooxidation. The rate of auto-oxidation also increases as pH increases, along with the formation of various intermediate products. During this process the solution turns yellow burn indicating a 400-425 nm spectrum. After this, its color changes to green in the beginning after several min's, which turns yellow after a few hours. Therefore, the autoxidation process was observed specifically in the initial stage and its rate was calculated when its absorbance increased linearly at 420 nm which persists for several minutes after 10 seconds after induction of the cycle. During this time, Superoxide anion radical (O2 acts as a catalyst for autoxidation of pyrogallol. Assay for SOD is a simple and non-complex process that is evaluated by its ability to prevent pyrogallol's autoxidizing capacity.

Reagents

1. Tris Hydrochloric buffer (pH 8.5)
2. Potassium phosphate buffer (50 mM/l, pH 7.4
3. Pyrogallol (24 mm)

Preparation of Reagents

A. Tris Hydrochloric acid buffer (pH 8.5): 788 mg Tris- Hydrochloric acid buffer and 186 mg Ethylene dynamine tetra acetate dissolved in 100 ml of DDWand adjusted pH to 8.5 with 1 N NaOH**.**

B. Potassium phosphate buffer (50 mM/l, pH 7.4): It was manufacture by mixing KH PO solution and K HPO solution of 1: 1.55.

C. Pyrogallol (24 mm): 15.1 mg of pyrogallol was dissolved at 10 mM HCl, 5 ml. At the time of assay the solution was freshly prepared.

Procedure

20.0 mg of liver tissue was homogenised in two ml of potassium phosphate sol'n. Homogeneous centriftion was conducted for twenty minutes at 10,000 rpm at 4°C in a cold centrifuge. Applying 100µl supernatant to three ml tris HCl buffer, pH 8.5 followed by 25µl pyrogallol and then thoroughly mixed. The absorbance change was reported at 420 nm at one mint interval for three mins.

Calculation

The amount of enzyme added causes 50 % pyrogallol autoxidation prevention in every three ml of the assay solution, it is described as one unit of SOD.

This can be equated as:

(A-B) × 100

Unit of SOD/ml of Sample = A × 50

Where, A = Change in a.p mint of Standard,

B = Change in a.p min of test Sample

Catalase Estimation[17]

Principle

Hydrogen peroxide exhibits a continuous rise in absorption with decreasing wavelength in the UV range. The rate of disappearance of hydrogen peroxide is shown by the rate ofdecrement in absorbance at a wavelength of 240 nm. There is a change in absorbance ΔA/minute which exhibits the appraisal of catalase activity.

Unit definition: 1 unit of catalase will decompose 1.0 µmole of H O p.m at pH 7.0 at 25°C.

Reagents

1. Potassium dihydrogen phosphate
2. Disodium hydrogen phosphate
3. Potassium phosphate buffer
4. Hydrogen peroxide

Procedure

The liver tissue was homogenized with a ratio of 1:10 w / v to potassium phosphate buffer (50 Mm, pH 7.4). At 10,000 rpm at 4 oC in cooling centrifuge for 20 min's centrifugation was done to the homogenate. 50 µl of supernatant was applied to the potassium phosphate buffer containing 2.95 ml of 19mM / L H2O2 solution. H2O2's disappearance was monitored for 3 mints at a wavelength of 240 nm at 1 minute interval.

Calculation

Catalase activity was calculated by using the following equation and the result was expressed as nano moles of H_2O_2 consumed/minute/mg protein.

$$\text{Catalase activity} = \frac{\Delta A/\text{minute} \times \text{total volume of assay}}{0.81 \text{ sample volume} \times \text{mg of protein}}$$

Statistical analysis

By using statistical software package (Graph Pad PRISM version 5.01, Graphpad Software, San Diego, CA), statistical analysis was performed semi automatically. Outcomes were expressed as mean ± standard mean error. Data comparison was made using ANOVA (variance analysis) accompanied by Dunnet's post hoc (multiple comparison) study. P values<0.05 were deemed statistical.

RESULT AND DISCUSSION

Effect of Allium-cepa Extract on Body weight

Treatment of Test Item formulations i.e., Allium-cepa Extract extract at the given doses did not cause any body weight loss. No difference in body weight was observed among the Test Item treated groups as compared with disease control group. Test item formulations treated groups were gained around ~8% when compared to disease control group. Hence, the Test item formulations i.e., Allium-cepa Extract are found to be safe at the tested dose and regimen.

Data is represented in Table 6.1 and Figure 6.1.

