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Nanosuspension Formulation of Curcumin in Advanced Drug Delivery System and its Potential Hepatoprotective Effect

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NANOCURCUMIN IS A PROMISING CANDIDATE FOR THERAPEUTIC APPLICATIONS IN ADVANCED DRUG DELIVERY SYSTEM

ABSTRACT
Curcumin is the primary bioactive substance in turmeric, it has numerous health benefits with potential pharmacological action as anti-inflammatory, regulate blood pressure, have potent anti-cancerous activity, anti-arthritis, prevent diabetes, reverse Alzheimer's disease, improve skin condition, anti-aging, prevent eye degeneration and also play a role in depression, rheumatoid arthritis, unusual signs of burnout and protect body from free radicals. Nanoencapsulation incorporate most of the drugs which are poorly water soluble, this help drugs administered through any route with much lesser irritability and much better absorption ratio. Considering the health benefits of curcumin and nanoparticles in drug delivery system, the attempt was made to convert curcumin to nanocurcumin and a successful incorporation of nanocurcumin in medicinal practices for various uses in several conditions. Evaporative precipitation method, where nanosuspension of the parent drug is formed by quick evaporation of solvent and antisolvent using Rotary evaporator, then the final residues were collected and lyophilized using Lyophilizer. The stability analysis performed for nanocurcumin for the period of 3 months was found to have good stability to the solution. The Z-average of curcumin particles was found to be 4706 nm and the resultant nanoparticle size of curcumin was found to be 69.30 nm. The resultant nanosuspension was found to have better solubility in water and PBS as compared to the parent curcumin.

Keywords: Curcumin, Nanocurcumin, Hepatoprotective, Z-average, Lyophilizer

I. INTRODUCTION

Curcumin is a natural phenolic compound extracted from the rhizome of turmeric (Curcuma longa). It has been widely used as a traditional herbal medicine for a variety of diseases. Curcumin contain methoxy groups and phenols which are responsible for its biological and pharmacological properties. Besides being anti-inflammatory and antimicrobial, curcumin can inhibit tumor growth, thus lowering the risk of cancer. The interest in use of curcumin as hepatoprotective has recently increased due to its anti-oxidant, anti-inflammatory and free radical scavenging properties. However, the pharmacodynamic actions of curcumin are limited by its unfavourable pharmacokinetic profile. In fact, curcumin showed low absorption due to its poor solubility in aqueous solutions and fast liver and intestinal metabolism leading to rapid excretion as well as susceptible to photochemical degradation. Additionally, it is highly unstable and rapidly hydrolyzed at physiological pH, and undergoes intense hepatic biotransformation. Improving oral bioavailability of curcumin is a major challenging factor. However, to overcome curcumin's unfavorable pharmacokinetic profile, Nano formulations have been proposed, including curcumin-loaded nanoparticles, complexes with phospholipids,micro emulsifying and association with drug bioenhancers in advanced drug delivery system.

The aim of our study is to increase the absorption of curcumin by preparing its nanosuspension and to effectively study its hepatoprotective effect in hepatotoxicity induced by alpha-amanitin.

II. METHODOLOGY

Technical grade curcumin was procured from HIMEDIA with CAS no. and purity. HPLC grade Alpha-amanitin pure compound was procured from Sigma Aldrich to experimentally induce liver toxicity in Wistar albino rats. Nanosuspension was prepared by Evaporative precipitation nanosuspension (EPN) method

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described by Kakran et al. (2012): the solution of parent curcumin compound solution was prepared in ethanol (solvent) and then nanosuspension was formed by adding antisolvent hexane. Curcumin nanosuspension was obtained by quick evaporation of the solvent and antisolvent under vacuum using a Rotary evaporator at 47°C. This was followed by vacuum drying of the nanoparticles to completely evaporate all the solvents. The curcumin concentration used were 5, 10, 15 mg/mL and the solvent to antisolvent (SAS) ratios were verified from 1:10, 1:15, 1:20 (v/v). Here, for 20 ml of curcumin solution in ethanol 200-400 ml hexane was used.

Protocol:

