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Toxicological investigation of malic acid-succinic acid-butane 1, 4-diol co-polyester on albino rats

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Abstract
Malic Acid-Succinic Acid-Butane 1,4-diol Co-polyester(MSBC) is a biodegradable co-polyester synthesized from malic acid and succinic acid in different mole combinations with 1 mole of butane 1,4-diol following Dean-stark apparatus using $FeCl_3$ (Approximately 0.4% of the total weight) as catalyst and o-xylene as the reaction medium at temperature 145-150 ^{0}C for about 7 hours. MSBC was administered intraperitoneally to albino rats at a dose of 300 µg $rat^{-1}day^{-1}$ for 21 consecutive days in order to assessing the toxic effect of MSBC. The studies included the gross observation such as changes in body weight, haematological profiles, biochemical parameter of blood and histopathology of liver, kidney, heart, lungs and spleen of control group, and experimental group of rats. The changes in body weight, haematological and biochemical parameters are statistically insignificant after administration of MSBC when compared to that of control group, and experimental group of rats. 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. This study preliminary infers that Malic Acid-Succinic Acid-Butane 1,4-diol Co-polyester is a safer biodegradable polymer expected to be usable as an enteric coating materials or other purposes where biodegradable polymers be needed to use safely.

Keywords: Malic acid-succinic acid-butane 1,4-diol co-polyester, sub-acute toxicity, hematological profile, biochemical parameter, histopathology

Introduction
Use of biodegradable polymers for biomedical applications has increased in recent decades due to their biocompatibility, biodegradability, flexibility, and minimal side effects [1 - 3]. The main advantage of biodegradable polymers is that the products of degradation are not toxic or are completely eliminated from the body by natural metabolic pathways [4], with minimal side effects [5 - 6]. 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 poly LA is limited because it is highly a crystalline polyester [7]. The 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 [8, 9]. 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 MSBC co-polyester from malic acid, succinic acid and Butane 1,4-diol. It was found that the co-polyester is expected to be usable as an enteric coating materials [9]. In order to develop and to establish the safety and efficacy level of a new drug, toxicological studies are very essential experiment in animals like mice, rat, guinea pigs, dog, rabbit, monkey etc. and no drug is used clinically without its clinical trial as well as toxicological studies.
In sub-acute toxicity studies, repeated doses of drug are given in sub-lethal quantity for a period of 14 to 21 days [10, 11, 12]. Sub-acute toxicity studies are used to determine the effect of drug on biochemical and haematological parameters of blood as well as to determine histopathological changes. However, in the biomedical area, the toxicologist is primarily concerned with adverse effects in human resulting from exposure to drugs and other chemicals as well as the demonstration of safety or hazard associated with their use.

Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. In this work, we report the toxic effect of the Malic Acid-Succinic Acid-Butne 1,4-diol Co-polyester (MSBC) in albino rats.

Experimental Section

Materials and Method

The sub-acute toxicity studies of the biodegradable polymer MSBC were performed on normal adult healthy Albino rats by giving a daily dose of 300 µg/rat intraperitoneally for 21 consecutive days. The rats were kept under keen observations throughout the treatment period [8].

Collection of Experimental Rats

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

Maintenance of Rats

The rats were kept properly in numbered iron cages individually and they were given ideal food [13]. 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 before experiment to adjust with food and environment and continued up to the end of the experiment.

Grouping of Rats

Rats were weighed individually and divided into two groups; group A (average body weight 132.67 gm rat^{-1}) and group B (average body weight 136.25 gm rat^{-1}), each comprising of 4 rats. Group A received vehicle only to act as control, while group B received MSBC.