Table 6.1: Mean Body Weight (Unit: g ± SEM).

Group		DAYS								
		1	3	7	10	14	17	21	24	28
G1 (Normal control, Standard diet, and water)	**Mean**	**360.01**	**322.47**	**365.46**	**368.09**	**373.21**	**374.17**	**382.49**	**383.84**	**391.65**
	SEM	*7.04*	*12.83*	*9.16*	*9.96*	*9.21*	*10.35*	*10.19*	*11.29*	*10.48*
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**Mean**	**348.58**	**313.84**	**290.49**	**298.50**	**294.77**	**293.54**	**295.11**	**287.67**	**287.35**
	SEM	*10.51*	*8.86*	*12.72*	*11.56*	*14.47*	*16.66*	*15.68*	*16.30*	*16.63*
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**Mean**	**346.48**	**306.51**	**300.05**	**301.52**	**302.92**	**302.49**	**305.32**	**299.14**	**305.39**
	SEM	*7.83*	*7.33*	*7.89*	*11.42*	*10.42*	*13.24*	*12.36*	*14.45*	*13.71*
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**Mean**	**364.80**	**326.27**	**321.42**	**325.02**	**325.38**	**323.13**	**325.54**	**325.16**	**326.45**
	SEM	*7.23*	*7.08*	*7.59*	*8.22*	*7.63*	*7.42*	*8.64*	*9.72*	*9.39*

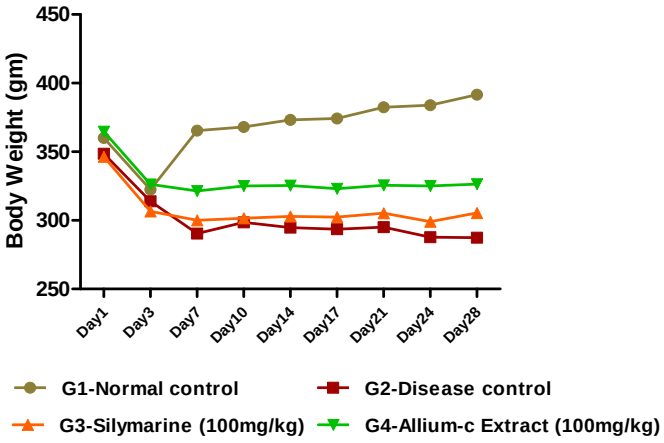


Figure 6.1: Body Weight (Mean ± SEM).

Effect of Allium-cepa Extract on feed Consumption

Non-significant change in cumulative feed consumption was found in all groups throughout the study period. Treatment of Test item formulations Allium-cepa Extract at given doses showed a similar pattern throughout the study.

Data is represented in Table 6.2 and Figure 6.2.

Table 6.2: Mean feed Consumption (Unit: g ± SEM).

Group		DAYS								
		1	3	7	10	14	17	21	24	28
G1 (Normal control, Standard diet, and water)	**Mean**	**25.54**	**38.52**	**28.02**	**31.69**	**31.06**	**31.49**	**30.64**	**30.86**	**25.54**
	SEM	*0.26*	*1.36*	*1.08*	*1.31*	*0.92*	*1.19*	*1.33*	*0.88*	*0.26*
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**Mean**	**25.76**	**39.33**	**29.01**	**37.45**	**42.85**	**38.62**	**41.22**	**36.61**	**25.76**
	SEM	*0.52*	*1.38*	*2.72*	*4.10*	*1.81*	*3.05*	*2.05*	*2.49*	*0.52*
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**Mean**	**25.71**	**37.28**	**31.89**	**41.23**	**41.19**	**39.72**	**40.91**	**40.24**	**25.71**
	SEM	*0.63*	*1.19*	*2.13*	*2.97*	*3.35*	*3.67*	*2.18*	*1.53*	*0.63*
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**Mean**	**24.18**	**35.11**	**29.78**	**43.04**	**37.27**	**36.87**	**37.65**	**38.98**	**24.18**
	SEM	*0.53*	*1.46*	*1.94*	*1.68*	*2.77*	*2.00*	*1.66*	*1.44*	*0.53*

