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Evaluation of the cytotoxic effect of Malic acid-Butane 1,4-diol Copolyester in Albino Rats

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Abstract
MBC biodegradable co-polyester was synthesized from malic acid and butane 1,4-diol following Dean-stark apparatus using p-toluene sulphonic acid (Approximately 0.4% of the total weight) as catalyst. This copolyester was administered intraperitoneally to the rats at a dose of 300 µg $rat^{-1}day^{-1}$ for 21 consecutive days in order to assessing the toxic effect of the polymer. The gross general observations such as changes of body weight, hematological profiles, biochemical parameters of blood and the histopathology of liver, kidney, heart, lungs and spleen were investigated both in control and experimental rats. The body weights of the rats were slightly increased. The changes of hematological and biochemical parameters were statistically insignificant. No abnormalities were found in the histopathology of the liver, kidney, heart, lung and spleen in the experimental group of rats when compared with control group of rats. From this study, it was inferred that malic acid-butane 1,4 -diol copolyester could be safely subjected to clinical trial for specialized application such as controlled and sustained release of drugs.

Keywords: Malic acid-butane 1,4-diol copolyester, sub-acute toxicity, haematological profile, biochemical parameter, histopathology

Introduction
Biodegradable polymers can be defined as polymers that are degradable in vivo, either enzymatically or non-enzymatically, to produce biocompatible or nontoxic by-products. The synthesis and development of these biodegradable polymers is one of the brilliant aspects of polymer science now-a-days. Many of the existing biodegradable carriers are linear polymers [1] (Heller, 1980) and are being used for specialized application such as controlled release drug formulation [2-5] insecticide and pesticide carriers as well as non-toxic surgical implant materials. Poly(L-lactic acid) is a biodegradable polyester having good biocompatibility, it has been utilized as an useful biodegradable material in the medical and pharmaceutical fields. But the application scope of polyLA is limited because it is highly a crystalline polyester [6]. Ongoing research in our laboratory is directed towards the synthesis and characterization of new biodegradable, flexible materials based on aliphatic polyester for controlled and sustained drug delivery [7-8]. Hydrolysis of labile ester linkages along the polymer backbone converts these materials into products that the human body can easily metabolize and eliminate them without adverse effects. Our aim is, therefore, to develop novel commercially viable polymers especially designed to degrade under controlled biological conditions and in this connection, we have attempted to synthesize MBC polymer from malic acid and butane 1,4-diol. It was found that the copolyester is expected to be usable as matrix for the controlled and sustained release of medicaments. In order to develop and to establish the safety and efficacy level of a new drug, toxicity studies are very essential and no drug is used clinically without its clinical trial as well as toxicity studies. In this work, we report the toxic effect of the malic acid-butane 1,4-diol copolyester (MBC) in albino rats.

Experimental
Materials and Methods
Synthesis, characterization and toxicological study of MBC on brine shrimp have been carried out. Subacute toxicity of MBC in albino rats is our present investigation.

Collection of experimental rats

Long Evan's rats of same sex (male) and age (adult) were collected from the Animal Resources Branch, International Center for Diarrhoeal Diseases Research, Bangladesh (ICDDRB).

Maintenance of the rats

The rats were kept properly in numbered iron cages individually and they were given ideal food [9]. They were kept in a clean animal house with an optimal room temperature (25-30 ^{0}C). The animals were maintained in this way for 15 days prior to administration of polymer and continued up to the end of the experiment.

Grouping of the rats

Rats were weighed individually and divided into two groups; group A (average body weight 132.00 gm rat^{-1}) and group B (average body weight 135.75 gm rat-1), each comprising of 4 rats. Group A received vehicle only to act as control, while group B received MBC.

Administration of sample

Malic acid-butane 1,4-diol copolyester were dissolved separately in distilled water with the help of polyoxyethylene 20 sorbitan mono laurate (Tween-20) in such a way that 0.3 ml of final preparation contained 300 μg of the polyester. The MBC was administered to the rats of group B intraperitoneally at a dose 300 μg rat^{-1}day^{-1} respectively for 21 consecutive days.