Administration of sample

Malic Acid-Succinic Acid-Butane 1,4-diol Copolyester (MSBC) was 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 μ g of the polyester. The MSBC 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 polymer administration

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

Monitoring the Change of Body Weight

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

Monitoring the Hematological Profiles

The haematological profiles of the experimental rats were done to check the abnormalities after administration of the polymer intraperitoneally. For this purpose, the following parameters were observed.

i) Total RBC count
ii) Total WBC count
iii) Different count of WBC
iv) Platelet count
v) Hemoglobin percentage and
vi) ESR (Erythrocytic Sedimentation Rate)

Experimental work plan or procedure are as follows

i) Blood was drawn from the tail veins of all the rats in group A and group B before the commencement of polymer administration.
ii) Blood smears were made on glass slides and stained with Leishmen reagent to performed TC, DC and platelet count with the use of capillary tubes, blood was drawn from each rat to estimate the hemoglobin percentage by Van Kampen-Zijlstra's method, which is the pre-hematological study on normal rats.
iii) The polymer MSBC was administered intraperitoneally regularly to the rats of group A, but group B received vehicle only.
iv) The tests were repeated on 7th, 14th and 21th day after the commencement of polymer administration following the same procedure as that done on normal rats.

Monitoring the Biochemical Parameters of Blood

The biochemical parameters such as SGOT (Serum glutamate oxaloacetate transaminase), SGPT (Serum glutamate pyruvate transaminase), SALP (Serum alkaline phosphatase) and serum bilirubin are associated with the condition of liver, serum level of creatinine and urea are associated with the functioning of kidney. Serum levels of these parameters change with the pathological changes of these organs. In case of hepatic narcosis, cirrhosis and obstructive jaundice the serum level of SGOT and SGPT may increase up to 200 IU/L. If a drug possesses any effect on kidney, several pathological changes may occur and ultimately serum level of these parameters alters.

Biochemical parameters of blood were checked for rats of group A and B to find abnormalities if any due to polymer treatment with respect to food control group and vehicle control group. The following parameters were checked:

i) Liver function tests

a) Serum Glutamate Oxaloacetate Transaminase (SGOT)
b) Serum Glutamate Pyruvate Transaminase (SGPT)
c) Serum Alkaline Phosphatase (SALP)
d) Serum Billirubin (SB)

ii) Kidney function tests

a) Creatinine
b) Urea

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 [14-18].

Collection of Serum

In the 21th day of treatment with the polymer, the rats of experimental and control groups were sacrificed with the help of a surgical blade no. 22 and the blood were collected in a plastic centrifuge tubes. These were then allowed to clot at 40 ℃ for 4 hours. After clotting the blood samples were centrifuge LABOR-50M. The clear straw color serum was then collected in vials with Pasteur pipette and stored at 20℃.

Histopathology of Liver, Kidney, Heart and lungs

Histopathology of liver, kidney, lungs and heart were performed to observe any change in the cellular structures (Degeneration and Regeneration) of the rats receiving polymers at a dose of 300 µg daily for 21 consecutive days with respect to food control and vehicle control group.

Reagents are as follows:

i) Formalin (10%)
ii) Absolute alcohol (Ethanol)
iii) Paraffin
iv) Xylene
v) D.P.X. mounting fluid
vi) Harris hematoxylin and eosin stain

Collection and Processing of the Tissues

The liver, kidney, heart and lungs of the experimental and control group of rats were collected after sacrificing them at the 21th day of observation. The tissues were sliced into pieces each measuring a few mm of thickness. The sliced tissues were then immersed in 10% formalin for three days. The tissues were then dehydrated in ascending order of ethanol and embedded in paraffin. The blocks were sectioned with the help of rotating microtome at 6 micron thickness and then processed, stained with Harris Haematoxylin and eosin reagent, mounted on glass slides with diphenyl xylene and observed under microscope.

Results and discussion

Gross general observation

The rats of group A, group B treated with vehicle and Malic Acid-Succinic Acid-Butane 1, 4-diol Copolyester(MSBC) respectively 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.

Monitoring the change in body weight

Average body weights of all rats before and after treatment were presented in Table-1 and Fig-1.

