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Effect of L-arginine amino acid on liver regeneration after hepatocyte damage in rats: An experimental study

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ABSTRACT

The aim of the work was to check the therapeutic effect of L-arginine for liver cell regeneration. L-arginine is a semi-essential amino acid works as a nitric oxide precursor. Sustained released matrix beads of L- arginine were developed and tested for its hepatoprotective effect in rats (paracetamol was used to induce liver toxicity). Various biochemical tests of hepatic damage like SGPT, SGOT, ALP and bilirubin were done in all groups of animals. A toxic dose of paracetamol enhanced the SGPT, SGOT, ALP and bilirubin levels followed by treatment with silymarin as standard and L-arginine as test drugs. Histopathological studies also supported the hepatoprotective effect, containing L-arginine.Animal group treated with L-arginine indicated a significant decrease in the levels of biochemical markers to the near normal levels. The liver section of toxicant animals showed degenerative changes, aggregates of mononuclear inflammatory cells while treatment with formulations containing L-arginine helped in regaining the normal hepatic architecture, hence it can be concluded that significant effect of L-arginine was observed in liver cell regeneration.

Keywords: Hepatoprotective, L-arginine, Silymarin, SGPT, SGOT.

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INTRODUCTION

The Liver is the largest vital organ in the body. In human being it is separated incompletely into lobes, covered on their external surfaces by a thin connective tissue capsule. Major functions of the liver are metabolism and detoxification of hepato-toxic substances which can cause damage to the liver during metabolic reactions [1]. Liver diseases, including hepatitis B virus and hepatitis C virus infections, alcoholic liver disease, nonalcoholic fatty liver disease and associated cirrhosis, liver failure and hepatocellular carcinoma, are major causes of illness and death worldwide [2]. It has been reported that liver diseases have become a global problem and about 20,000 deaths has been reported every year due to liver disorders [3].

Arginine is a semi-essential amino acid involved in multiple areas of human physiology and metabolism. Nitric Oxide produced from arginine is helpful in improving outcomes in various diseases. L-arginine is readily available over the counter and is popular as a nutritional supplement. Several researches has been proved that L-arginine had a therapeutic effect in numerous acute and chronic disease states, including sickle cell chest crisis, pulmonary artery hypertension, coronary heart disease, pre myocardial infarction. Hence present work was aimed to determine the effect of L-arginine on liver regeneration after hepatocyte damage in rats.

MATERIALS AND METHODS

L-Arginine was purchased from SD Fine Chemicals Ltd. India, paracetamol from Sigma-Alderich Ltd. India, Silymarin form Sigma Chemical Co., India were purchased from the local supplier. Diagnostic kits for the estimation of SGOT, SGPT, SALKP, and serum bilirubin were purchased from a local supplier (Sai chemicals, India).

Animals used

Animals Studies were carried out using Wistar albino rats (150-180 g) of either sex. They were obtained from the animal house, Indian Veterinary Research institute (IVRI), Bareilly, India. The animals were grouped and kept in poly acrylic cages with not more than six animals per cage and

maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (14/10 hr). They were allowed free access to standard dry pellet diet and water. The rats were acclimatized to laboratory condition for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the Animal ethical Committee (CPCSEA registration number: 715/PO/Re/S/02/CPCSEA).

Preparation of Microbeads

The microbeads were prepared by using ionic gelation method as mentioned in previous work of formulation development [4]. SA solution (4 % w/v) was prepared by dissolving 8 g of SA in small amount distilled water in a mortar pestle. When a clear solution was formed, the volume made up to 100 mL. L-Arginine was added into SA solution with 15 m stirring on magnetic stirrer to form a clear solution. Chitosan (0.5%) and calcium chloride (6%) were dissolved in 1% acetic acid solution. The pH of the solution was adjusted to 5 using a 0.1 N NaOH solution and was stirred for a further 30 min. Drug containing SA solution was then dropped drop wise through 21 gauge needle into 100 mL solution of CC solution and microbeads were formed. The gelation time of 30 m was allowed to complete the curing reaction in the CC solution containing chitosan and then microbeads were collected, filtered through wattman filter paper and washed thoroughly with water [5, 6].