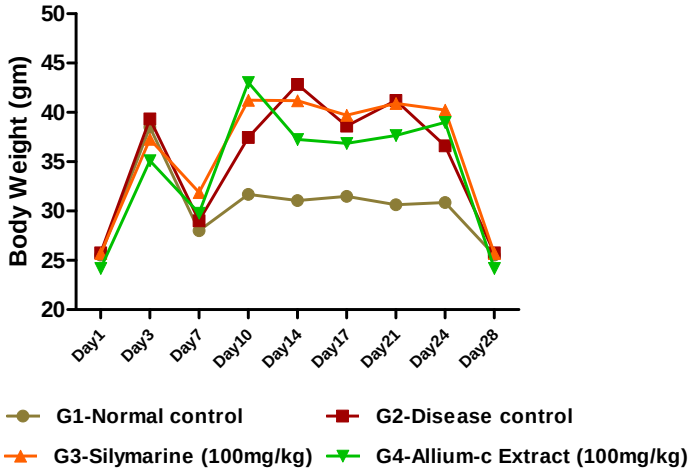


Figure 6.2: Feed Consumption (Mean ± SEM).

Antioxidant Assay

There is a Significant Activity ($p<0.01$), ($p<0.001$), ($p<0.001$) & ($p<0.001$) is observed in G2 (Disease Control) compared to Normal control (G1) on Superoxide dismutase (SOD), Lactoperoxidase (LPO), Catalase activity and L-Glutathione (GSH) respectively. Silymarine (100mg/kg) oral) is a Significant Activity ($p<0.01$) & ($p<0.05$) is observed in G3 (Silymarine) compared to Disease control (G2) on Catalase activity and L-Glutathione (GSH) respectively. No significant activity has been observed in other parameters. Even though there is no significant activity is observed but there is scientific trend has been observed seen in graph and tables.

Table 6.3: Summary of Level of Antioxidant Enzymes (MEAN ± SEM).

Group		SOD Activity (U/mL)	Lactoperoxidase (LPO) (µM)	Catalase Activity (µmol/min/mL)
G1 (Normal control, Standard diet, and water)	**Mean**	**649.41**	**6.00**	**16.77**
	SEM	52.39	0.78	1.42
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**Mean**	**427.65****	**17.63*****	**6.05*****
	SEM	47.07	1.56	0.70
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**Mean**	**506.46**	**14.40**	**13.53****
	SEM	25.39	1.12	2.42
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**Mean**	**496.07**	**16.58**	**11.21**
	SEM	28.23	1.26	1.01

Table 3: Conti…

Group/Treatment		Malondialdehyde (MPO) (ng/ml)	GSH (µM)	GPx Activity (nmol/min/mL)
G1 (Normal control, Standard diet,and water)	**Mean**	**6.061**	**116.91**	**4.59**
	SEM	1.880	1.92	0.34
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**Mean**	**9.447**	**24.53*****	**3.87**
	SEM	0.524	1.21	0.38
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**Mean**	**8.762**	**37.90***	**4.86**
	SEM	0.384	4.92	0.49
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**Mean**	**8.684**	**27.79**	**3.63**
	SEM	0.377	1.04	0.21

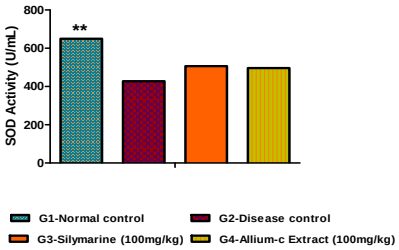


Figure 6.3(a): SOD (Mean ± SEM).

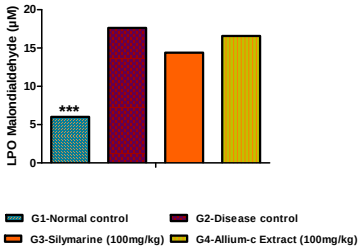


Figure 6.3(b): LPO (Mean ± SEM).

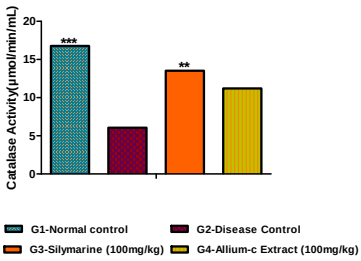


Figure 6.3(c): Catalase (Mean ± SEM).

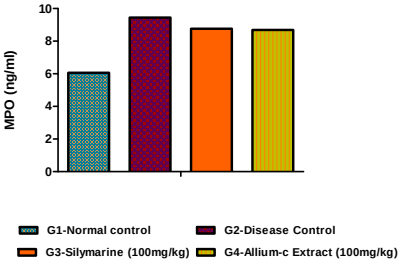


Figure 6.3(d): MPO (Mean ± SEM).

