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BIODISTRIBUTION AND TOXICOLOGICAL EVALUATION OF NANOCONSTRACT OF BENDAMUSTINE

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ABSTRACT

The present works deals with the biodistribution and toxicological evaluation of nanoconstruct of Bendamustine. Bendamustine is a most common alkaliting agent used in treatment of cancer. As per the previous studies indicates that anticancer drugs produce toxicity, by affecting on normal cells. Bendamustine is a well stabilized drug for cancer treatment with various formulations. Main objectives of present work were to, Improve biodistribution parameters and reduce toxicological parameters of the nanoconstruct of bendamustine. Were prepared of nanoconstruct of bendamustine with emulsification method by using chitosan and mannose were chitosan work as biodegradable polymer and mannose as ligand. Were characterizations, toxicological evaluation and biodistribution performed in prepared nanoconstruct. Were the characterization of nanoconstruct by SEM, Zitasizer, toxicology and biodistribution were performed by using Albino wistar rat in different groups. The result show that a prepared nanoconstruct was less toxic and it's also alters the biodistribution parameters as compared pure drug of bendamustin.

KEYWORDS: Biodistribution, Toxicology, Nanoconstruct, Biodegradeble Polymer, Bendamustine.

1. INTRODUCTION

Biodistribution is distribution of a compound within a biological system or within organism. Method of tracking where compounds of interest travel in an experimental animal or human subject. Biodistribution studies are necessary to provide preclinical safety evaluation of novel drug delivery system.[1] In anticancer drug main disadvantages that the drug effect on normal cells also which simultaneously causes organ toxicity. Various studies suggested that Polymeric nanostructure are promising drug delivery systems for drugs with low side effects.[2] They can be used as slow release carriers or they can have ligands for site-specific delivery. Nanostructure could increase solubility, decrease degradation during circulation and concentrate the drug at the desired site of action, while decreasing unwanted side effects and all these features can be incorporated in one nanostructure.

Nanoconstruct having various properties that suggested as best delivery system:

- Decrease drug resistance[6,7]
- Decrease toxicity[10]
- Enhance oral bioavailability[11]
- Enhance rate of dissolution[8,9]
- Enhance solubility[12]
- Increase the stability of drug and formulation[13]
- Increase drug targeting ability[14,15]
- Increase patient compliance[16]
- Increase surface area[7]
- Reduce the dose needed[17]

In present work were nanoconstruct of bendamustine is prepared by biodegradable polymer and ligand were the chitosan as polymer and ligand as mannose.

Bendamustine is an intravenously administered alkylating agent that was approved by the FDA following a priority review for treating patients with chronic lymphocytic leukemia (CLL) and others.[19] It is metabolized via multiple pathways, has a short effective *t*1/2 (~40 min) with the maximum plasma concentration (*C*max) typically reached near the end of the infusion period (~1 h), and a low ratio of concentration at 12 h to *C*max (mean 1:25,000).[18] Approximately 95 % of bendamustine is protein bound (mostly to albumin).

Chitosan is a molecule with a carbohydrate backbone structure similar to cellulose, which consists of two types

of repeating units, *N*-acetyl-D-glucosamine and D-glucosamine, linked by (1-4)-β-glycosidic linkage. It is a biopolyaminosaccharide cationic polymer that is obtained from chitin by alkaline deacetylation and characterized by the presence of a large numbers of amino groups on its chain. Although chitosan is obtained from chitin, the applications of the latter compared to chitosan are limited because it is chemically inert.[20] There are some medical applications as antitumour compounds, for example, their degradation products are preferred, as they have a lower viscosity and a better solubility in water. The antitumour activity of chitin/chitosan is manifested by the stimulation of the immune system (production of lymphokines, including interleukins 1 and 2, stimulation of NK.[21] chitosan have great economic value because of their versatile biological activities such as biocompatibility, biodegradability, non-toxicity and adsorptive abilities, as well as chemical applications, mainly in the medical and pharmaceutical fields.[20] In particular, the use of biodegradable nanoparticles for lung delivery is an attractive proposition because of the following advantages: uniform particle distribution in the lung, local administration of vaccine antigens or therapeutic drugs, sustained delivery of macromolecules, improved patient compliance associated with noninvasive immunization and administration of fewer doses, and avoidance of first-pass metabolism, among others.[23,24]

Basic properties of d- mannose as legends are enhance the absorption of drugs, increase the cell site specificity, allow reduced toxicity, and enhance the permeability of drugs.[22,23]

2. MATERIALS AND METHODS

Chitoasn (shrimp shell mw 150kDa) purchased from HIMEDIA D-Mannose purchased from HIMEDIA Sodium triacetoxyborohydride purchased from Sigma Aldrich Methanol 99.9%HPLC Grade purchased from LOBA chem. Aqueous acitic acid purchased from LOBA chem. NaCl purchased from LOBA chem. KCl purchased from LOBA chem. Disodium hydrogen ortho phosphate purchased from LOBA chem. Potassium hydrogen ortho phosphate anhydrous purchased from Molychem Tween 80 purchased from LOBAchem Dialysis tube purchased from Himedia, India Water HPLC grade purchased from LOBAchem Stearic Acid purchased from LOBAchem.