Paracetamol-induced liver damage in rats

The rats were randomly distributed in five groups (six rats in each group):

Group I (control group): received a normal diet.

Group II (Paracetamol-intoxicated group): received Paracetamol 1000 mg/kg of body weight in distilled water to induce hepatotoxicity and received no other treatment which is severed as a positive control.

Group III (protection group): Received Paracetamol 1000 mg/kg (1 ml tween/kg body weight) of body weight in 1ml of distilled water. After confirmation of hepatotoxicity, animals were treated orally with pure L-arginine (100 mg/kg of body weight).

Group IV (curative group): Received Paracetamol 1000 mg/kg of body weight in 1ml of distilled water. After confirmation of hepatotoxicity, animals were treated orally with prepared L-arginine loaded alginate microbeads (equivalent to 50 mg/kg of body weight).

Group IV (curative group): Received Paracetamol 1000 mg/kg of body weight in 1ml of distilled water. After confirmation of hepatotoxicity, animals were treated orally with prepared L-arginine loaded alginate microbeads (equivalent to 100 mg/kg of body weight).

Prepared formulations were administered by oral route using a plastic catheter.

Biochemical studies

The blood was obtained from all animals by puncturing retro-orbital plexsus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various bio-bio-chemical parameters namely SGPT and SGOT [7,8].

Estimation of Transaminase

Serum Glutamate Pyruvate Transaminase (SGPT)

The SGPT catalyzes the transfer of an amino group from L - Alanine to L - Katogthta rate with the formation of Pyruvate and glutamate at a pH 7.4. The pyruvate so formed is allowed to react with 2, 4 DWPH to produce 2:4 definitionPhenylehydrazonederivative which is brown colored in alkaline Medium. The absorbance of this hydrazone derivative is correlated to SGPT activity by plotting a Calibration curve using Pyruvate [9,10].

Serum Glutamate Oxaloacetate Transaminase (SGOT)

SGOT Catalyzes transfer of an amino group from L. aspartate to L - Kalogluterate with the formation of Oxaloacetate and glutamate. The Oxaloacetate so formed, is allowed to react with 2, 4, DNPH to form 2: 4 dinitroPhenylehydrazone derivative which is brown colored is alkaline medium. The absorbance of this hydrazone derivative is correlated to SGOT activity by plotting a calibration curve using pyruvate standard [9, 10].

Estimation of Serum Alkaline Phosphatase (SALP)

Serum SALP hydrolyzes phenyl phosphate into phenol and disodium hydrogen phosphate at OH 10.0. The phenol so formed acts with 4-Aminoantipyrine in alkaline medium in presence of oxidizing agent potassium ferricyanide to form a red colored complex whose absorbance is proportionate to the enzyme activity [11,12].

Estimation of Total Bilirubin

Bilirubin reacts will diazotize sulfanilic acid in acidic medium to form azobilirubin, a purple colored complex whose absorbance is proportional to bilirubin concentration. The total bilirubin (Direct and Indirect) the diazotization is carried out in the presence of an activator[13]. Direct bilirubin, being water soluble is allowed to react with diazotized sulfuric acid in the absence of an activator.

Liver Weight and Volume

The liver was carefully excised and washed in ice-cold normal saline solution and pressed between filter paper pads and weighed. A portion of the liver (one animal of each group) was preserved in 10% neutral formalin for histopathology studies [14]

Histopathological Studies

On the eight day surviving animals from each group were sacrificed by decapitation and the histopathologic techniques [15]. The liver was carefully dissected out, extraneous tissue was cleaned off and then wet weight and volume were noted. The Part of the liver was fixed in formol-saline and processed for microtome sectioning at 5-micron thickness, stained with harmatoxylin and eosin. The damaged produced in the liver structure in the form of degeneration, necrosis and fibrosis was graded [16].