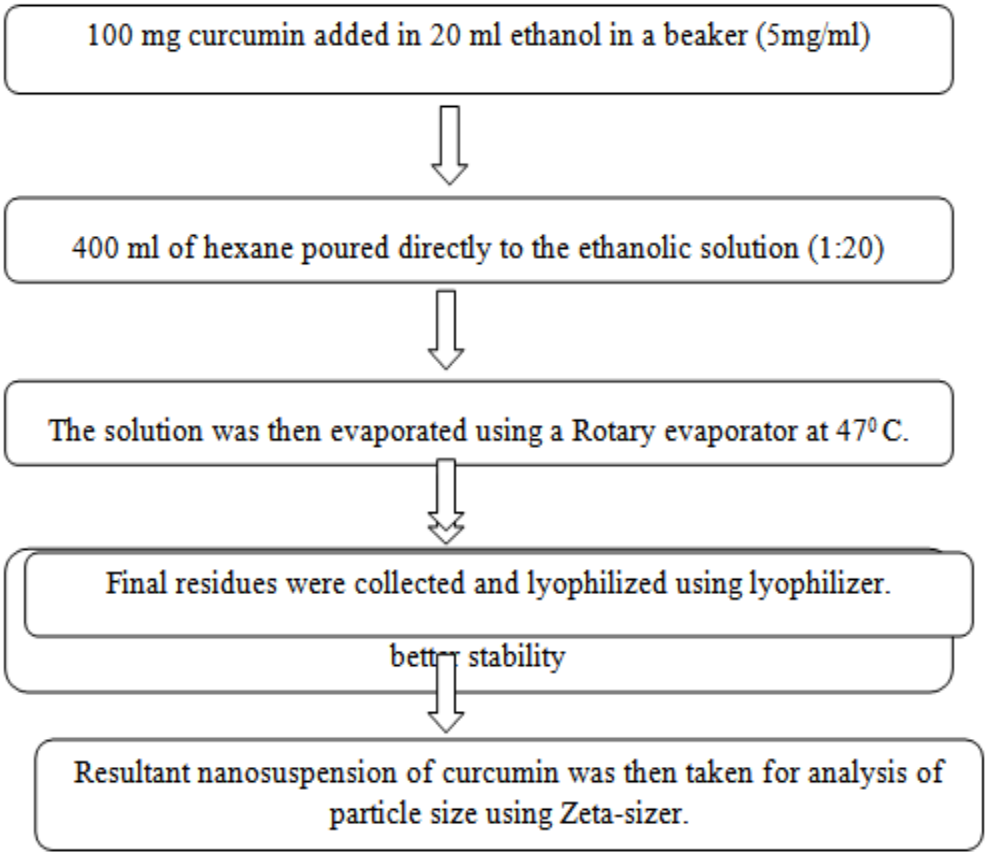

Fig 1: Protocol of preparation of Nanosuspension of curcumin as per Kakran et al. 2012

The stability analysis was performed for nanocurcumin by centrifuging 10% nanosuspension at 3,500 rpm for 30 minutes. Alternatively, the same assay was also performed by storage of nanosuspension for 3 months, to check for better stability to the solution. Hepatoprotective effect of Nanocurcumin was studied invivo by experimentally inducing hepatotoxicity by alpha-amanitin in Wistar albino rats and further confirmed by serum biochemical test and histopathology.

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III. RESULTS

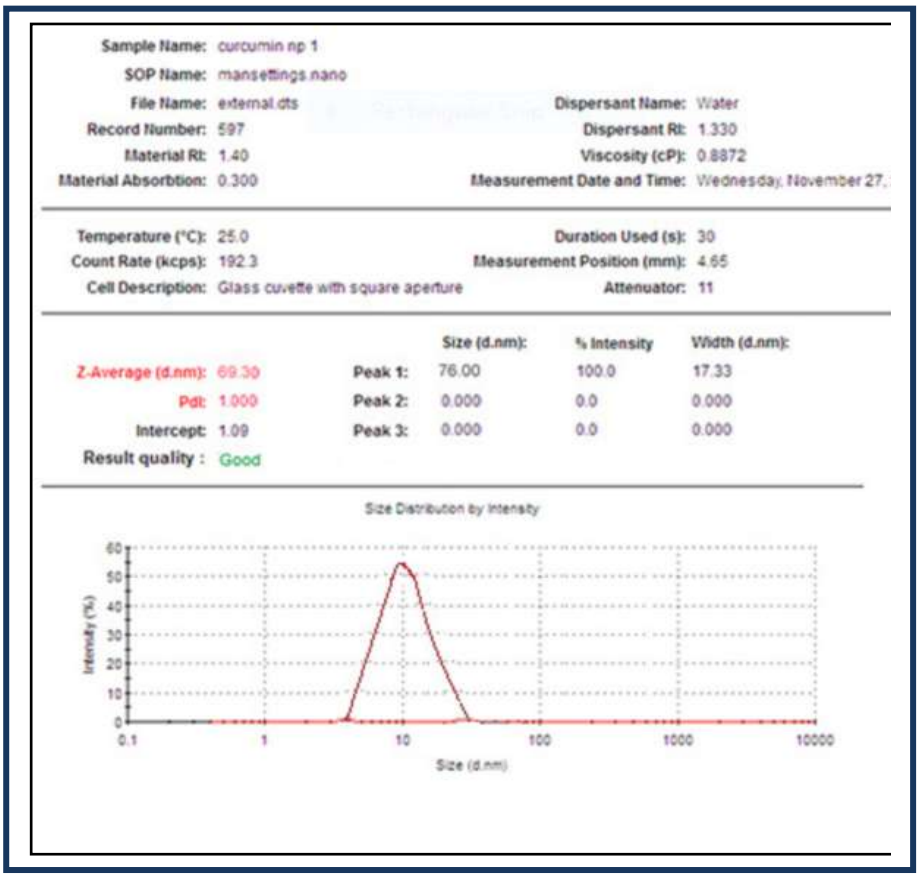

Fig 2a: Z-average of nanocurcumin: 69.30 nm obtained by Zeta-sizer (with EPN method)