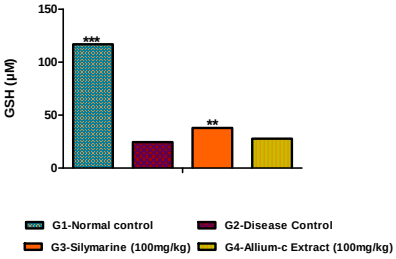


Figure 6.3(e): GSH (Mean ± SEM).

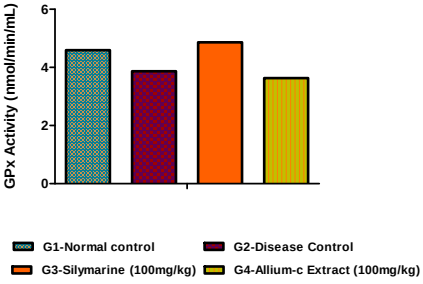


Figure 6.3(f): GPx Activity (Mean ± SEM).

Figure 6.3: Antioxidant Enzymes.

Effect of Cactus & Ladyfinger Extract on Hematological

There is a Significant Activity ($p<0.001$) & ($p<0.01$) is observed in G4 (Allium cepa extract) compared to Disease control (G2) on Basophils and MCHC respectively.

Silymarine (100mg/kg) oral) (G3) is a Significant Activity ($p<0.001$) is observed in when compared to Disease control (G2) on MCHC. No significant activity has been observed in other parameters. Even though there is no significant activity is observed but there is scientific trend has been observed seen in graph and tables.

Table 6.4 Hematology (Mean ± SEM).

Group		Hematology				
		TLC ($X10^3/mm^3$)	**Neutrophils (%)**	**Lymphocyte (%)**	**Monocyte (%)**	**Eosinophils (%)**
G1 (Normal control, Standard diet, and water)	**Mean**	**9.01**	**11.77**	**80.27**	**4.67**	**2.57**
	SEM	1.54	1.86	3.16	0.98	0.46
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**Mean**	**6.73**	**13.50**	**82.62**	**2.06**	**1.28**
	SEM	0.67	1.19	1.17	0.20	0.17
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**Mean**	**7.18**	**10.93**	**82.15**	**3.70**	**2.73**
	SEM	1.05	1.07	1.85	0.94	0.59
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**Mean**	**5.24**	**24.53**	**68.50**	**3.47**	**2.63**
	SEM	1.23	8.62	6.85	0.96	0.79

Table 6.4 Conti.

Group		Hematology			
		Basophils (%)	RBC ($X10^6/mm^3$)	Hb (g/dl)	Hct(%)
G1 (Normal control, Standard diet, and water)	**Mean**	**0.73**	**7.96**	**12.10**	**38.07**
	SEM	0.27	0.42	0.20	0.98
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**Mean**	**0.54**	**7.42**	**11.40**	**36.70**
	SEM	0.09	0.67	0.68	2.03
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**Mean**	**0.50**	**8.13**	**12.13**	**37.50**
	SEM	0.07	0.31	0.18	0.58
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**Mean**	**0.50*****	**8.13**	**12.13**	**37.50**
	SEM	0.07	0.31	0.18	0.58

Table 6.4 Conti.

Group		Hematology				
		MCH (pg)	MCHC (g/L)	RDW-CV (%)	Platelets ($X10^3/mm^3$)	MPV (fL)
G1 (Normal control, Standard diet, and water)	**Mean**	**15.30**	**31.87**	**13.87**	**723.83**	**4.7**
	SEM	0.65	0.38	0.27	368.08	0.26
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**Mean**	**15.56**	**30.98**	**15.42**	**1107.40**	**4.6**
	SEM	0.57	0.28	1.39	97.34	0.07
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**Mean**	**14.98**	**32.38***	**14.25**	**1017.75**	**4.4**
	SEM	0.42	0.17	1.09	110.46	0.12
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**Mean**	**15.60**	**33.00****	**16.07**	**1411.33**	**4.3**
	SEM	0.60	0.42	1.44	92.03	0.15