Statistical methods employed

To achieve a meaningful conclusion the data were analysed by standard statistical procedures Snedecor and Cochran (1967). Average and standard errors were calculated at different days after treatment and also in different treatment groups, for each of the parameter studied. In order to investigate the effect of days of treatment (0 - 7 days) as well as the effect of the treatment groups on different biochemical constituents and enzymes one way ANOVA was carried out Whenever an effect was found to

be significant the critical difference (CD) test were carried out in order to compare the subclass means for significance. All experiments were repeated for three times. Results are reported as means ± S.E.M. ANOVA was used to evaluate differences between groups. P < 0.05 considered as significant.

RESULTS

Biochemical studies

Estimation of Transaminase (Serum Glutamate Pyruvate Transaminase)

(A) Control set

The SGPT activity in group1 (normal) was recorded to be 84.17 ± 6.20 IU/L

(B) Test set

SGPT activity after administration of paracetamol 500 mg/kg for 7 days was found to be 268.92. ± 2.32 IU/L while 78.23± 4.20; 96.04± 4.77 and 85.44± 5.12 IU/L respectively for group 3, 4 &5 (table 1 & figure 1).

Serum Glutamate Oxaloacetate Transaminase

(A) Control set

The SGOT activity in group1 (normal) was recorded to be 355.92. ± 1.65 IU/L.

(B) Test set

SGOT activity after administration of paracetamol 500 mg/kg for 7 days was found to be 465.92. ± 5.11 IU/L while 380.51.± 4.65; 393.65.± 4.45 and 386.42 ± 4.78 IU/L respectively for group 3, 4 &5 (table 1 & figure 1).

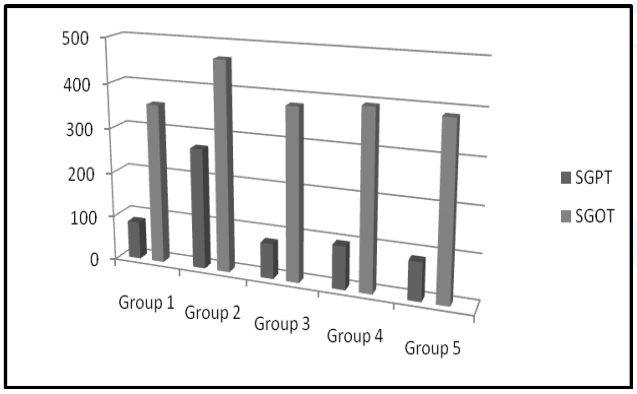

Fig. 1: SGPT and SGOT level of various groups

Serum Alkaline Phosphate (SALP)

(A) Control set

The mean serum alkaline phosphate (SALP) of group1 (normal) was recorded to be 159.74±6.21IU/L.

(B) Test set

After administration of paracetamol 500 mg/kg for 7 days serum alkaline phosphate (SALP) was found to be 485.22 ± 7.10 IU/L while 315.77 ± 5.65; 336.25 ± 5.33and 322.53 ± 4.96 IU/L respectively for group 3, 4 &5 (table 1 & figure 2).

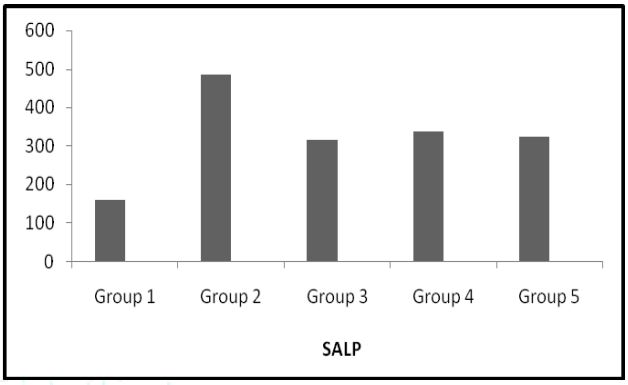

Fig. 2: Serum albumin phosphate of various groups

Data are expressed as mean ± S.E.M from six rats and analyze by one way ANOVA followed by Tukey tests. *P<0.05, **P<0.01 as compared to normal group animals

Total Serum Bilirubin

The results of total serum bilirubin in group1, 2, 3, 4 & 5 have been depicted in table 6.1. The unit is expressed in mg/dL.