Gross general observation after drug administration

The rats were observed daily very keenly to note the following features: Behaviour, CNS excitation, CNS depression, Food intake, Salivation, Diarrhoea, Muscular weakness.

Prior to sacrificing the animals, the body weights of each rat of groups A and B were measured before administration of the drug and after completion of the treatment.

Study of haematological profiles, biochemical parameters of blood and histopathology of liver, kidney, lung, heart and spleen

For haematological studies, blood was drawn from the tail veins of each rat in group A and B before the commencement of polymer administration. Blood smears were made on glass slides and stained with Leishmen reagent to perform TC, DC and platelet count. With the use of capillary tubes, blood was drawn from each rat to estimate the haemoglobin percentage by a hemocytometer. The tests were repeated on 7th, 14th and 21st days after the compound administration.

For the determination of SGOT (Serum-glutamate-oxaloacetate-transaminase), SGPT (Serum-glutamate-pyruvate-transaminase), SALP (Serum alkaline phosphatase), bilirubin, creatinine and urea, blood samples were collected separately from each of the control and experimental rat from their throat vein after sacrificing at the end of 21 days of polymer administration. The samples were then analyzed for biochemical parameters using the procedures and reagents as described in Enlehringer Mannheim GmbH Diagnostica [10-13].

For histopathological studies of liver, kidney, heart, lungs and spleen were collected separately, sliced into pieces, fixed in formalin (10%) for two days, processed, stained with Harris Haematoxylin and eosin reagent, mounted on glass slides with diphenyl xylene and observed under microscope at the Department of Genetics and Breeding, Rajshahi University, Bangladesh.

Results and discussion

Gross general observation

The group A (control) and group B (experimental) rats showed no signs of tremor, convulsions and reflex abnormalities. No muscular numbness of the hind and four legs, salivation or diarrhoea was observed. The food intake per day was also found normal. However, the body weights of all the rats were increased after administration of MBC and the changes of body weights were found to be statistically insignificant which are shown in Table 1.

Table 1: Effect of malic acid- butane 1,4-diol copolyester on body weight of rats after intraperitoneal administration.

Group	Dose level μg rat^{-1}day^{-1}	Body weight (in gm) before drug treatment $M_1 \pm SD_1$ n = 4	Body weight (in gm) after drug treatment $M_1 \pm SD_1$ n = 4	Percentage change	Calculated 't' value	't' value at 5% level of significant	Remark
A Control	300 μL of Vehicle	132.0	133.5	+1.022	+ 1.288	2.447	NS
		130.2	131.5				
		131.5	132.8				
		134.3	135.6				
		132.00 ± 1.48	133.35 ± 1.48				
B MBC Copolyester	300 μgm of Copolyester	135.0	136.2	+ 1.046	+ 1.606	2.447	NS
		137.5	138.8				
		136.5	138.1				
		134.0	135.6				
		135.75 ± 1.34	137.17 ± 1.31				

M_1 and M_2 = Sample mean value, SD_1 & SD_2 = Standard deviations of control and experimental group respectively, N = Number of rats, + = Increase, – = Decrease, NS = Not significant

Haematological profiles

The haematological profiles of the experimental rats were studied after intraperitoneal administration of the polymer to check the haematological disorders. Haematological profiles like total counts of RBC and WBC, differential count of WBC, platelet count and haemoglobin percentage were found normal before treatment and after 7th, 14th and 21st days of treatment. No detectable changes were observed in the values of these parameters compared to that of the control groups. The results are shown in Tables 2 and 3.