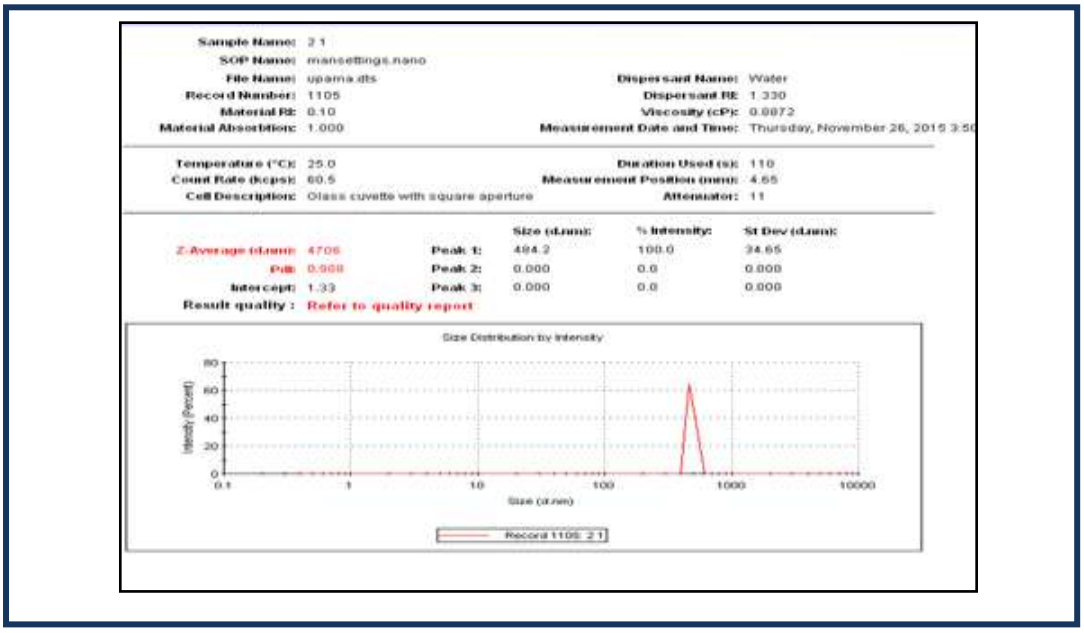

Fig 2a: Z-average of Curcumin: 4706 nm obtained by Zeta-sizer

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Fig 3: Solubility of Curcumin and Nanocurcumin in water

Table 1: Changes in various serum parameters in rats exposed to Alpha-Amanitin, Silymarin, Curcumin and Nanocurcumin

Test groups	AST	ALT	Total Protein	Urea	BUN	Creatinine
Control group (con grp)	86.18±3.97	70.02±1.38	6.90±0.32	23.51±1.44	10.96±0.68	0.98±0.08
Alpha-amanitin treated group (a-ama grp)	1357±322.2	1494±439.0	3.76±0.91	41.62±1.57	19.44±0.73	9.65±2.25
Standard/ standard treated group (std grp)	173.4±47.95	352.4±105.8	6.81±0.11	23.76±1.47	11.09±0.68	1.54±0.16
Curcumin treatment group (cur grp)	716±170.1	693.4±169.9	5.68±0.42	33.04±2.14	15.43±1.00	5.23±0.12
Nanocurumin treatment group @200mg/kg (n-cur 200)	648.8±146.5	568.5±133.9	5.71±0.27	29.92±3.44	13.97±1.60	5.29±0.74
Nanocurumin treatment group @400mg/kg (n-cur 400)	464.9±108.9	477.3±118.4	6.49±0.59	24.99±2.28	11.67±1.06	3.93±0.68

Data expressed as mean±SEM (n=6)

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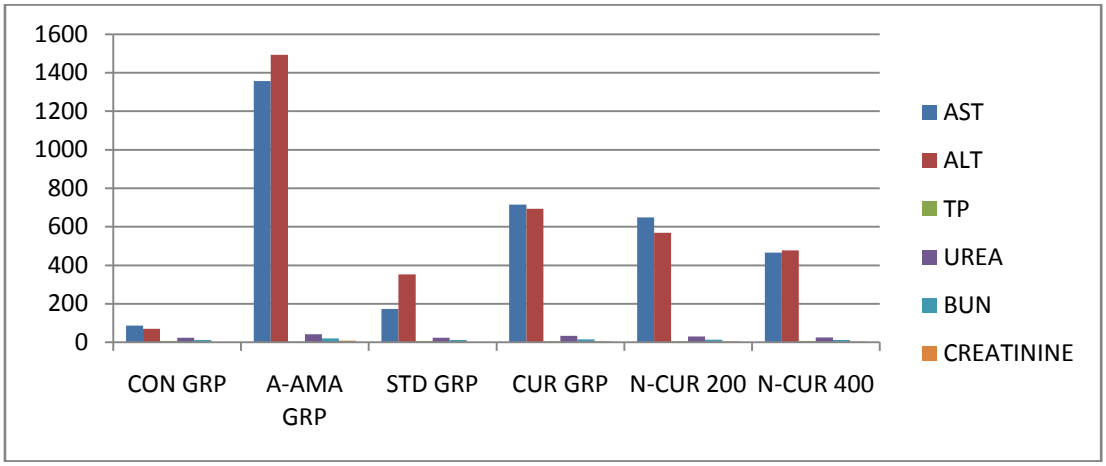

Fig 4: Graphical Representation of Serum Parameters of Rats exposed to Alpha-amanitin, Silymarin, Curcumin and Nanocurcumin

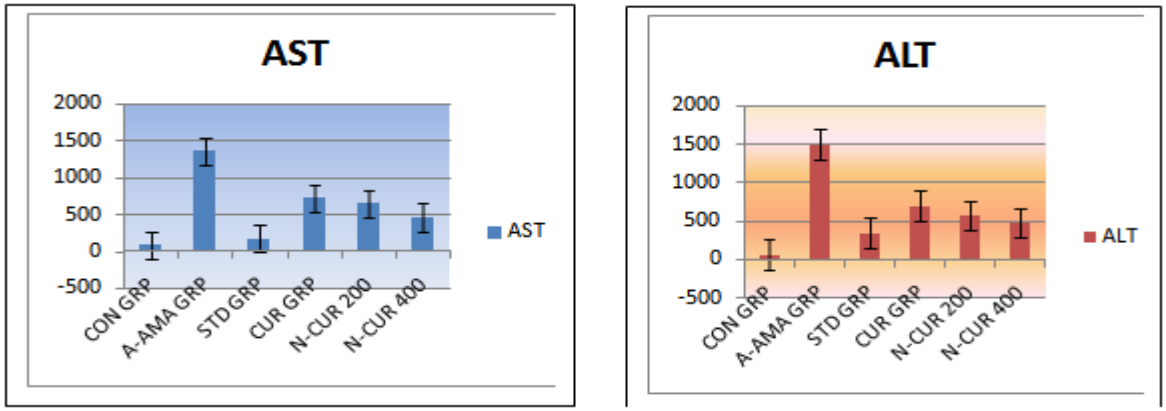

Fig 5a & b: Graphical Representation of Liver enzymes (AST,ALT) of Rats exposed to Alpha-amanitin, Silymarin, Curcumin and Nanocurcumin