(A) Control set

The mean total serum bilirubin of group 1(normal) was recorded to be 0.41 ± 0.2o mg/dL.

(B) Test set

After administration of paracetamol 500 mg/kg for 7 days total serum bilirubin was found to be 7.22 ± 11.12 mg/dL while 0.45 ± 0.09; 0.35 ± 0.21 and 0.32 ± 0.13mg/dL respectively for group 3,4&5.

Data are expressed as mean ± S.E.M from six rats and analyze by one way ANOVA followed by Tukey tests. *P<0.05, **P<0.01 as compared to normal group animals

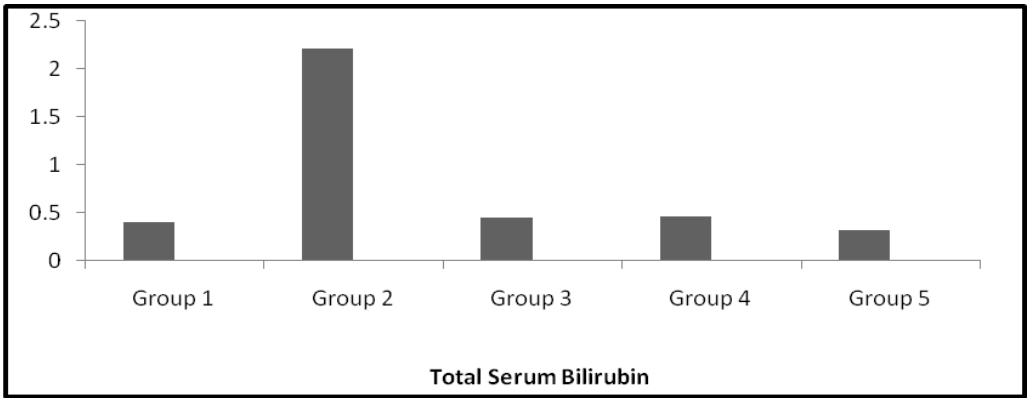

Fig. 3: Total serum bilirubin group of various groups

Table 1: Effect of *L-arginine* on serum parameters for paracetamol induced hepatotoxicity

Groups		SGPT(IU/L)	SGOT(IU/L)	SALP(IU/L)	Total bilirubin (mg/dL)	Liver weight (mg/100gm body weight)
Group 1	Normal	84.17 ± 6.20	355.92. ± 1.65	159.74±6.21	0.41±0.20	3.435±0.134
Group 2	Paracetamol; (500mg/kg)	268.92. ± 2.32	465.92. ± 5.11	485.22 ± 7.10	2.22 ± 11.12	4.693±0.122
Group 3	Silymarin + Paracetamol (100 mg/kg p.o.)	78.23± 4.20**	380.51. ± 4.65**	315.77 ± 5.65**	0.45 ± 0.09**	3.785±0.210**
Group 4	L-arginine + Paracetamol (50mg/kg)	96.04± 4.77*	393.65. ± 4.45*	336.25 ± 5.33*	0.47 ± 0.21*	4.005±0.105*
Group 5	L-arginine + Paracetamol (100mg/kg)	85.44± 5.12**	386.42 ± 4.78**	322.53 ± 4.96**	0.32 ± 0.13	3.86±1.021**

Data are expressed as mean ± S.E.M from six rats and analyze by one way ANOVA followed by Tukey tests. *P<0.05, **P<0.01 as compared to normal group animals. Serum Glutamate Pyruvate Transaminase (SGPT), *Serum Glutamate Oxaloacetate Transaminase (SGOT)*, *Estimation of Serum Alkaline Phosphatase (SALP)*

Histopathology Studies

Histological samples were categorized based on the extent of hepatic injuries like necrosis, inflammation, fibrosis, vascular characteristics and overall injury (figure 4-8) [17].