2.1 Formulation of Nanoconstruct

2.1.1 Synthesis of M-chitosan conjugation

M-chitosan was synthesized as described by Yalpani and Laurance (1984) with little modification. Mannose conjugation was carried out by reductive amination of chitosan with d- mannose in the presence of sodium triacetoxyborohydride [NaBH(OAc)3] (reductive amination reagent). In brief, chitosan was dissolved with stirring in 1% aqueous acetic acid of pH 5.5. An aqueous solution of d-mannose and sodium triacetoxyborohydride was prepared. This solution was then, added to the resulting viscous solution of chitosan while stirring slowly and left at room temperature for 2 days. The products were dialyzed against double distilled water in a dialysis tube (MWCO 12-14 kDa; Himedia, India) for 72 h followed by lyophilization.[27]

2.1.2 Preparation of Nanoconstruct

The nanoconstruct were prepared and characterized by reported method of *et. al.* Abather A. sadiq with modification. Of emulsification method with ratio of 50mg: 500mg: 2.5% of drug with chitosan, stearic acid and tween 80 concentrations. were stearic acid melt at their melting point 75°c after the melting of stearic acid , adding of tween 80 drop wise with continuously stirring after the added of tween 80, prepared the solution of bendamustine with m-chitosan, were the solution added in stearic acid solution drop wise with continuously stirring to formed milky form, than sample were centrifuged by cold centrifugation upto2000 rpm for 30 min were the prepared sample was sonicated up to 10 minutes, by Probe Sonicator, than samples were lyophilized for 72hrs. Finally the product were collected, stored at cold temprature and characterized sample.[27]

2.2 Characterization and morphology of Nanoconstruct

2.2.1 Scanning Electron Microscopy (SEM) The size of the nanoparticles was examined using a high Scanning Electron Microscopy (SEM).[3,4]

2.2.2 Zeta potential is an indirect measure of the surface charge. It can be obtained by evaluating the potential difference between the outer Helmholtz plane and the surface of shear. Thus zeta potential of colloidal based dispersion assists in directly evaluating its storage stability. Z Zeta potential values can be utilized in evaluating surface hydrophobicity and the nature of material encapsulated within the nanocapsules or coated onto the surface.[5]

2.3 Toxicological studies

2.3.1 Experimental Design

Albino Wistar Rats (either sex) were used. Total 24 rats were taken and divided into 4 groups, each groups contain 6 rats. As per OECD guideline 423 and 407 The animals were housed in the polypropylene cages. All animals were kept in experimental room. The room was well - ventilated (> 10 air changes per hour) with 100% fresh air. A 12 hour light/dark photoperiod was maintained. Room temperature and relative humidity was set to be maintained between 22 ± 2◦C and 40 -80% respectively according to CPCSEA guidelines. The animals had free access to pelleted feed of standard composition (Ashirwad Industries, Chandigarh, India). Water from (Aqua guard on-line water filter-cum-purifier) was provide ad libitum. The animals were examined at regular intervals for behavioural abnormalities if any. All the experiments were carried out after the protocol was approved by the Institutional Animal Ethics Committee.

2.3.1.1 Test animals
Rat is the preferred rodent species, as a regulatory requirement. Normally females (nulliparous and non-pregnant) generally being slightly more sensitive than males, are used. However, if males have been shown to be more sensitive with structurally related substances then this sex should be used and adequate justification is to be provided.

2.3.1.1.1 Age: Healthy young adult animals should be of age between 8 and 12 weeks at the time of dosing.

2.3.1.1.2 Weight: The weight should fall in an interval within + 20 % of the mean weight of any previously dosed animals.

2.3.1.1.3 Housing and Feeding Conditions

Temperature	22±3ºC
Relative Humidity	50-60% (However, humidity should not be below 30% or exceed 70% at any given point of time).
Lighting	12 hours light and dark cycle
Diet and water	Standard laboratory diet specific to the species and filtered water, free from contamination.

2.3.1.1.4 Number of animals
Six animals are used for each group.

2.3.1.1.5 Allocation of Animals to Various Groups
The animals should be acclimatized to the laboratory conditions for at least 5 days by keeping in individual cages prior to dosing.

Table no. 01.

S. No.	Group name (n=6)	Identification
1	Group I	Control
2	Group II	Pure drug
3	Group III	Formulation
4	Group VI	Placebo

2.3.1.2 Acute Toxicity Study
The acute toxicity of Nanoconstruct was evaluated as per OECD Guideline-423. The six groups are selected and each group in six animals is present. Animals were received dose of Nanoconstruct in rat of 15.3 mg/kg body weight of various formulation groupI I pure drug, group III formulation and group VI placebo. Administration of drugs given on tail vein route The general behaviour of the animal was continuously monitored for ½ hr, 1 hr, 2 hr and 3 hr after dosing, periodically during the first 24 hr (with special attention given during the first 4 hr) and same treatment were followed for 7 days.[28,29]

2.3.1.2.1 Cage-side observations
The detailed cage-side observations were conducted including changes in eyes and mucous membranes, skin and fur, respiratory, circulatory, Autonomic and Central nervous system, and also somatomotor activity and behaviour pattern. Special attention was directed to observations, tremors, diarrhoea, salivation, lethargy, sleep and coma. All rats were observed for toxic signs and any pre-terminal deaths daily.

2.3.1.2.2 Body weight, food and water intake
Food and water intake was recorded daily and average weekly consumption was calculated. Individual body weights were recorded once in a week.

2.3.1.3 Repeated dose 28 days Dose Toxicity
A repeat toxicity study of the same drug was conducted on Albino Wistar Rats of Either for a period of 28 days according to the OECD-407 Guideline. The animals were divided in 6 groups Nanoconstruct in rat of 15.3 mg/kg body weight of various formulation groupI I pure drug, group III formulation and group VI placebo. By tail vein route. While the drug was orally administered using gavage to test groups, saline water was administered to the Normal group for 28 days. All animals were supplied with standard food and water ad libitum during the testing periods. All rats were observed daily for toxic manifestations and mortality. Body weight, water and food intake were measured on a daily basis.[30]

2.3.1.3.1 Cage-side observations
The detailed cage-side observations were conducted including changes in eyes and mucous membranes, skin and fur, respiratory, circulatory, Autonomic and Central nervous system, and also somatomotor activity and behaviour pattern. Special attention was directed to observations, tremors, diarrhoea, salivation, lethargy, sleep and coma. All rats were observed for toxic signs and any pre-terminal deaths daily.

2.3.1.3.2 Body weight, food and water intake
Food and water intake was recorded daily and average weekly consumption was calculated. Individual body weights were recorded once in a week.