Table 2: Haematological profile of group-A (Rat treated with vehicle)

Haematological parameters		Normal rats	Rats Treated with vehicle only		
		1st day $M_1 \pm SD_1$	7th day $M_1 \pm SD_1$	14th day $M_1 \pm SD_1$	21st day $M_1 \pm SD_1$
i. Total RBC count (million/cc)		4.8	4.9	5.1	5.4
		5.1	4.7	5.4	5.5
		5.4	5.0	5.6	5.7
		5.5	5.1	5.7	5.8
		5.20 ± 0.273	4.92 ± 0.147	5.45 ± 0.229	5.60 ± 0.158
ii. Total WBC count (Thousand/cc)		12.40	12.80	12.60	13.10
		13.10	13.20	13.70	13.80
		14.20	14.60	14.00	14.70
		13.90	13.80	13.70	14.20
		13.40 ± 0.703	13.60 ± 0.678	13.5 ± 0.533	13.95 ± 0.585
iii. Differential count of WBC in %	a. Neutrophil	45	46	40	46
		47	45	43	44
		40	47	45	43
		41	40	46	42
		43.25 ± 2.861	44.50 ± 2.692	43.5 ± 2.291	43.75 ± 1.479
	b. Lymphocyte	50	52	53	52
		53	50	50	51
		52	55	51	53
		51	51	50	53
		51.50 ± 1.118	52.00 ± 1.870	51.00 ± 1.224	52.25 ± 0.829
	c. Monocyte	5	2	4	3
		3	1	3	4
		4	3	2	2
		3	2	3	1
		3.75 ± 0.829	2.00 ± 0.707	3.00 ± 0.707	2.50 ± 1.118
	d. Eosinophil	2	2	4	2
		3	2	3	1
		0	1	3	2
		1	1	0	1
		1.50 ± 1.118	1.5 ± 0.500	2.50 ± 1.50	1.50 ± 0.500
iv. Platelet count (million/cc)		3.30	3.00	3.50	3.70
		3.40	3.20	3.70	3.40
		4.00	4.40	3.50	3.50
		4.10	4.00	3.60	3.70
		3.700 ± 0.353	3.650 ± 0.572	3.575 ± 0.829	3.570 ± 0.129
v. Haemoglobin (%)		76	73	77	75
		74	70	75	73
		70	71	73	74
		75	72	72	71
		73.75 ± 2.277	71.50 ± 1.118	74.25 ± 1.920	73.25 ± 1.479
vi. ESR (mm/1st hour)		14	16	14	15
		10	15	13	13
		13	14	15	13
		12	13	13	12
		12.25 ± 1.479	14.50 ± 1.118	13.75 ± 0.829	13.25 ± 1.089

Table 3: Haematological profile of group-B (Rats treated with malic acid- Butane 1,4-diol copolyester)

Haematological parameters		Normal rats	Rats Treated with MBC only		
		1st day $M_1 \pm SD_1$	7th day $M_1 \pm SD_1$	14th day $M_1 \pm SD_1$	21st day $M_1 \pm SD_1$
i. Total RBC count (million/cc)		4.1	4.2	4.1	4.1
		4.3	4.0	4.2	4.5
		4.0	4.2	4.4	4.3
		4.5	4.4	4.6	4.4
		4.25 ± 0.192	4.20 ± 0.141	4.32 ± 0.192	4.32 ± 0.147
ii. Total WBC count (Thousand/cc)		13.40	13.50	13.00	13.20
		13.80	13.80	13.10	13.70
		13.60	13.70	13.80	13.30
		13.20	13.90	13.40	13.50
		13.50 ± 0.223	13.72 ± 0.147	13.32 ± 0.311	13.42 ± 0.192
iii. Differential count of WBC in %	a. Neutrophil	45	43	42	44
		42	43	40	43
		41	44	41	42