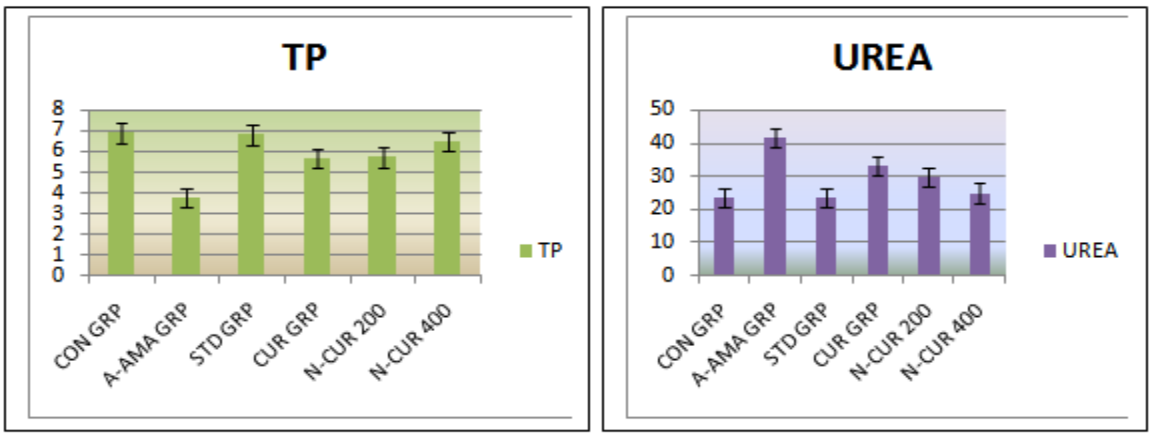

Fig 5c & d: Graphical Representation of Total Protein (TP) & UREA of Rats exposed to Alpha-amanitin, Silymarin, Curcumin and Nanocurcumin

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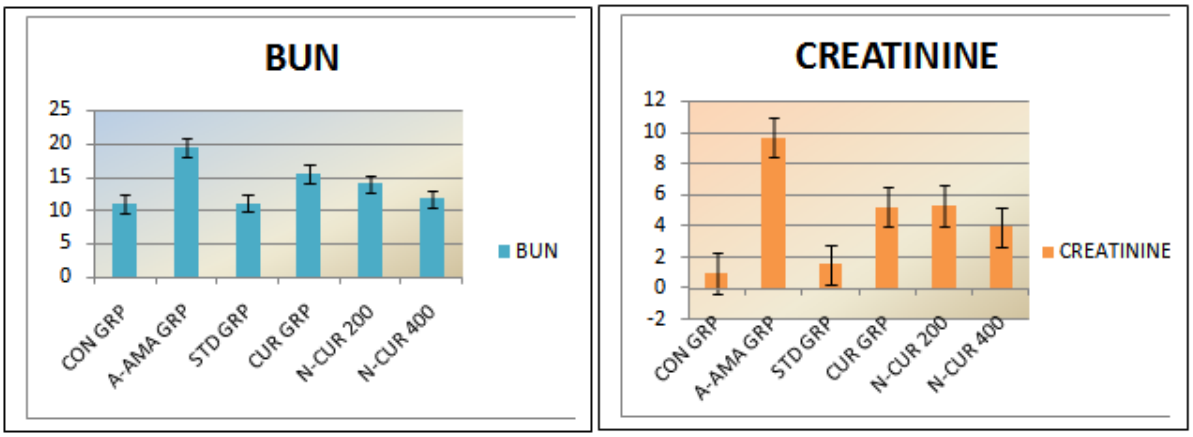

Fig 5 e & f: Graphical Representation of BUN&Creatinine of Rats exposed to Alpha-amanitin, Silymarin, Curcumin and Nanocurcumin

IV. DISCUSSION

The resultant nanoparticle size of curcumin obtained by Evaporative precipitation of nanosuspension (EPN) methods measured by particle size analyzer i.e Zeta-sizer was found to be 69.30 nm (Figure 2a), whereas the parent curcumin compound was found to have particle size of 4706 nm (Figure 2b) Debbarma et al., 2021. The stability analysis performed for nanocurcumin for the period of 3 months was found to have good stability to the solution (Fig 3). The resultant was also found to have better water solubility.

International organization of Standardization (ISO, 2008) defined nanoparticles as a discrete nano-object where all the three dimensions are less than 100nm and the size can range from 1 nm to 100nm. Hencewe can conclude that the resultant nanocurcumin prepared will come under that range specified by ISO and can be used for further studies by considering the advantages of nano-drug preparations in drug delivery system and as a very good hepatoprotective agent.

The solubility of the nanocurcumin showed better solubility than the parent curcumin. curcumin is less soluble in water but nanocurcumin is highly soluble in water and that property increases the absorption of nanocurcumin. This solubility study was compared with Bhawana et al. (2011), where unlike curcumin, nanocurcumin was found to be freely dispersible in water in the absence of any surfactants.