2.3.1.3.3 Haematology
Blood was collected by Retro orbital plexus from the overnight fasted animals. Investigation of whole blood for following was done: Red blood cells (RBCs), White blood cells (WBCs), Haemoglobin (Hb), Platelet count, Haematocrit (HTC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), RDW-SD, RDW-CV, PDW, MPV, P-LCR, PCT, Neutrophil (N), Lymphocytes (L), Monocytes (M), Eosinophil's (E), Basophils (B).[32]

2.3.1.3.4 Clinical Biochemistry
Determination in serum or plasma should include: Sodium, potassium, glucose, total cholesterol, urea,

blood urea nitrogen, creatinine, total protein and albumin, Alkaline Phosphatase (ALP), Alanine aminotransferase, Aspartate aminotransferase, Gamma glutamyl transpeptidase, Sorbitol dehydrogenase, Bile acids.[32]

2.3.1.3.5 Biodistribution study

Sacrifices the animal humanly and isolate the organ brain, kidney, liver, lungs, heart and blood respectively than weight the organ and record the weight were wash with the 0.9% w/v normal saline to clean the organ were the individual organ were triturated carefully than placed on epidrop tube adding 100úg were centrifuge in cooling centrifugation temptature 4^{c} at 6000rpm for 5min after the supernatant are collected by help of syringe labeled its and optimized the drug contained in each by the help of HPLC method were collected the data and prepared a report.[34]

2.3.1.3.6. Histopathology

Liver, Kidney, stomach, Intestine, Spleen, Pancreas, Adrenal, Lungs, Heart, Brain and Gonads were fixed immediately in 10% formalin for routine Histopathological examination. The tissues were embedded in paraffin and then sectioned, stained with Haematoxylin and Eosin and were examined under light microscope.[31,32,33]

2.3.2 Statistical Data Analysis

All studies were performed in triplicate and the values were expressed in mean ± SD. The data was analysed by one way analysis of variance (ANOVA) Graph Pad Prism Instat Software (version 6.00, Graph Pad Software), using one way ANOVA followed by student test. ANOVA was done to show that the work done is statistically significant a value of $P < 0.05$ was considered to be statistically insignificant & significant

3. RESULT

3.1 Characterization of nanoparticles

3.1.1 Scanning Electron Microscopy (SEM) the SEM image recorded the presence of sphere shape and well dispersed chitosan nanoparticles and average size was 90nm.

Fig. no 01: Scanning Electron Microscopy (SEM) SEM of nanoparticles of bendamustine Optimized Respectively Size range – 90 nm taken at 20000 X Magnification.

3.1.2 Particle charge analysis: Particle size analyzed by zetasizer and zetasizer analysis graph recorded the optimum range size of nanoparticles found to be.

Table no 02: Particle charge analysis.

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -24.1	Peak 1:	-24.1	100.0	14.8
Zeta Deviation (mV): 14.8	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.566	Peak 3:	0.00	0.0	0.00
Result quality : Good				

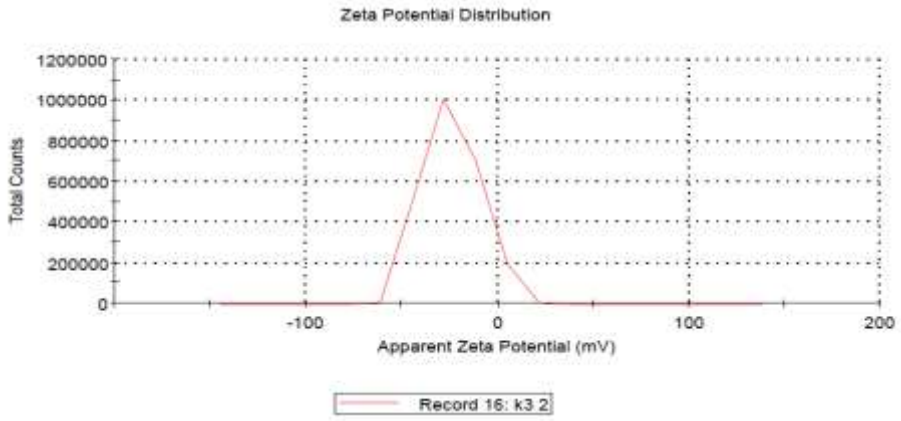

Fig no. 02: Zetasizer analysis graph.

3.2 Toxicological study

3.2.1 In vivo study

3.2.1.1 Acute toxicity study: There were no treatment related death or signs of toxicity developed in the control, pure drug, nanoparticles formulation and placebo treated animals throughout the study. The animals were divided into 4 groups and each containing six animals. Group I for control maintained with normal saline. Group II to VI were treated as test. Animals were used different dose from 15.3mg/kg body of pure drug, formulation and placebo respectively. And time interval should be taken for examine 1/2hr, 2hr, 6hr and 24hr.

3.2.1.1.1 Behavioral observation Behavioral observation of different dose the results should recorded that the animal in all groups were in good condition no unusual change in behavior or in locomotors activity during 24hr shown table no

Table no. 03: Effect of prepared nanoparticles on behavioral observation in rats for 24hrs oral toxicity study.

Observation	Control	Pure drug	Formulation	Placebo
Respiration	Normal	Normal	Normal	Normal
Tremor	No effect	No effect	No effect	No effect
Temperature	Normal	Normal	Normal	Normal
changes in eye	No effect	No effect	No effect	No effect
Urination	Normal	Normal	Normal	Normal
Sedation	No effect	No effect	No effect	No effect
skin colors	No effect	No effect	No effect	No effect
Coma	No effect	No effect	No effect	No effect
Death	Alive	Alive	Alive	Alive

3.2.1.2 Repeated dose 28 days (Repeated) dose toxicity Repeated dose toxicity studies of prepared nanoparticles were examined for 28 day and different time interval of 1st day, 7th day, 14th day, 21st day and 28th day respectively. Animals were divided into as mention section 3.2.1.1.