		42	42	43	41
		42.55 ± 1.880	43.25 ± 0.829	42.10 ± 1.581	42.65 ± 1.479
	b. Lymphocyte	53	55	53	52
		55	53	54	53
		54	54	56	54
		52	51	54	51
		53.75 ± 1.379	53.25 ± 1.479	54.25 ± 1.089	52.50 ± 1.118
	c. Monocyte	5	5	4	3
		2	3	4	4
		6	7	3	5
		3	1	3	2
		4.00 ± 1.5812	4.00 ± 2.236	3.50 ± 0.500	3.50 ± 1.188
	d. Eosinophil	3	4	2	4
		2	2	3	2
		3	5	4	3
		1	2	1	1
		2.25 ± 0.829	3.25 ± 1.299	2.50 ± 1.188	2.50 ± 1.188
iv. Platelet count (million/cc)		3.80	3.60	3.50	3.70
		3.50	3.40	3.45	3.40
		3.40	3.40	3.60	3.70
		3.10	3.10	3.10	3.20
		3.42 ± 0.324	3.37 ± 0.178	3.41 ± 0.118	3.39 ± 0.192
v. Haemoglobin (%)		54	56	55	54
		57	55	56	57
		57	53	55	56
		54	51	54	54
		55.55 ± 1.890	54.10 ± 2.176	55.15 ± 0.819	55.25± 1.299
vi. ESR (mm/1st hour)		11	13	12	10
		11	10	10	12
		9	11	8	9
		10	12	11	10
		10.25 ± 0.829	11.50 ± 1.188	10.25 ± 1.479	10.25 ± 1.089

Monitoring the biochemical parameters

Biochemical parameters of blood e.g. SGOT, SGPT, SALP, serum bilirubin, serum creatinine, urea of both experimental and control rats were determined to check any change of these parameters due to the administration of polymer (MBC) with respect to control rats. The results are presented in Table 4. It was found that most of the parameters were slightly increased with respect to that of the control groups but remained within the normal range.

From the Table 4, it was found that the changes are also statistically insignificant. These results indicated that the compound has no adverse effects on liver and kidney functioning.

Table 4: Effect of malic acid-butane 1,4-diol copolyester on biochemical parameters of rat's blood after i.p. administration of 300 μg rat^{-1}day^{-1} for 21 consecutive days.

Biochemical Parameters	Group-A, n=4 $M_1 \pm SD_1$	Group-B, n=4 $M_1 \pm SD_1$	Percentage Change	Calculated't' value	't' value at 5% level of significant	Remark
Serum Glutamate Oxaloacetate Transaminase (SGOT) (IU/L)	10	11	+ 2.38	+ 0.269	2. 447	NS
	11	9				
	12	13				
	9	10				
	10.50 ± 1.118	10.75 ± 1.479				
Serum Glutamate Pyruvate Trans-aminase (SGPT) (IU/L)	12	10	+ 2.27	+ 0.460	2.447	NS
	10	11				
	11	11				
	11	13				
	11.00 ± 1.089	11.25 ± 0.866				
Serum Alkaline Phosphatase (SALP) (IU/L)	50	52	+ 0.990	+ 1.146	2.447	NS
	52	50				
	51	51				
	49	51				
	50.50 ± 1.118	51.00 ± 0.707				
Serum bilirubin (mg/dl)	0.39	0.32	+ 3.225	+ 0.641	2.447	NS
	0.36	0.34				
	0.30	0.32				
	0.28	0.31				
	0.31 ± 0.029	0.32 ± 0.012				
Creatinine	1.29	1.23	+ 2.089	+ 0.251	2.447	NS
	1.20	1.25				

(mg/dl)	1.12	1.03				
	0.95	1.07				
	1.142 ± 0.159	1.167 ± 0.120				
Urea (mg/dl)	22	26	+ 1.904	+ 0.010	2.447	NS
	28	30				
	25	24				
	30	27				
	26..25 ± 3.03	26.75 ± 2.165				

M1 and M2 = Sample mean value, SD1 and SD2 = Standard deviations
n = Number of rats, + = Increase
NS = Non significant

Histopathological studies

The histopathological studies of liver, kidney, heart and lung of both control and experimental rats were performed after intraperitoneal administration of the drugs for 21 consecutive days (Fig.1-8). No detectable differences in the histopathology of these organs of control and drug treated rats were observed when viewed under oil immersion objective. This indicates that the tested polymer MBC has no effect on cellular structures, i.e, the polymer does not cause degeneration of the cells of these organs.