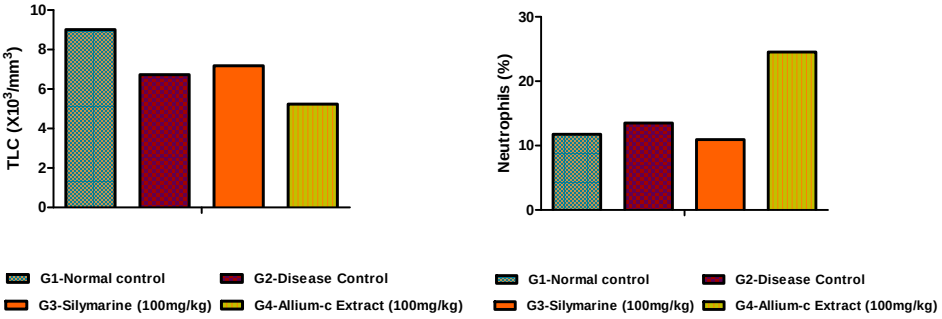


Figure 6.4(a): TLC (Mean ± SEM).

Figure 6.4(b): Neutrophils (Mean ± SEM).

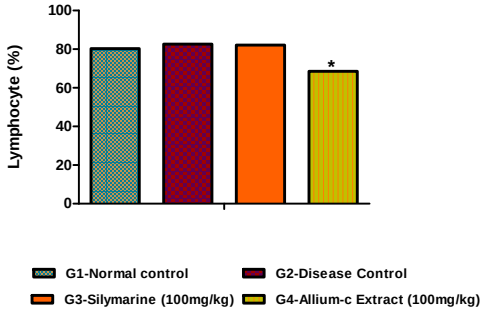


Figure 6.4(c): Lymphocytes (Mean ± SEM).

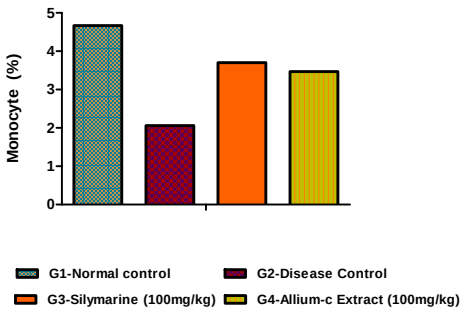


Figure 6.4(d): Monocytes (Mean ± SEM).

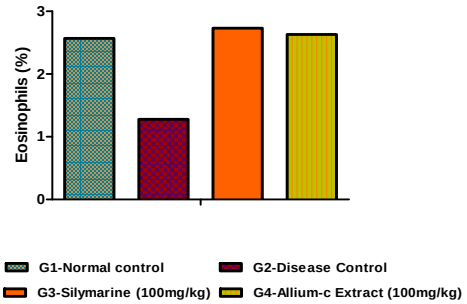


Figure 6.4(e): Eosinophils (Mean ± SEM).

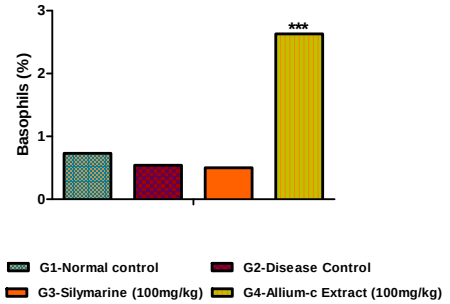


Figure 6.4(f): Basophils (Mean ± SEM)

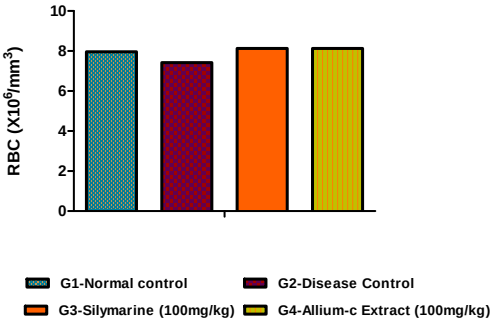


Figure 6.4(g): RBC (Mean ± SEM).

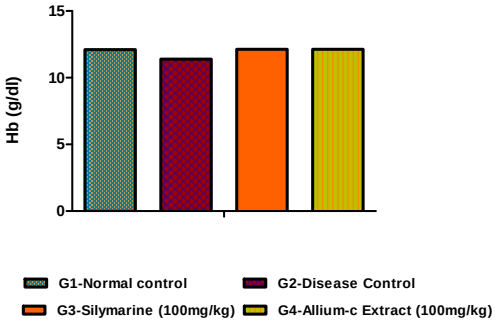
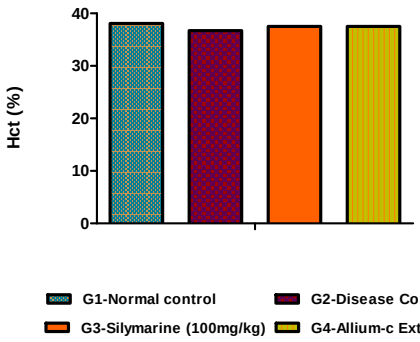


Figure 6.4(h): Hemoglobin (Mean ± SEM).