The results of biochemical examination are summarized in Table 1. The obtained resultsrepresent a contribution to a better understanding of liver toxicity caused by alpha-amanitin and its attenuation by nanocurcumin

Invivo Hepatoprotective Study

In this study, serum AST, ALT, Urea, BUN and Creatinine activities were greatly increased in rats exposed to alpha-amanitin as compared to Control group. The increased serum levels of hepatic markers have been attributed to the liver injury because these enzymes are mainly placed in cytoplasmic area of the cell and are released into circulation seen incase of cellular damage (Kucera et al., 2006; Kume et al., 2006). Treatment with nanocurcumin attenuate the toxic effects of alpha-amanitin and the above marker enzyme restored towards the normal level. This effect might be due to the free radical scavenging activity of nanocurcumin and the results obtained in this study was in agreement with the earlier findings of Park et al., 2000; Hismiogullariet al., 2014; Zhao et al., 2006; Mahmoud et al., 2015; Gamal et al., 2016 who had reported the protective effect of curcumin against CCl_4 induced hepatotoxicity and concluded that curcumin lowered the activity of serum aspartate aminotransferase (AST) enzyme in rats intoxicated with CCl_4. The liver marker enzymes (AST, ALT) used as the most common biochemical markers to evaluate liver injury (Kozeret al.,2003; Girishet al., 2009) because these enzymes occur in the cytoplasmic area of the cell and released into circulation in case of cellular damage (Brent and Rumack, 1993). Thus, the activities of these enzymes in serum reflected the severity of liver injury (Zhang et al., 2005).

The Total protein levels for the alpha-amanitin treated group were found to be decreased and subsequently all the animals of this group died. Reduction in total protein content can be deemed as a useful index as the severity of hepatocellular

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damage (Kumar et al., 2007). In the present study, amanitin intoxication reduced the serum total protein level which is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of cytochrome P-450 enzymes leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (Bishayeeet al., 2009).

Histopathological Examination

The histopathological result of the present study revealed that treatment of alpha-amanitin induced hepatotoxicity in rats with nanocurcumin showed moderate improvement of necro-inflammatory changes and fatty changes caused by alpha-amanitin. These results suggested that treatment with nanocurcumin attenuated the severity of inflammation and necrosis induced by alpha-amanitin, which might be due to their antioxidant effect.

These histological changes were in agreement with previous reports of alpha amanitin studies in mice (Kaya et al., 2014; Wills et al., 2005; Zhao et al., 2006).Massive necrosis of the hepatocytes, granularity of the cytoplasm of the hepatocytes were increased indicating necrosis. In addition, moderate proliferation of biliary epithelial cells around the portal with formation of new bile ducts were observed. Moreover, histopathological hepatic damage in laboratory animals is similar to that found in humans after Amanita phalloides intoxication featuring hepatic massive centrilobular necrosis and vacuolar degeneration (Fineschiet al., 1996).