3.2.1.2.1 Behavioral observation Behavioral observation of different dose the results should recorded that the animal in all groups were in good condition no unusual change in behavior or in locomotors activity during 1st day, 7th day, 14th day, 21st and 28th day shown table no.

3.2.1.2.2 Body weight, food and water intake Body weight, food and water intake all animal showed normal weight gains during study periods. And there was no significant difference in increassing food and water intake between the control group and test groups. Indicates that no effect of nanoparticles on weight of animal.

Table no 04: Effect of prepared nanoparticles on body weight in rats for 28-day repeated dose toxicity study.

Duration	Control	Pure drug	Formulation	Placebo
0 Day	173.33±3.55	174.33±3.71	178.83±4.45	176.33±4.04
7 Days	181.00±4.81	183.83±5.28	187.50±5.89	185.50±5.56
14 Days	190.50±6.39	193.00±6.80	197.66±7.58	194.16±6.99
21 Days	202.16±8.32	201.50±8.21	207.66±9.24	203.00±8.46
28 Days	212.33±10.01	214.33±10.35	217.33±10.85	212.83±10.10

Value are Mean±SEM. (n=6)

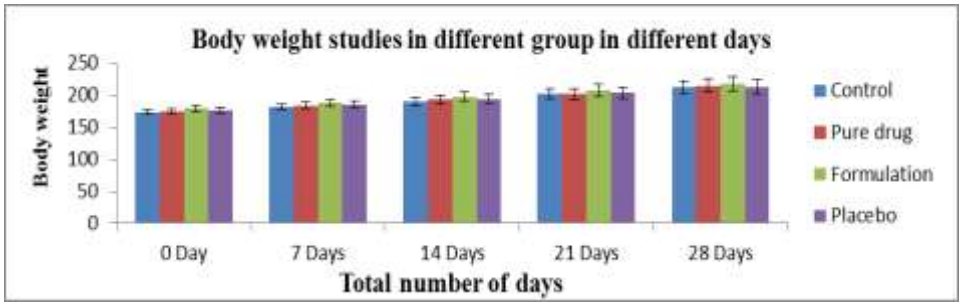

Fig no. 03: Body weight studies in different group in different days.

Table no 05: Effect of prepared nanoparticles on food and water consumption in rats for 28-day repeated dose toxicity study.

Duration	Control	Pure drug	formulation	Placebo
0 Day	83±1.57	84±1.73	82±1.42	84±1.73
7 Days	84±1.73	85±1.89	86±2.04	86±2.04
14 Days	88±2.36	88±2.36	89±2.52	89±2.52
21 Days	96±3.67	96±3.67	94±3.34	94±3.34
28 Days	107±5.48	106±5.32	107±5.48	106±5.32

Value are Mean±SEM. (n=6)

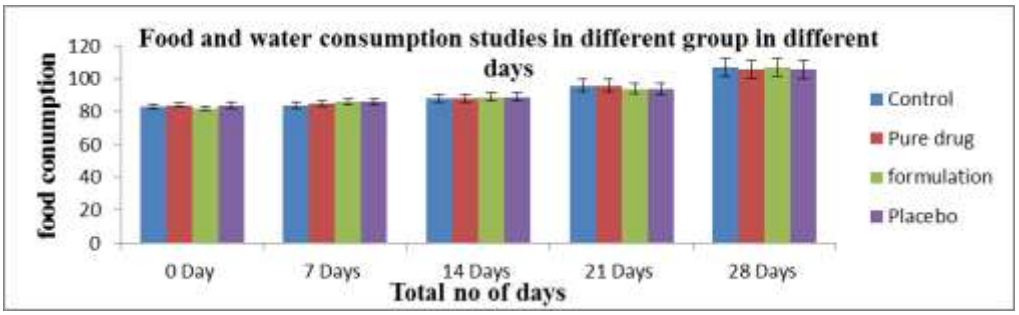

Fig No. 04: Food and water consumption studies in different group in different days.

3.2.1.2.3 Hematological toxicity Hematological studies having various type of parameter haemoglobine, total RBCs count, lymphocyte count and eosinophil count had been investigated result shown below.

Table no 06: Total haemoglobin count in different group before and after treatment.

Haemoglobine	Control	Pure drug	Formula	Placebo
0 day	14.5± 0.054	13.2±1.41	13.5±1.30	13.7±0.23
7th day	14.5±0.054	12.3±1.43	12.4±1.36	13.7±0.20
14th day	14.6±0.056	11.7±1.51	11.2±1.24	13.5±0.24
21st day	14.5±0.054	10.4±1.65	10.9±1.41	13.6±0.26
28th day	14.6±0.056	9.7±1.58	10.2±1.10	13.2±0.48

Value are Mean±SEM. (n=6)

Table no. 07: Lymphocyte count determination in different group after and befor the treatment.

Lymphocyte	Control group	Pure drug	formulation	Placebo
0 day	44±0.54	45±4.32	43±2.54	42±0.83
7th day	43±0.52	49±4.34	44±2.58	40±0.84
14th day	43±0.51	52±4.29	46±2.51	40±0.82
21st day	44±0.52	54±4.38	48±2.56	41±0.87
28th day	43±0.54	56±4.35	49±2.57	41±0.89

Value are Mean±SEM. (n=6)

Table no. 08: Eocinophil count in different group before and after treatment.

Eosinophiles	Control	Pure drug	Formulation	Placebo
0 day	5±0.44	6±0.41	5±0.42	5±0.44
7th day	5±0.41	6.2±0.44	5.2±0.41	6±0.41
14th day	5±0.44	6.4±0.41	5.7±0.42	5±0.42
21th day	5±0.43	6.9±0.42	5.8±0.43	5±0.43
28th day	4±0.44	7±0.43	6±0.44	4±0.41

Value are Mean±SEM. (n=6)

Table no 09: RBCs count in different group befor and after the treatment.