The results of histopathological studies of normal rat liver showed normal hepatocytes, sinusoids in control group (Group 1.). Liver section of rat treated with Paracetamol exhibited severe necrosis, disappearance of hepatocytes and areas of inflammation with increased sinusoidal spaces.

Liver section of rat treated with Arginine (50mg) and paracetamol exhibited mild degree of necrosis, reduced sinusoidal dilation and less inflammation in group 4. Liver section of rat treated with arginine (100mg) and paracetamol showed normal hepatocyte appearance with normal sinusoids with no inflammation in group 5. Liver section of rat treated with silymarin (100mg) and paracetamol exhibited normal hepatocytes in group 3. The results are given in Fig. 4 to 8.

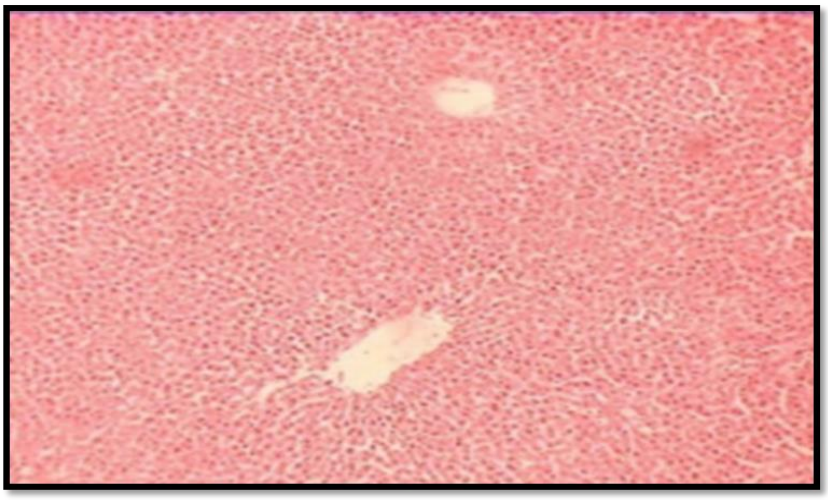

Fig. 4: Histopathological photomicrograph in the experimental rats liver (group 1)

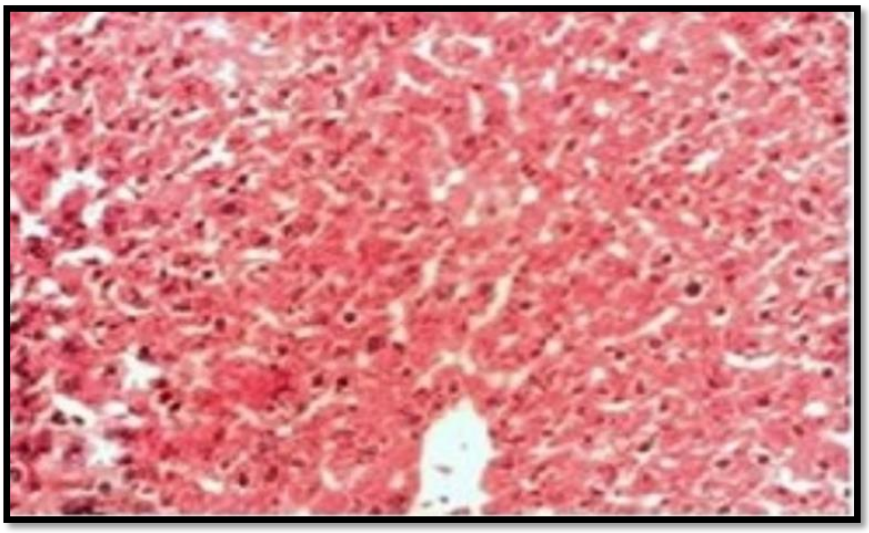

Fig. 5: Histopathological photomicrograph in the experimental rats liver (group 2)