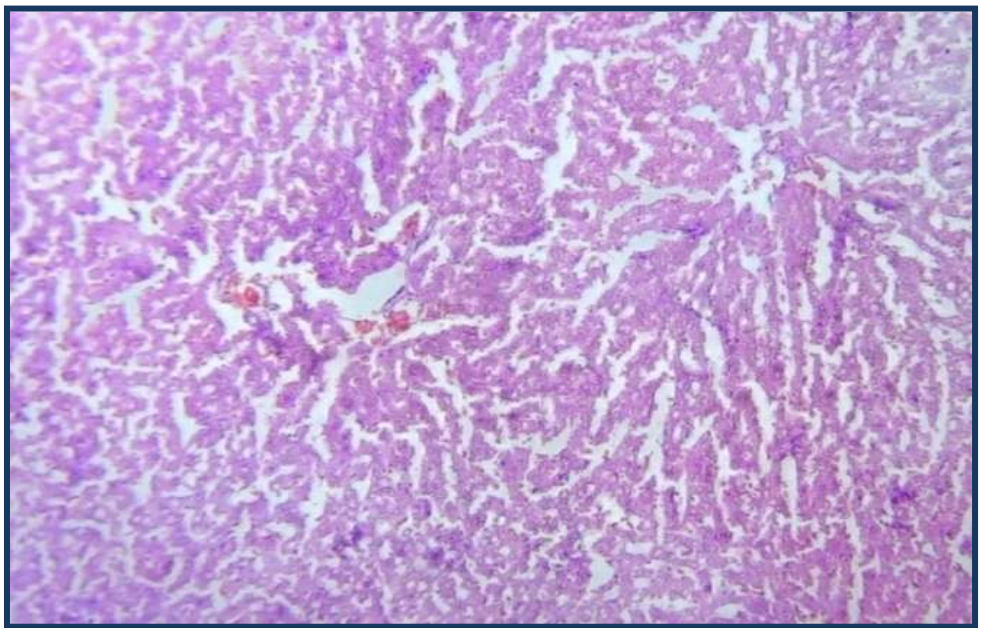

Fig 6: Normal liver,Control group, H&E, ×10

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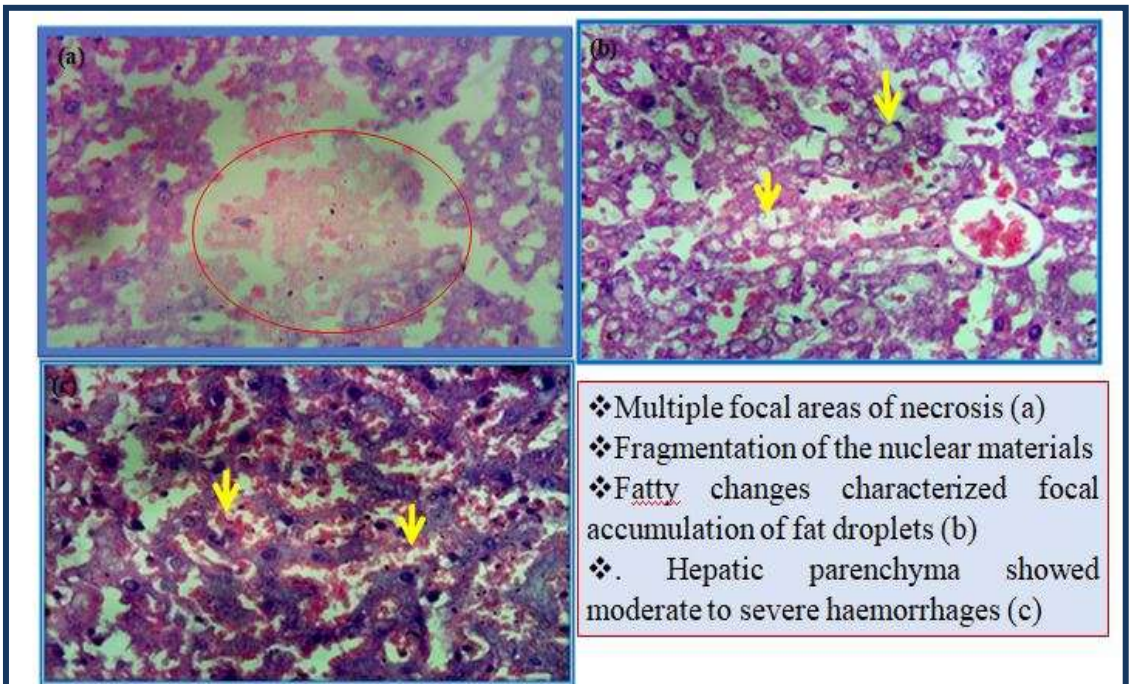