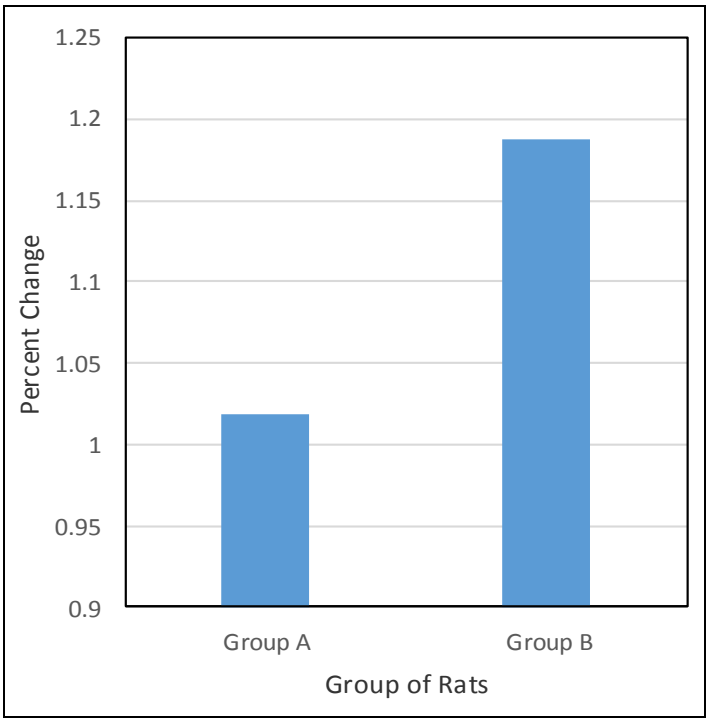

Fig 1: Percentage change in body weight.

After 21 days treatment group A was gained weight 1.019% and experimental group B gained weight 1.188%. The change in body weight for group A and group B were found to be statistically insignificant.

Table 1: Effect of Malic Acid-Succinic Acid-Butane 1,4-diol Co-polyester 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	121.4 122.3 122.5 124.5 122.67 ± 1.13	122.6 123.6 123.4 126.1 123.92 ± 1.31	+1.019	+ 1.386	2.447	NS
B MSBC (Polymer)	300 μgm of Polymer	124.7 126.3 127.3 126.6 126.22 ± 0.95	126.3 127.8 128.6 128.2 127.72 ± 0.87	+ 1.188	+ 1.688	2.447	NS

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

Monitoring the 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 mentionable 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)		5.5 5.8 5.7 5.9 5.72 ± 0.147	5.3 5.6 5.5 5.8 5.55 ± 0.180	5.4 5.5 5.8 5.9 5.65 ± 0.206	5.4 5.7 5.7 5.8 5.65 ± 0.150
ii. Total WBC count (Thousand/cc)		12.50 13.20 14.30 14.10 13.52 ± 0.722	12.40 13.90 13.80 13.50 13.40 ± 0.595	12.40 13.90 13.60 13.90 13.45 ± 0.618	12.50 14.30 13.90 14.20 13.72 ± 0.722
iii. Differential count of WBC in %	a. Neutrophil	50 47 48 46 47.75 ± 1.479	52 46 45 44 46.75 ± 3.112	54 44 49 43 47.5 ± 4.387	53 46 46 45 47.50 ± 3.201
	b. Lymphocyte	47 51 50 52 50.00 ± 1.870	46 51 51 51 49.75 ±2.165	46 53 50 53 50.50 ± 2.872	45 52 50 53 50.00 ± 3.082
	c. Monocyte	3 4 4 2 3.25 ± 0.829	2 2 3 3 2.50 ± 0.500	1 3 2 2 2.00 ± 0.816	2 1 2 3 3.00 ± 1.154
	d. Eosinophil	0 2 0 2 1.00 ± 1.000	1 2 2 1 1.50 ± 0.500	2 3 2 0 1.75 ± 1.089	3 2 1 0 1.50 ± 1.118
iv. Platelet count (million/cc)		3.55 3.65 3.50 3.65 3.587 ± 0.064	3.45 3.50 3.75 3.60 3.575 ± 0.114	3.20 3.35 3.65 3.55 3.437 ± 0.174	3.45 3.45 3.55 3.65 3.525 ± 0.082
v. Haemoglobin (%)		65 71 63 69 67.00 ± 3.162	63 69 67 70 67.25 ± 2.680	67 72 65 71 68.75 ± 2.861	64 73 66 71 68.50 ± 3.640
vi. ESR (mm/1st hour)		14 12 14 13 13.25 ± 0.829	13 14 13 14 13.50 ± 0.500	12 13 14 12 12.75 ± 0.829	14 12 13 13 13.00 ± 0.707