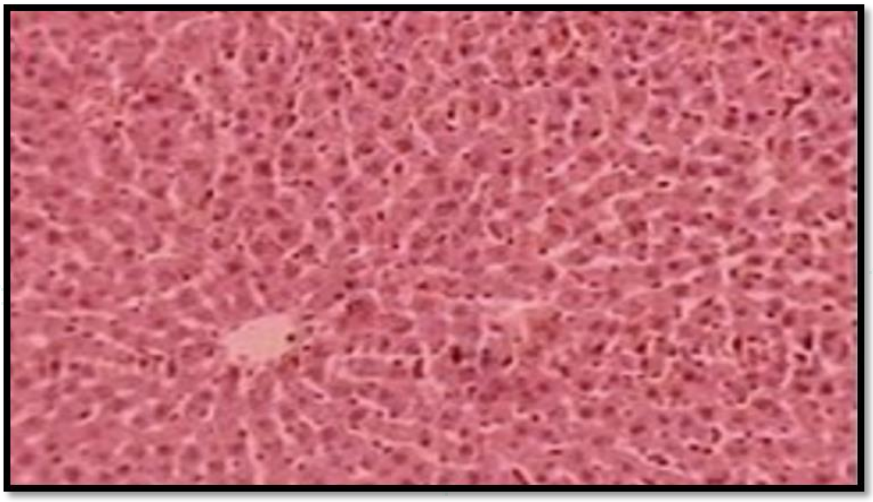

Fig. 6: Histopathological photomicrograph in the experimental rats liver (group 3)

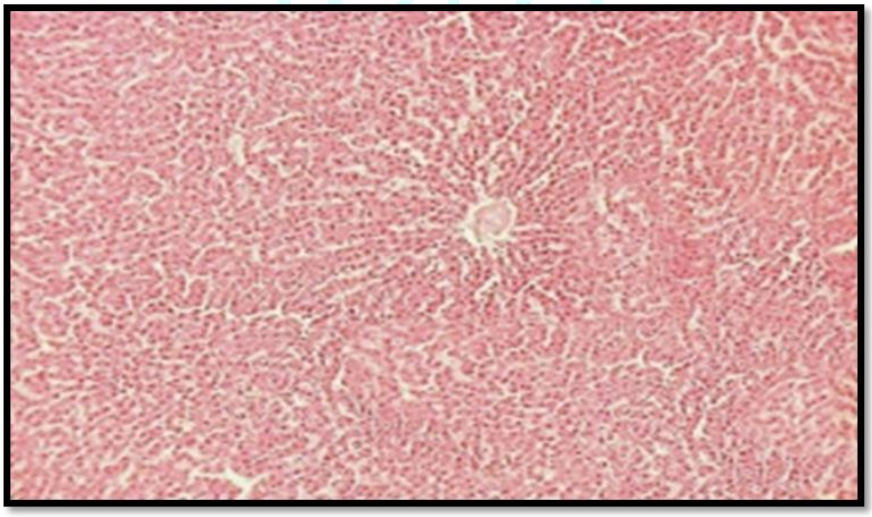

Fig. 7: Histopathological photomicrograph in the experimental rats liver (group 4)