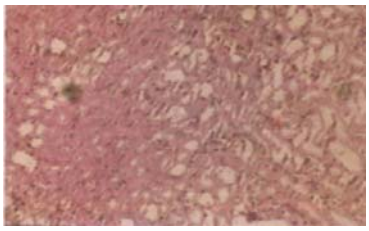

Fig 1: Microscopic view of kidney tissues of control rat group-A after 21 days.

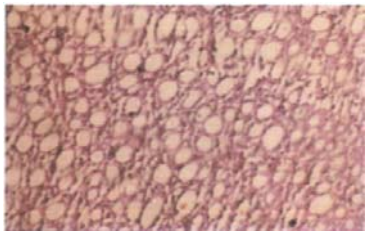

Fig 2: Microscopic view of kidney tissues of experimental rat group-B after 21 days.

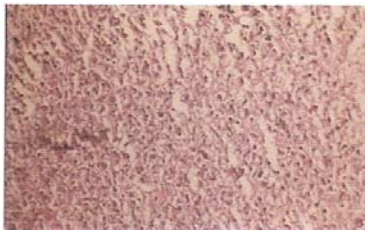

Fig 3: Microscopic view of liver tissues of control rat group-A after 21 days.

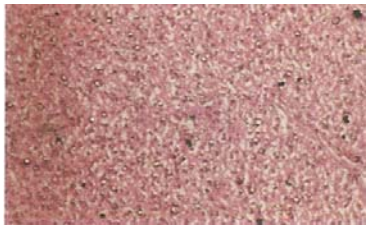

Fig 4: Microscopic view of liver tissues of experimental rat group-B after 21 days.

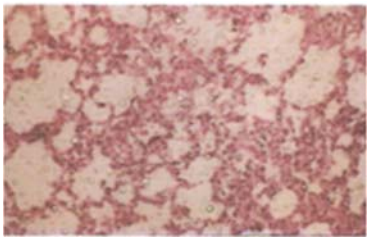

Fig 5: Microscopic view of lung tissues of control rat group-A after 21 days.

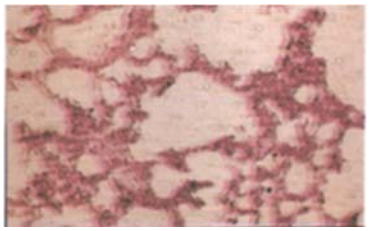

Fig 6: Microscopic view of lung tissues of experimental rat group-B after 21 days.

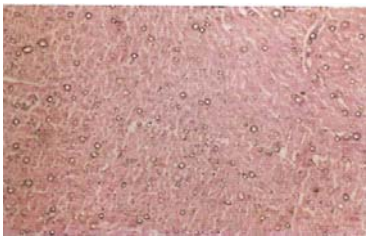

Fig 7: Microscopic view of heart tissues of control rat group-A after 21 days.

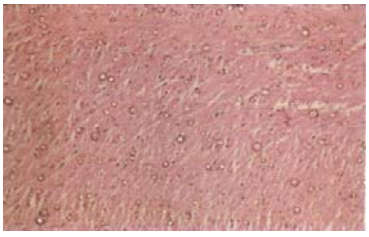

Fig 8: Microscopic view of heart tissues of experimental rat group-B after 21 days.

Conclusion

The use of malic acid-butane1,4-diol copolyester (MBC) as medical devices for delivery systems appears to be promising. The use of biodegradable materials will grow as new technologies have been developed to supplement traditional treatments. The study of haematological profiles, biochemical parameters of blood and histopathology of liver, kidney, lung and heart indicates that the biodegradable material MBC might be used for the clinical purposes.

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