RBCs count million/cmm	control	Pure drug	Formulation	Placebo
0 day	4.1±0.27	5.1±0.62	6.1±0.21	6.1±0.10
7th day	4.2±0.21	5±0.61	6.1±0.24	6.1±0.14
14th day	4.8±0.26	4.3±0.64	6.2±0.26	6.3±0.13
21st day	4.2±0.24	4±0.61	6±0.21	6.1±0.14
28th day	4.5±0.21	3.7±0.63	5.7±0.23	6±0.10

Value are Mean±SEM. (n=6)

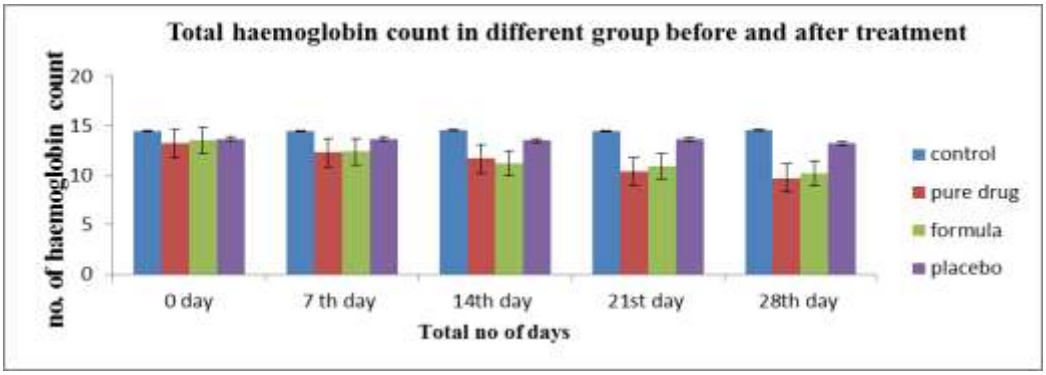

Fig No. 05: Total haemoglobin count in different group before and after treatment.

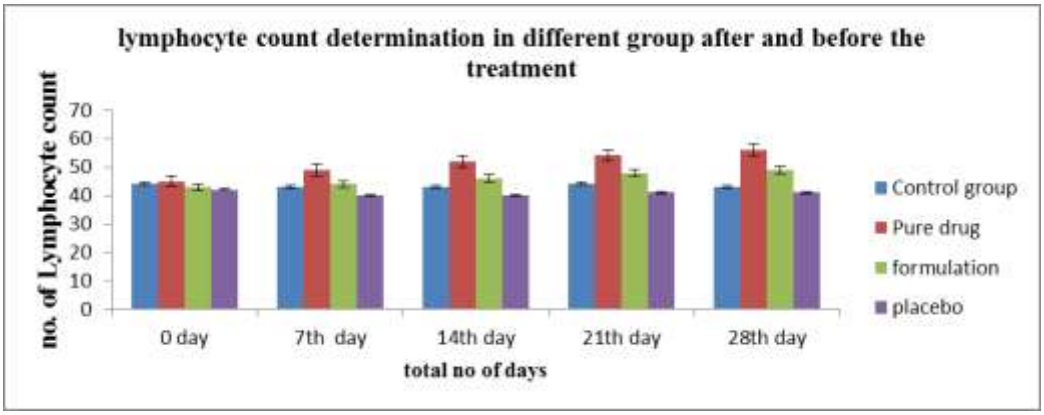

Fig No. 06: Lymphocyte count determination in different group after and before the treatment.

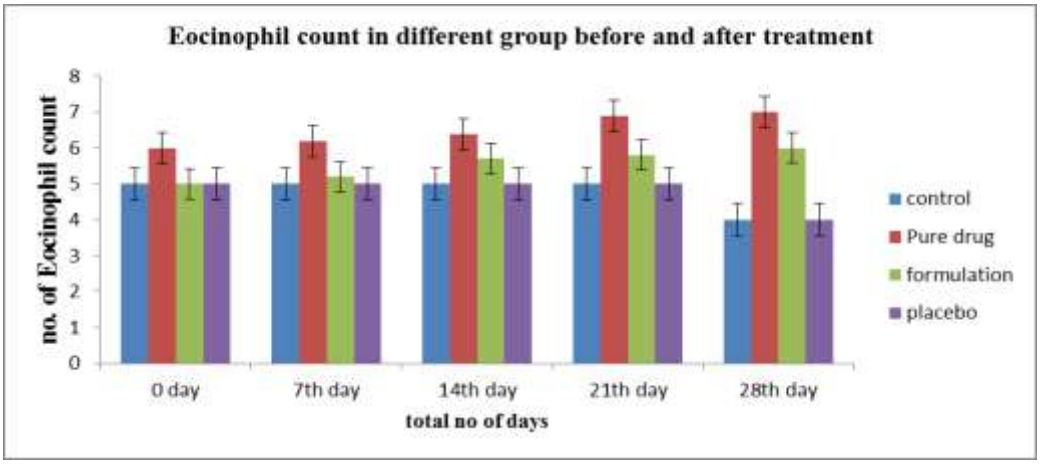

Fig No. 07: Eocinophil count in different group before and after treatment.

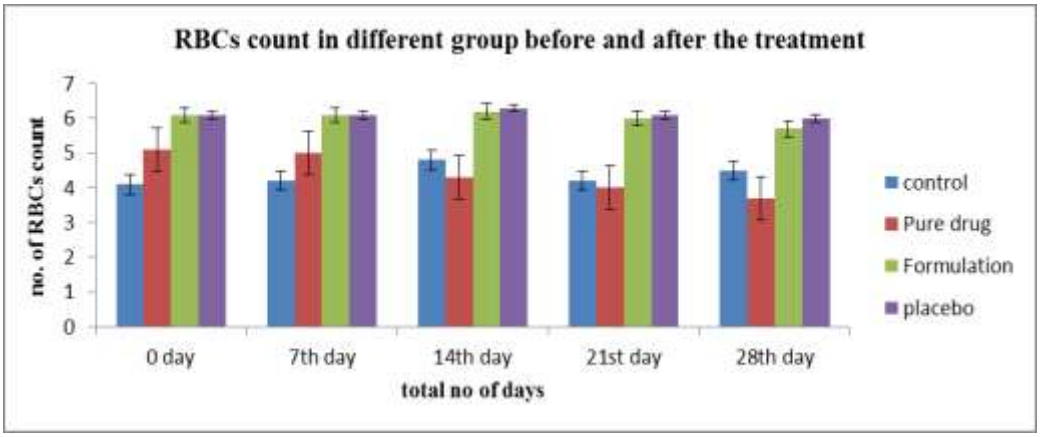

Fig No. 08: RBCs count in different group before and after the treatment.