Fig 7a, b, c: AMA-treated group, H&E, ×10

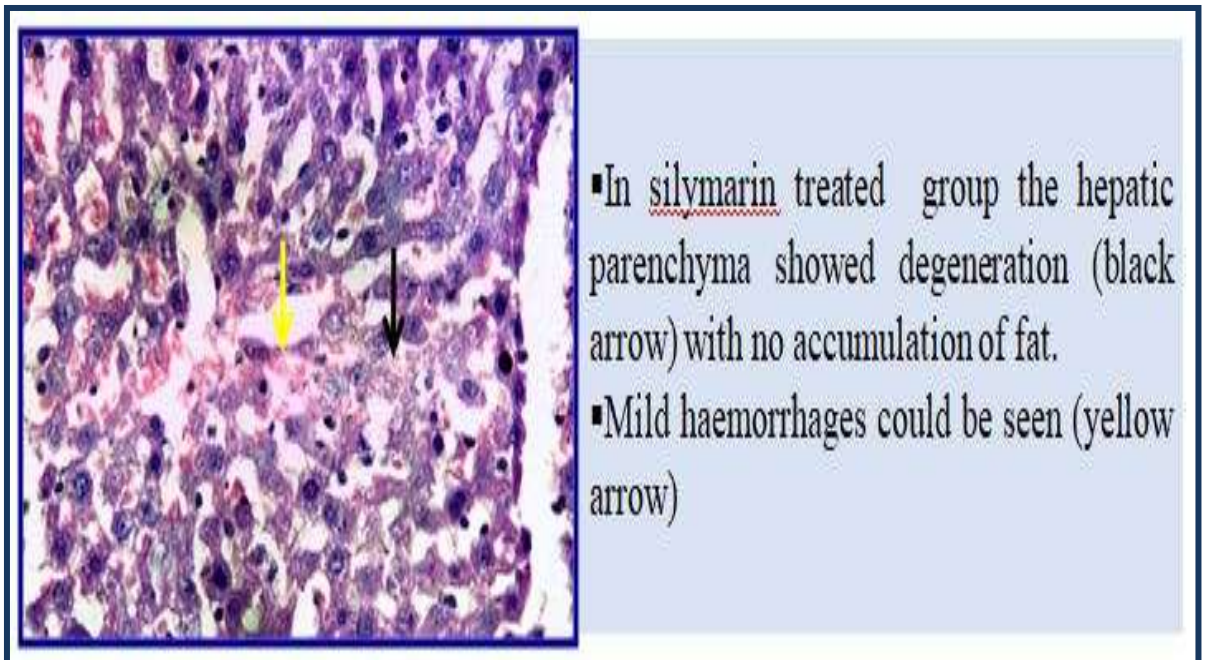

Fig 8: Silymarin-treated (Standard) group, H&E, ×10

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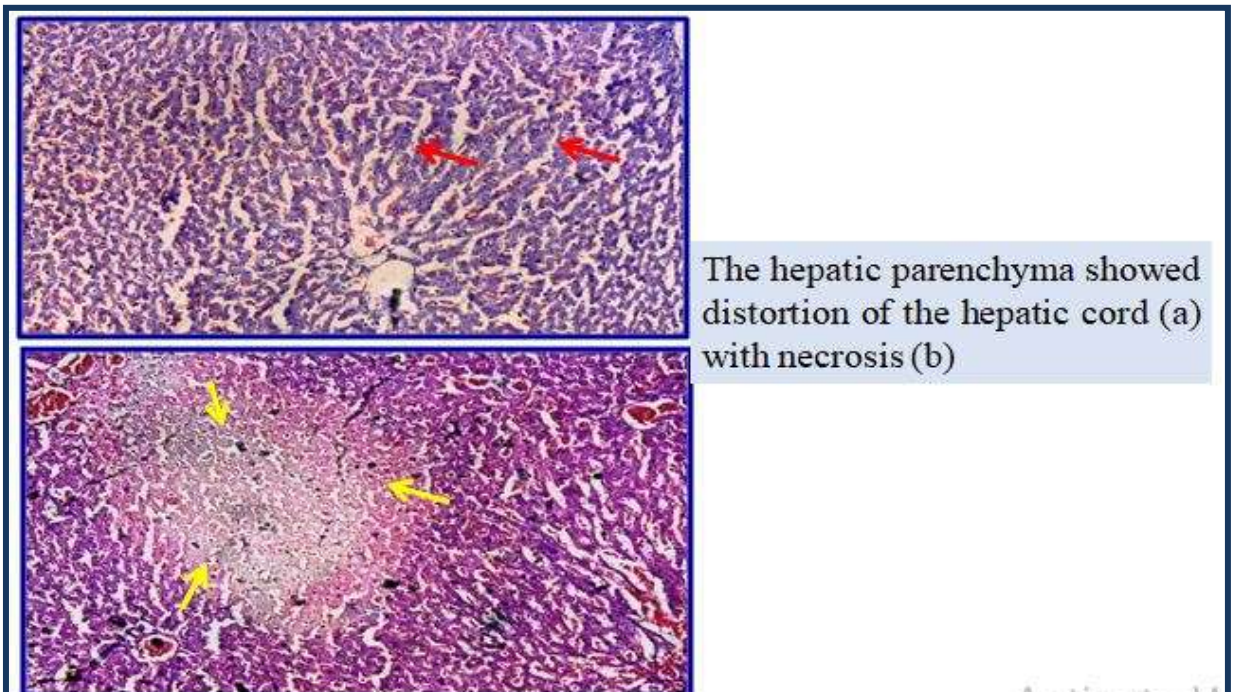

Fig 9a, b: Curcumin-treated group, H&E, ×10

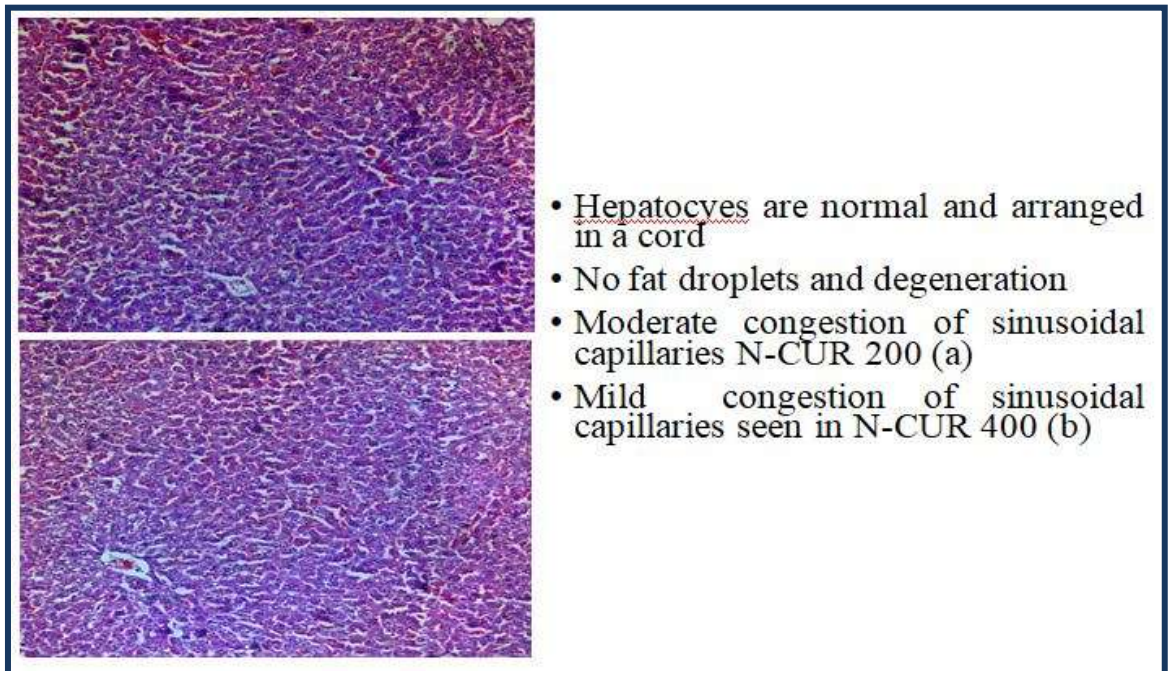

Fig 10a, b: Nanocurcumin-treated (200 mg, 400mg) group, H&E, ×10

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The results of the present study demonstrated that curcumin and nanocurcumin both have protective effect on liver in alpha-amanitin induced hepatic damage in rats and nanocurcumin showed dose dependent hepatoprotective effect: as seen at the dose of 200 mg/kg body weight showed protective effect, whereas at the dose rate of 400 mg/kg showed subsequently greater effect comparatively (highly significant) as shown by the improved serum parameters.

Histopathological study revealed that nanocurcumin protects the liver from alpha-amanitin induced toxicity.So therefore, from the present study data we can be concluded that, nanocurcumin have better efficacy than curcumin with respect to hepatoprotective effect.

Acknowledgement
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Conflict of Interest
Authors do not have any conflict of Interest.