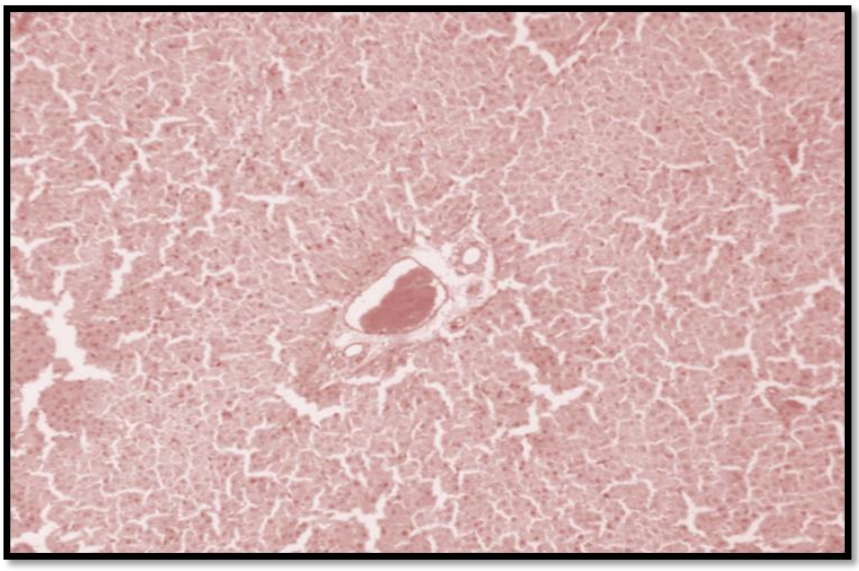

Fig. 8: Histopathological photomicrograph in the experimental rats liver (group

DISCUSSION

Hepatoprotective activity of L-arginine was evaluated in paracetamol treated albino Wistar rats. The activity was assessed by measuring serum marker enzymes, bilirubin, alkaline phosphate and histopathological changes in the liver. Enhanced levels of serum marker enzymes, bilirubin, and alkaline phosphate are an indication of liver damage.

Paracetamol is a well-known analgesic and antipyretic drug moiety, but an overdose of this may produce centrilobular hepatic necrosis. More than 90% of the Paracetamol is excreted out after glucuronidation/sulfation and a small amount undergoes metabolism by cytochrome P450 enzyme to form reactive intermediate N-acetyl-p-benzoquinone amine. This intermediate is readily detoxified by GSH, but the saturation of glucuronidation/sulfation occurs when paracetamol is administered at higher doses. This leads to excessive production of N-acetyl-p-benzoquinone amine, which in turn deplete the GSH completely and binds to proteins to form adducts. These adduct cause impairment in the function of cellular proteins. The overdose of paracetamol may also lead to lipid peroxidation and pyridine nucleotide oxidation, which may induce liver.

In the present study, significant hepatic damage was observed in the toxicant group (group 2, paracetamol) animals as evidenced by the elevated levels of serum markers. The alteration in levels reflects the structural integrity of hepatocytes. An enhanced Serum glutamic oxaloacetic transaminase (SGOT) level is usually associated with increased levels of Serum glutamic pyruvic transaminase (SGPT) and conversion of amino acids to keto acids is effected by an enhanced level of these enzymes. The treatment with formulations containing L-arginine equivalent to 50mg and 100 mg; significantly lowered the level of SGPT and SGOT and it indicates that formulations are able to protect the cell membrane integrity of liver. The synthesis of SALP gets increased due to enhanced biliary pressure. Both formulations containing L-arginine equivalent to 50mg and 100 mg significantly reduced the levels of SALP and bilirubin compare to the positive control group. Improvement in the hepatic secretory mechanisms IS clearly indicates the effective control of SALP and bilirubin levels by L-arginine formulations. Both formulations containing L-arginine equivalent to 50mg and 100 mg enhanced serum protein levels and reduced liver weight in their respective groups. Histopathological studies also supported the hepatoprotective effect of both formulations, containing L-arginine equivalent to 50mg and 100 mg. Histopathological studies also supported the results observed by biochemical studies where L-arginine administration for reduced the hepatic injury score of inflammation, apoptosis and necrosis. Oral administration of L-arginine significantly improved hepatic injury. Hestopathographsalso indicated that L-arginine improved the resistance of hepatic cells to be damaged by toxic dose of paracetamol. Improved level of Nitric oxide (NO) produced by L-arginine may play an important role in the metabolism as well as removal of paracetamol.

The liver section of toxicant animals showed degenerative changes, aggregates of mononuclear inflammatory cells with congested sinusoids. Treatment with formulations containing L-arginine equivalent to 50mg and 100 mg helped in regaining the normal hepatic architecture. Reduction in the levels of SGPT and SGOT reflects the stabilization of plasma membrane and the repair of hepatic tissue. An elevated level of liver wt, serum marker enzymes, and total bilirubin was observed in paracetamol treated group 1. Both formulations containing L-arginine equivalent to 50mg and 100 mg significantly altered the levels of all the parameters and almost brought back to the normal level. Possible mechanisms responsible for hepatoprotective effect maybe free radical scavenging and intercepting those radicals involved in the metabolism of paracetamol. The histopathological changes associated with the hepatoprotective activity of both the formulations containing L-arginine equivalent to 50mg and 100 mg support the result of biochemical estimations.