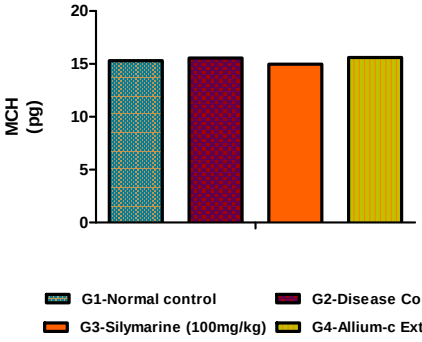
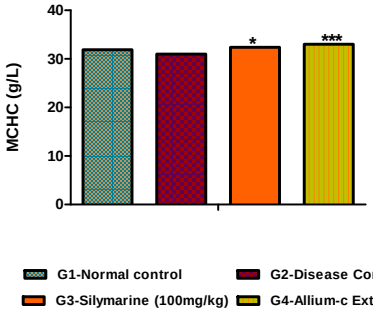


Figure 6.4(i): Hematocrit test (Mean ± SEM). Figure 6.4(g): Mean Corpuscular Hemoglobin (Mean ± SEM).



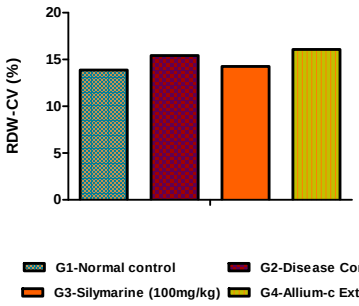


Figure 6.4(h): Mean Corpuscular Hemoglobin Concentration (Mean ± SEM). Figure 6.4(l): Red Cell Distribution Width (Mean ± SEM).

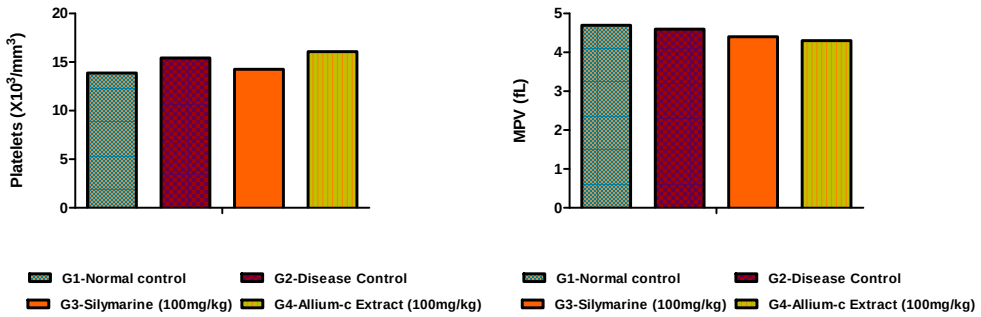


Figure 6.4(j): Platelets (Mean ± SEM), Figure 6.4(k): Mean Platelets Volume (Mean ± SEM)
Figure 6.4: Hematology.

Effect of Allium-cepa Extract on Biochemical Parameters

There is a Significant Activity ($p<0.01$) & ($p<0.001$) is observed in G2 (Diabetic control) when compared to Normal control (G1) on SGOT and Glucose respectively. Ladyfinger Extract (100mg/kg) (G5) is a Significant Activity ($p<0.01$) is observed in when compared to Diabetic control (G2) on Glucose. No significant activity has been observed in other parameters. Even though there is no significant activity is observed but there is scientific trend has been observed seen in graph and tables.

Table 6.5 Biochemistry (Mean ± SEM).