3.2.1.2.4 Biochemical studies: Clinical biochemistry parameters were examined by following the procedure as mentioned in section 5.15.8.4 the collected plasma sample were examined for Sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, creatinine, total protein and albumin, Alkaline Phosphates (ALP), Alanine aminotransferase, Aspartate aminotransferase, Gamma glutamyl transpeptidase, Sorbitol Dehydrogenase, Bile acids.

Table no. 10: After Determine blood glucose level in different group before and after treatment.

Blood glucose	Control	Pure drug	formulation	Placebo
0 day	87±0.84	84±0.51	85±0.52	89±0.56
7th day	87±0.87	78±0.89	83±0.58	87±0.84
14th day	86±0.64	74±0.74	80±0.49	87±0.21
21st day	85±0.87	68±0.65	78±0.94	85±0.64
28th day	85±0.81	54±1.07	76±0.89	84±0.14

Value are Mean±SEM. (n=6)

Table no. 11: SGPT determination in different group before and after treatment.

SGPT	Control	Pure drug	Formulation	Placebo
0 day	32±0.55	34±0.86	31±0.56	32±0.87
7th day	32±0.54	39±0.87	33±0.58	32±0.24
14th day	33±0.57	48±0.81	35±0.24	33±0.74
21st day	33±0.58	51±1.54	37±0.58	34±0.98
28th day	33±0.59	54±1.25	38±0.98	34±0.84

Value are Mean±SEM. (n=6)

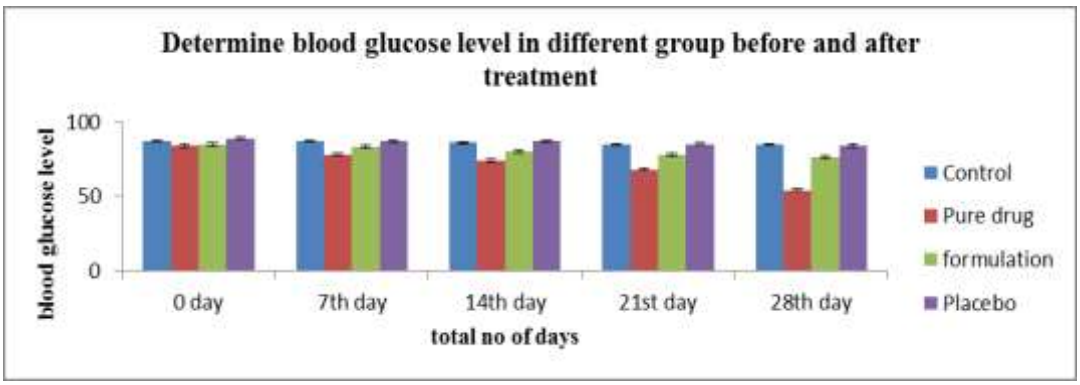

Fig no 09: Determine blood glucose level in different group before and after treatment.

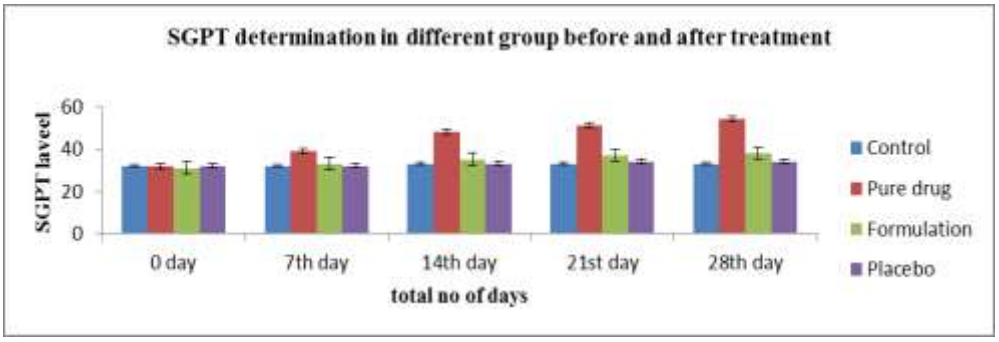

Fig No. 10: SGPT determination in different group before and after treatment.

Table no. 12: Biodistribution studies after 28 days of treatment with pure and formulated drugs.

Groups	Brain (mg)	Lungs (mg)	Liver (mg)	Kidneys (mg)	Heart (mg)	Blood (mg)
Pure bendamustine	5.53±0.388	10.57±0.467	5.57±0.321	4.74±0.241	4.00±0.123	5.91±0.148
Formulation	2.36±0.124	6.02±0.328	3.26±0.256	4.56±0.147	4.06±0.234	4.39±0.128

Value are Mean±SEM. (n=3)

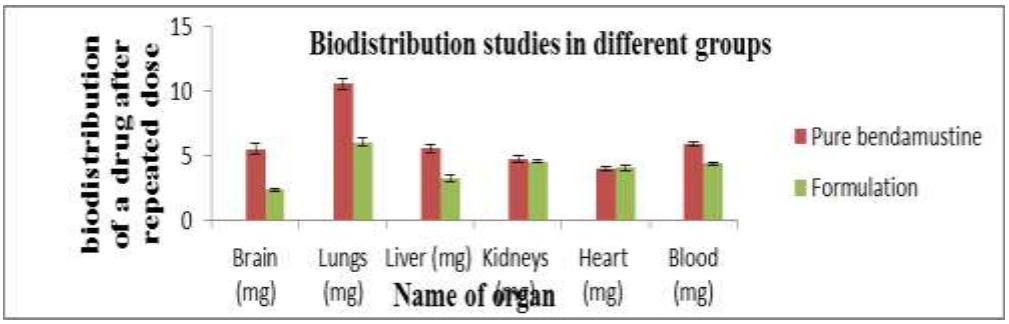

Fig no. 11: Biodistribution studies in different groups.