Table 3: Haematological profile of group-B (Rats treated with Malic Acid-Succinic Acid-Butane 1, 4-diol Co-polyester)

Haematological parameters		Normal rats	Rats Treated with MSBC 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)		4.2 4.3 4.4 4.3 4.30 ± 0.070	4.4 4.2 4.3 4.4 4.32 ± 0.082	4.5 4.4 4.5 4.3 4.42 ± 0.082	4.3 4.3 4.6 4.5 4.42 ± 0.129
ii. Total WBC count (Thousand/cc)		14.70 14.90 14.10 14.70 14.60 ± 0.300	14.50 13.80 14.40 13.60 14.07 ± 0.383	14.60 13.90 13.90 13.90 14.07 ± 0.303	14.70 13.70 13.70 13.80 13.97 ± 0.420
iii. Differential count of WBC in %	a. Neutrophil	40 43 42 43 42.00 ± 1.224	41 43 45 43 43.00 ± 1.414	43 41 44 44 43.00 ± 1.224	42 41 41 43 41.75 ± 0.829
	b. Lymphocyte	54 53 53 51 52.75 ± 1.089	50 51 53 53 51.75 ± 1.299	51 54 53 54 53.00 ± 1.224	52 55 53 54 53.50 ± 1.118
	c. Monocyte	4 4 5 4 4.25 ± 0.433	5 5 3 3 4.00 ± 1.000	4 4 3 2 3.25 ± 0.687	4 3 4 3 3.50 ± 0.500
	d. Eosinophil	1 2 0 2 1.25 ± 0.829	4 3 2 2 2.75 ± 0.829	4 3 3 2 2.75 ± 0.829	4 4 2 3 3.25 ± 0.829
iv. Platelet count (million/cc)		3.45 3.35 3.45 3.50 3.43 ± 0.054	3.55 3.45 3.35 3.45 3.45 ± 0.070	3.60 3.40 3.45 3.55 3.50 ± 0.079	3.65 3.55 3.35 3.75 3.57 ± 0.147
v. Haemoglobin (%)		62 63 57 65 61.75 ± 2.947	60 59 62 63 61.00 ± 1.581	62 60 59 61 60.50 ± 1.118	61 62 56 60 59.75 ± 2.277
vi. ESR (mm/1st hour)		16 17 18 17 17.00 ± 0.707	15 17 15 16 15.75 ± 0.829	16 15 17 15 15.75 ± 0.829	14 16 16 17 15.75 ± 1.089

Monitoring the biochemical parameters

Biochemical parameters of blood both experimental and control rats were determined to check any change of the parameters e.g. SGOT, SGPT, SALP, serum bilirubin, serum creatinine, urea due to the administration of polymer MSBC with respect to control rats. The results are presented in Table 4 and Fig. 2. It was found that most of the parameters were slightly changed with respect to that of the control groups but remained within the normal range.