Group		Biochemistry				
		SGOT (U/L)	SGPT (U/L)	Total Bilirubin (mg/dl)	Glucose (mg/dl)	Creatinine (mg/dl)
G1 (Normal control, Standard diet, and water)	**Mean**	**129.96**	**0.32**	**0.11**	**94.20**	**26.84**
	SEM	*5.11*	*0.02*	*0.01*	*3.97*	*1.43*
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**Mean**	**396.71****	**0.21**	**0.15**	**579.78*****	**150.13**
	SEM	*57.06*	*0.02*	*0.02*	*25.96*	*35.63*
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**Mean**	**428.28**	**0.29**	**0.14**	**532.44**	**250.80**
	SEM	*70.71*	*0.06*	*0.03*	*33.22*	*84.02*
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**Mean**	**495.01**	**0.24**	**0.19**	**560.30**	**122.86**
	SEM	*45.91*	*0.02*	*0.04*	*22.06*	*20.27*

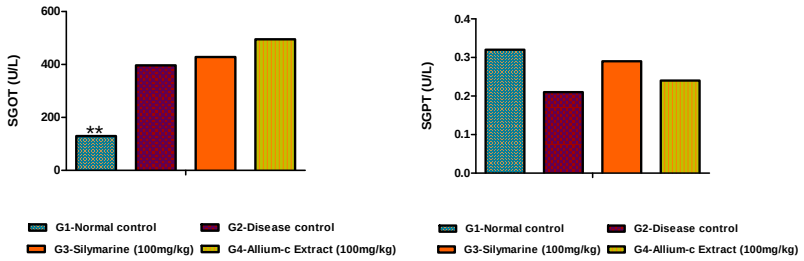


Figure 6.5(a): SGOT (Mean ± SEM), Figure 6.5(b): SGPT (Mean ± SEM)

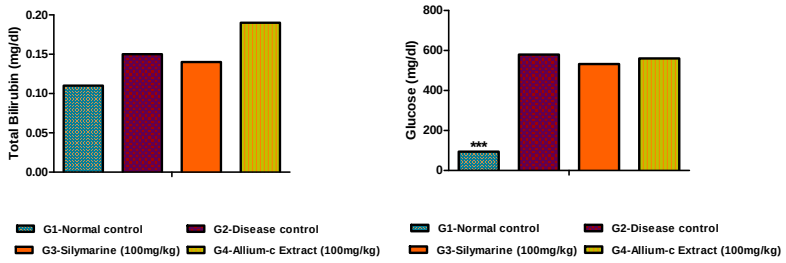


Figure 6.5(c): Total Bilirubin (Mean ± SEM), Figure 6.5(d): Glucose (Mean ± SEM)

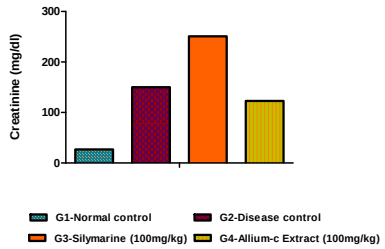


Figure 6.5(e): Creatinine (Mean ± SEM)

Effect of Cactus on Histopathological analysis in Pancreases

Histology data are presented in table 11 and 12 and figure 7

Gross Pathological Examination

All the treatment group animals (G2 to G4) except normal control (G1) found to be weak. No treatment-related gross pathological findings were observed in all the animals. The organ weights were recorded, and relative organ weight was calculated. Gross observation was done, and the absence of treatment related abnormalities revealed that the test items were well tolerated.

Summary and individual animal data for relative organ weight data and gross pathological results are presented in Table 10, Figure 6 and Appendix 7, 8.

Table 6.6: Gross observation of vital organs of individual animals.

Groups	Organs					
	A No.	Heart	Kidney	Lungs	Liver	Spleen
G1 (Normal control, Standard diet, and water)	**1**	NAD	NAD	NAD	NAD	NAD
	2	NAD	NAD	NAD	NAD	NAD
	3	NAD	NAD	NAD	NAD	NAD
	4	NAD	NAD	NAD	NAD	NAD
	5	NAD	NAD	NAD	NAD	NAD
	6	NAD	NAD	NAD	NAD	NAD
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	**1**	NAD	NAD	NAD	AD	NAD
	2	NAD	NAD	NAD	AD	NAD
	3	NAD	NAD	NAD	AD	NAD
	4	NAD	NAD	NAD	AD	NAD
	5	NAD	NAD	NAD	AD	NAD
	6	NAD	NAD	NAD	AD	NAD

NAD: *No Abnormality Detected,* ***AD:*** *Abnormality Detected*

Table 6.6 Conti.