3.2.1.2.5 Histopathological studies: Histopathological studies performed after 28 days of treatment on this studies comparison of the histological parameters of different organ liver, kidney and lungs respectively had been studied in different four group which are already mansion previously and result was shown below.

3.2.1.2.5.1 Histological studies of liver

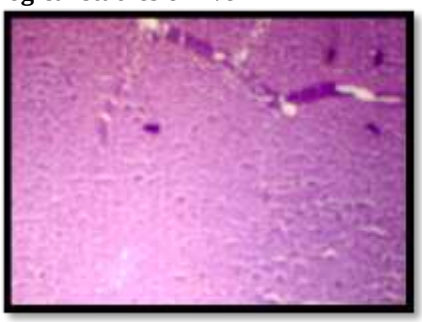

Fig 12: Group I (Control).

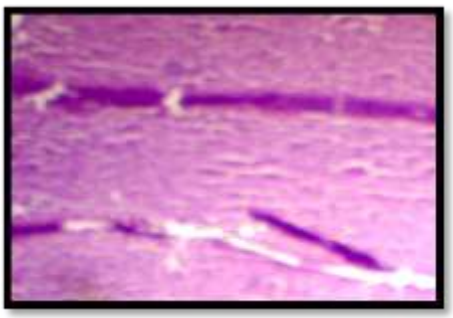

Fig 13: Group IV(placebo).

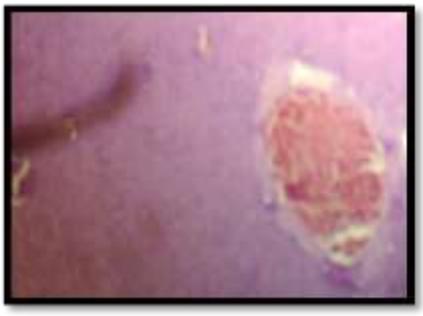

Fig 14: Group II (Pure drug).

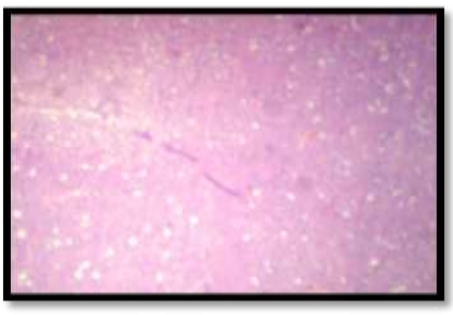

Fig 15: Group III (formulation).

3.2.1.2.5.2 Histological studies of kidney

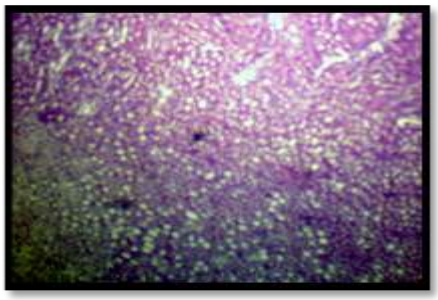

Fig 16: Group I (control).

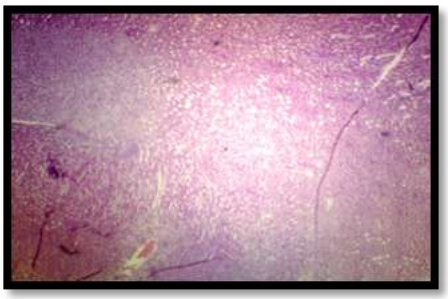

Fig 17: Group IV (placebo).

Fig 18: Group II (pure drug).

Fig 19: Group III (formulation).

3.2.1.2.5.3 Histological studies of lungs

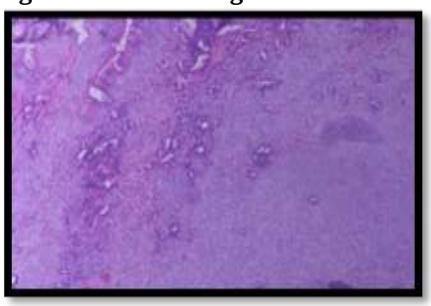

Fig 20: Group I (control).

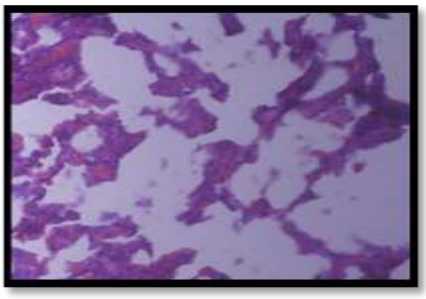

Fig 21: Group IV (placebo).

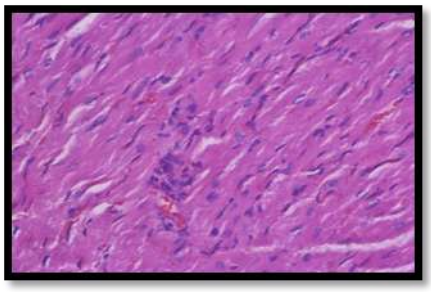

Fig 22: Group II (pure drug).

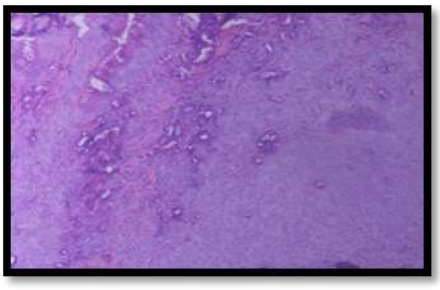

Fig 23: Group III (formulation).