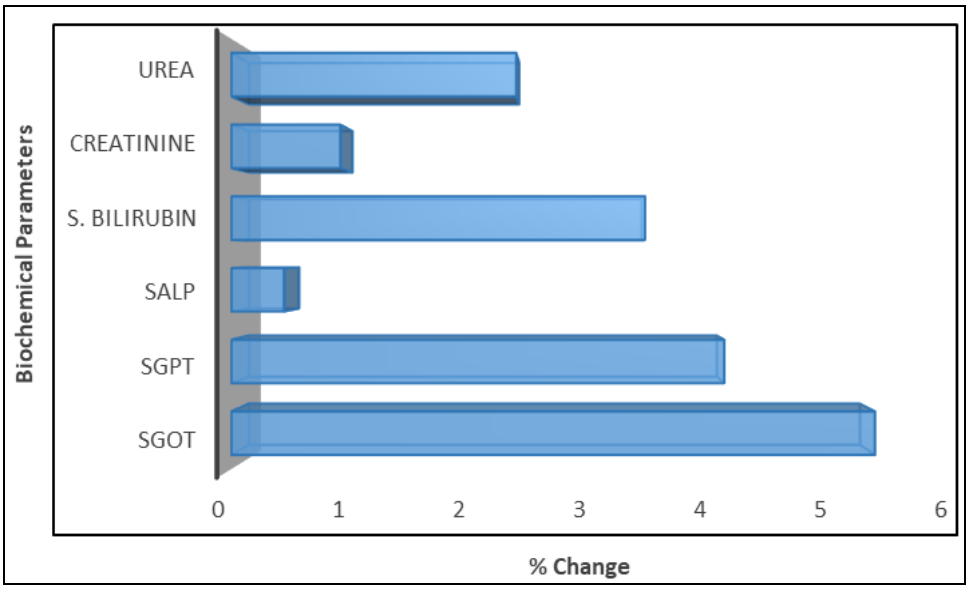

Fig 2: Effect of MSBC on biochemical parameters of blood between Group A and Group B

From the Table 4 and Fig. 2, 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 MSBC on biochemical parameters of blood between group A & group B

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)	12 14 15 13 13.50 ± 1.118	13 15 15 14 14.25 ± 0.829	+ 5.555	+ 1.736	2. 447	NS
Serum Glutamate PyruvateTrans-aminase (SGPT) (IU/L)	10 14 12 11 11.75 ± 1.479	11 14 13 11 12.25 ± 1.299	+ 4.255	+ 1.460	2.447	NS
Serum Alkaline Phosphatase (SALP) (IU/L)	57 56 52 54 54.75 ± 1.920	58 55 54 54 55.00 ± 1.870	+ 0.456	+ 1.115	2.447	NS
Serum bilirubin (mg/dl)	0.27 0.29 0.30 0.26 0.28 ± 0.015	0.28 0.29 0.31 0.28 0.29 ± 0.015	+ 3.571	+ 1.065	2.447	NS
Creatinine (mg/dl)	1.03 1.00 1.09 1.12 1.06 ± 0.047	1.04 1.01 1.08 1.14 1.07 ± 0.048	+ 0.943	+ 0.874	2.447	NS
Urea (mg/dl)	32 31 29 30 30.50 ± 1.118	33 30 31 31 31.25 ± 1.089	+ 2.459	+ 1.326	2.447	NS

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

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 at a dose of 300 µg/rat/day. No detectable abnormalities in the histopathology of these organs of control and drug treated rats were observed when viewed under oil immersion objective. The results are presented in Table 5. The result indicates that the tested MSBC has no effect on cellular structures, i.e., the polymer does not cause degeneration of the cells of these organs.

Table 5: Effect of malic acid-succinic acid-butane 1, 4-diol copolyester on histopathology of rat's kidney, heart, lung, liver and spleen tissue i.p. administration of 300 μg rat^{-1}day^{-1} for 21 consecutive days

Group	Dose (i.p.)	Histopathological Change Observed			
		Kidney	Heart	Lung	Liver
A - Control	300μL rat^{-1}day^{-1} (Vehicle)	NAD	NAD	NAD	NAD
B - MSBC	300μg rat^{-1}day^{-1} (MSBC)	NAD	NAD	NAD	NAD

NAD indicates no abnormality detected.

Conclusion

The result of sub-acute toxicity studies have shown no abnormalities on body weight, haematological and biochemical parameters of blood and on histopathological slides. The biological effect of MSBC and its present toxicological studies suggest that, MSBC can be safely subjected to clinical trial for specialized application such as control release drug formulation. The use of malic acid-succinic acid-butane 1,4-diol co-polyester as medical devices or 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, heart and spleen indicates that the biodegradable material MSBC might be used for the clinical purposes.

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