CONCLUSION

The toxic dose of paracetamol enhanced the SGPT, SGOT, ALP and bilirubin levels. Treatment with silymarin as standard and L-arginine as test drugs shows a significant decrease in the levels of biochemical markers to the near normal levels as compared with the control group. Histopathological studies also supported the hepatoprotective effect, containing L-arginine. The liver

section of toxicant animals showed degenerative changes, aggregates of mononuclear inflammatory cells while treatment with formulations containing L-arginine helped in regaining the normal hepatic architecture.Hence, results observed during present work indicated that, administration of L-arginine amino acid showed significant hepatoprotective and hepatocurative effects against paracetamol induced induced hepatotoxicity in rats.

REFERENCES

1. Giannelli G, Quaranta V, Antonaci, S. Tissue remodelling in liver diseases. Histol Histopathol 2003; 18:1267–74.
2. Fu-Sheng W, Jian-Gao F, Zheng Z, Bin G, Hong YW. The global burden of liver disease: The major Impact of China. Hepatol 2014; 60:2099–108.
3. Latha TB, Srikanth A, Kumar EK, Srinivasa MSK, Rao Y, Bhavani B. Comparative hepatoprotective efficacy of Kumaryasava and liv fit against carbon tetrachloride induced hepatic damage in rats. Pharmacol Online. 2009; 1:1127–34.
4. Manna A, Ghosh I, Goswami N, Ghosh LK, Gupta BK. Design and evaluation of an oral controlled release microparticulate drug delivery system of nimesulide by ionotropic gelation technique and statistical optimization by factorial analysis. J Sci Indus Res 1999; 58: 717 - 22.
5. George P, Nikolaos B. Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads. Int J Pharm 2006; 323: 34-42.
6. Sharma V, Singh L, Verma N. Embracing the quality using QbD: a systematic development of optimized arginine beads. Inter J Cur Adv Res 2017; 6:6292-99.
7. Bergmeyer HU, Scheibe P, Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin Chem 1978; 24:58-61.
8. King J. The hydrolases-acid and alkaline phospatase, Practical Clinical Enzymology, Van, D London: Nostrand company Ltd; 1965. p. 191-208.
9. Retiman, S. and Frenkel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Path 1957; 28:56-63.
10. Tietz NW. Carl Burtis & David Brun, 6th edition, Fundamentals of clinical Chemistry. 1970. p. 447.
11. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Path 1954;7: 322-6.
12. Varley R, Practical clinical Bio-chemistry, 4th edition, Interscience Publisher.1975. p. 453-455.
13. Jendrassik L, Grof P. Comparison of five routine methods with the candidate reference method for the determination of bilirubin in neonatal serum. Biochem 1938; 297: 81.
14. Syamasundar KV, Singh B, Thakur RS, Husain A, Kiso Y, Hikino H. Antihepatotoxic principles of Phyllanthus niruri herbs.J Ethnopharmacol 1985;14:41-4.
15. Luna L.G., Manual of Histologic Staining methods of the Armed Forces Institute of Pathology. McGraw Hill Book Company, New York. 1968. p.258.
16. Ilavarasan R, Vasudevan M,Sockalingam A.Hepatoprotective activity of Thespesia populnea bark extracts against carbon tetrachloride-induced liver toxicity in rats. Natu Pro Sci 2003;9:83-86.
17. Nanji AA., Jokelainen K., Rahemtulla, A., Thomas P., Tipoe GL. Increased sevirity of ethanolic liver injury in female rats: role of oxidative stress, endotoxin and chemokines. Am. J. Physiol. Gastrointest. Liver Physiol 2001; 281:1348-56.