Table no. 13: Histophatological report of different groups.

Organ	Control	Pure bendamustine	Formulation	Placebo
Kidney	Normal	Several degenerative changed and hemorrhages	Slightly necrosis in renal tubules	Normal
Liver	Normal	Conjugation, necrosis in liver and dilation of sinusoid	Normal	Normal
Lungs	Normal	Accumulation of transudate in the alveoli of lungs and degeneration of alveoli in lung	Normal	Normal

4. DISCUSSIONS

Toxicity produced with treatment of anticancer drug is vast problem in ancient to till behalf of these many researchers try to establish various approaches to reduced the toxic effect of anticancer drugs. So hypothisis suggested the Nanoconstructs have vast potential in the field of medical, therapeutic applications as well as diagnostic purpose to improve the quality performance of many products. Bendamustine is one of the alkylating agents used as anticancer drug that have various type of toxicity produced on chemotherapy session. These work using for reduced toxicity and change biodistribution patron of bendamustine. Nanoconstruct prepared by the natural polymers which are inert in biochemical environment and help to increases the site specific. Polymer increases the site specific that's promote the change the biodistribution patron directly. There is many type of natural and synthetic polymer is present which help out to formulate the nanoconstruct. Chitosan is one of the natural biodegradable polymers. It a quit safe for human application and it is very significant to know about the biocompatibility and metabolism of these nanoparticles. Also mannose is used as very good ligand for nanoconstructs that having potency to site specific. After the formulation of nanoconstructs it's necessary to characterized and evaluated the toxicity of nanoconstructs for it's *in vio* and *in vitro* stability of formulation. On this work the characterization parameters of nanoconstruct had been found the morphology size of the particles found to be 90nm that mansion good for nanoconstructs and zetapotency was found to be -24mV it's important for the ionization and that formed compatible interaction with biological membrane. In nanoconstruct the entrapment efficiency was important parameters for the stability of formulation the entrapment efficiency found to be 42%. After the characterization of nanoconstruct toxicity and biodistribution parameter had been studies for the *iv vivo* studies of formulation. In toxicity studies were rats are used as exparimantal animal and divided into four groups were mansion previously. Parameters are behavioral, food and water intact, body weight, heamatological, biochemical, histopathological and biodistribution studies respectively.

Behavioral studies were recorded there is on 1st day after administration there is no change in behavior as compare to control, all parameters are same in group I, II, III, and IV as well but after repeated dose of 28 days there is some changes in urination in group II increases urination behavioral shown on table no. 04.

There was no statistically significant difference (*P* >0_05) in increase in body weight between the control group and experimental group shown on table no. The consumption of food and water by the animals followed a similar pattern indicating a normal metabolism of the animals. The animals showed no difference in food conversion efficiency, indicating that the feed intake and utilization was not affected due to intake of nanoconstruct results shown on table no. 05.

Haematological parameters viz; haemoglobin concentration, red blood corpuscles count, white blood corpuscles count, erythrocyte sedimentation rate and differential leukocyte count in both control and experimental groups were conducted for 28 days. There is some significant difference in control group and experimental group (*P* >0_05) that were calculated Value are Mean±SEM. (n=6) there in group II heamoglobin count was reduced and lymphocyte count was increase as compare to group I the increases and decrease the parameter of hematological that indicates the drug produced hematological toxicity. Our work is to reduced that toxicity of drug by formulate nanoconstruct. By report the parameters are which are increases in Group II must reduced on Group III shown on table no.06, 07, 08 and 09 that may suggested the hematological toxicity were reduced by the formulation of nanoconstruct.

Biochemical parameters the biochemical tests are used to diagnosis diseases of heart, liver, kidney, etc. They are also widely used in monitoring the response of animals to toxic materials. If the organs do not function properly there will be increase in the enzyme levels. The elevated levels indicate the occurrence of liver damage, ischemic heart disease, and acute coronary syndromes, in the present study administration of pure drug formulation and placebo cause any significant difference in the control group and the experimental (*P* >0_05) group SGPT (serum glutamic-pyruvic transaminase) was increase and blood glucose were decreases in Group II as compare to Group I. The biochemical parameter which were affected significant change in Group III Mean±SEM. (n=6) shown on table no. 10 and 11Results suggested reduced the biochemical toxicity by the formulated nanoconstructs.

Organ weight had been calculated after 28th day of treatment shown on table no. 21 investigated shows that weight of liver is reduced as compare to control group by liver toxicity. Histopathological studies were carried out to record the effect of pure drug and formulated nanoconstruct on the vital organs.

There was some histopathological difference in the control and pure drug and formulated nanoconstruct administered Groups I, II, III and IV. Our findings indicate that formulated nanoparticles reduced the toxicity which produced by pure drug were the 28-day study experimental period shown on table no. 13 therefore report suggested that formulation on nanoconstruct were quite safer as compare the pure drug.

Biodistribution studies were studied for the investigated the distribution of drug in body organ and protein binding properties pure drug of bendamustine having higher degree of protein binging. By the formulated

nanoconstruct it was reduced shown on table no.12 therefore report suggested that the biodistribution pattern were modified.

5. CONCLUSION

It was concluded that formulation of nanoconstruct could be exploited in order to reduce the toxicity and change biodistribution pattern incorporation of bendamustine into chitoan, manose nanoconstruct by emulsification method. A technical advantage of this because bendamustine anticancer drug belongs to III category of BCS demonstrated the high solubility and low permeability. Here it is notable that, by the formulation of nanoconstruct of bendamustine its improve the permeability pattern of pure drug by using mannose as ligand and improve the zetapotential of drug by formulating nanoconstruct of chitosan. It's also suggested the site specificity of drug that increases mechanical strength and its ability to be formulated as a drug nanoconstruct. Studies suggested that the formulation of nanoconstruct is reduced the toxicity and modified biodistribution pattern.

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