Groups	Organs					
	A No.	Heart	Kidney	Lungs	Liver	Spleen
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	**1**	NAD	NAD	NAD	NAD	NAD
	2	NAD	NAD	NAD	NAD	NAD
	3	NAD	NAD	NAD	NAD	NAD
	4	NAD	NAD	NAD	NAD	NAD
	5	NAD	NAD	NAD	NAD	NAD
	6	NAD	NAD	NAD	NAD	NAD
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	**1**	NAD	NAD	NAD	NAD	NAD
	2	NAD	NAD	NAD	NAD	NAD
	3	NAD	NAD	NAD	NAD	NAD
	4	NAD	NAD	NAD	NAD	NAD
	5	NAD	NAD	NAD	NAD	NAD
	6	NAD	NAD	NAD	NAD	NAD

Histopathology

On histopathological examination, structural damage was observed in liver in all the Anti-TB Drug induced Hepato-toxicity in animals. Uncontrolled Hepato-toxicity can lead to many complications including nephropathy, cardiovascular diseases, and male impotency. Histopathological lesions observed can be considered as chronic complications of Anti-TB Drug induced Hepato-toxicity which is a common finding in Anti-TB Drug induced Hepato-toxicity rat model. Photographs of the

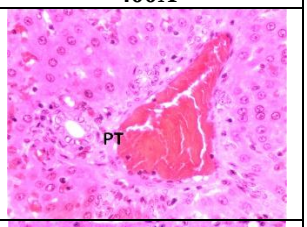
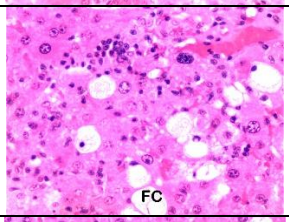
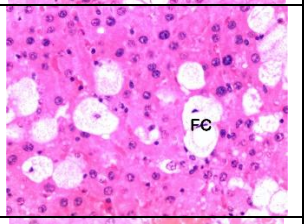
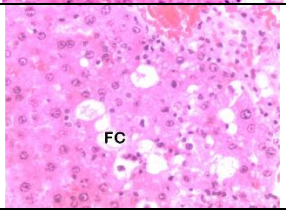
microscopic sections of the organs are presented in Histopathological photographs. Histopathological investigation revealed, maximum cellular damage in all the liver of various treatment groups. Tissue damage was histologically scored. Maximum injury was observed in the organs like liver etc. Maximum sum score was calculated in Hepato-toxicity stress group (G2). All the treatment groups showed relatively less damage score when compared to G2 group. Based on the gross and histopathological data, Allium-cepa treated was found to be well tolerated, and there is no safety and toxicity concern.

Table 6.7: Individual Histopathology Findings.

Animal No.	Organs*	Findings	Grade	Unilateral(U)/Bilateral (B)
Group G1	Liver	Fatty changes/ Bollooning Degeneration	-	-
		Inflammatory cell infiltration	-	-
		Necrosis	-	-
Group G2	Liver	Fatty changes/ Bollooning Degeneration	5+	
		Inflammatory cell infiltration	3+	
		Necrosis	3+	
Group G3	Liver	Fatty changes/ Bollooning Degeneration	1+	
		Inflammatory cell infiltration	4+	
		Necrosis	4+	
Group G4	Liver	Fatty changes/ Bollooning Degeneration	2+	
		Inflammatory cell infiltration	2+	
		Necrosis	5+	

keys: *The other organs/tissues mentioned in study plan did not show any lesions on histopathological examination, MNC- Mononuclear cells, NAD - No Abnormality Detected, 1+ - Minimal, 2+ Mild, 3+ - Moderate, 4+ Marked and 5+ Severe

Figure 6.6 Histopathological photographs.

Groups	400X
G1 (Normal control, Standard diet, and water)	
G2 (Disease control, Anti-TB Drug+ Standard diet and water)	
G3 (Anti-TB Drug + Silymarine (100mg/kg) oral)	
G4 Anti-TB Drug + Allium-cepa Extract (100mg/kg)	

SUMMARY AND CONCLUSION

In *in-vivo*, Allium-cepa extract no significant decrease in Anti-TB Drug induced Hepato-toxicity by not reducing the SGPT, SGOT and no significant activity has been observed in other parameters.Even though there is no significant activity is observed but there is scientific trend has been observed. Histological observations maximum sum score was calculated in

Disease control group (G2). All the treatment groups showed relatively less damage score when compared to G2 group. Based on the gross and histopathological data, Allium-cepa extract treated was found to be well tolerated and there is no safety and toxicity concern. There is no systemic toxicity has been observed in Biochemical and Hematological parameters in the study. The study has been concluded that Allium-cepa extract no potent therapeutic effect on treatment of liver damage (Hepato-toxicity)

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Human and animal rights: Not applicable.
Consent for publication: Not applicable.
Code availability: Not applicable.
Author contribution: All authors have contributed equally

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