Author(s) Contribution
Project proposal and idea by Jadav Sarma, overall work was done on supervision of Himangshu Baruah, technical work practically and manuscriptsubmission by Khumtya Debbarma.

REFERENCES

[1]. Bhawana; Basniwal, R.K.; Buttar, H.S.; Jain, V.K. and Jain, N. (2011). Curcumin nanoparticles: preparation, characterization and antimicrobial study. Journal of Agricultural and Food Chemistry,**59**(5): 2056-2061.

[2]. Bishayee, A. and Dhir, N. (2009). Resveratrol-mediated chemopreventive of diethyl nitrosamine-initiated hepatocarcinogenesis: inhibition of cell proliferation and induction of apoptosis. Chem. Biol. Interact.,**179**: 131-144.

[3]. Brent, J.A. and Rumack, B.H.(1993). Role of free radicals in toxic hepatic injury. II. Are free radicals the cause of toxin-induced liver injury?J.Toxicol. Clin. Toxicol.,**31**(1): 173-96.

[4]. Debbarma, K.; Sarma, J.; Roy, R. K.; Baruah, H. and Dutta, B. (2021). Role of Nanocurcumin on experimentally induced alpha-amanitin toxicity in rats. The Pharma Inovation, **10**(11): 799-806.

[5]. Fineschi, V.; Di Paolo, M. and Centini, F. (1996). Histological criteria for diagnosis of amanita phalloides poisoning. J. Forensic Sci., **41**(3): 429-432.

[6]. Gamal, A.; Abd-allah, K.A.; EI-Bakry, Mohamed, H.B.; EI-Shymaa, R.; EI-Shymaa R. EI-Khodary. (2016). Protective effects of curcumin and ginger onliver cirrhosis induced by carbon tetrachloride in rats. Int. J. Pharmacol., **12**(4): 361-369.

[7]. Girish, C.; Koner, B. C.; Jayanthi, S.; Rao, K. R.; Rajesh, B. and Pradhan, S. C. (2009). Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. Ind. J. Med. Res.,**129**: 105-114.

[8]. Hismiogullari, S.E.; Hismiogullari, A.A.; Sunay, B. and Paksoy, S. (2014). The protective effect of curcumin on carbon tetrachloride induced liver damage. Revue de medicine veterinaire,**165**(7): 194-200.

[9]. ISO. 2008. ISO/TS 27687 Nanotechnologies – Terminology and definitions for nano-objects-Nanoparticle, nanofibre and nanoplate.

[10]. Kakran, M.; Sahoo, N.; Tan, L.I.; Li, L. (2012). Preparation of nanoparticles of poorly water-solubleantioxidant curcumin by antisolvent precipitation methods.J.Nanopart. Res., **14**: 757.

[11]. Kaya, E.; Surmen, M.G.;Yaykasli, K.O.; Karahan, S.; Oktay, M. and Turan, H. (2014). Dermal absorption and toxicity of alpha amanitin in mice. Cutaneous and Ocular Toxicology, **33**(2): 68-73.

[12]. Kozer, E.; Evans, S.; Barr, J.; Greenberg, R.; Soriano, I. and Bulkowstein, M. et al. (2003). Glutathione, glutathione-dependent enzymes and antioxidant status in erythrocytes from children treated with high-dose paracetamol. Br. J. Clin. Pharmacol.,**55**: 234-240.

[13]. Kucera, O.; Cervinkova, Z.; Lotkova, H.; Krivakova, P.; Rousar, T. and Muzakova, V. (2006). Protective effect of sadenosylmethionine against galactosamine-innduced injury of rat hepatocytes in primary culture. Physiol. Res., **55:** 551-560.

[14]. Kumar, S. V.; Sujatha, C.; Syamala, J.; Nagasudha, B. and Mishra, S. H. (2007). Hepatoprotective activity of extracts from Pergulariadaemia against Carbon

tetrachloride induced toxicity in rats. Pharmacol. Mag.,**3**: 11.

[15]. Kume, H.; Okazaki, H. and Sasaki, H. (2006). Hepatoprotective effect of whey protein on D-galactosamine-induced hepatitis and liver function. Biosci. Biotechnol. Biochem.,**70**(5): 1281-1285.

[16]. Mahmoud, M.; Salem, H.G.; Abd El-Rasheid.; Ahmed N.M.; (2015). Therapeutic effects of curcumin and royal jelly as natural antioxidants on some biochemical parameters in hepatotoxicity induced by carbon tetrachloride (CCl4) in male albino rats. International Journal of Advanced Research.,**3**(11): 520- 535.

[17]. Park E.J.; Jeon, C.H.; Ko, G.; Kim, J.; Sohn, D.H. (2000). Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. J Pharm Pharmacol., **52**: 437–40.

[18]. Wills, B.K.; Haller, N.A.; Peter, D. and White, L.J. (2005). Use of amifostine, a novel cytoprotective, in alpha-amanitin poisoning. Clin. Toxicol. (Phila), **43**(4): 261-267.

[19]. Zhang, J. S.; Wang, H. L.; Yan, X. X. and Zhang, L. D. (2005). Comparison of short-term toxicity between Nano-se and selenite in mice. Life Sci.,**76**: 1099-1109.

[20]. Zhao, J.; Cao, M.; Zhang, J.; Sun, Q.; Chen, Q. and Yang, Z. (2006). Pathological effects of mushroom toxin alpha amanitin on BALB/C mice. Peptides, **27**: 3047-3052.

[21]. Zhao, J.; Cao, M.; Zhang, J.; Sun, Q.; Chen, Q. and Yang, Z. (2006). Pathological effects of mushroom toxin alpha amanitin on BALB/C mice. Peptides, **27**: 3047-3052.