Sub-Acute Toxicological Studies on Malic Acid-Butane-1, 4-Diol-Glycerol Co-Polyester

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Abstract - Malic acid butane-1, 4-diol-glycerol co-polyester (MBGC) is synthesized from malic acid and butane-1, 4-diol with 5% glycerol of total weight as a crosslinking agent using Dean-Stark apparatus with ferric chloride (Approximately 0.4% of the total weight) as catalyst and o-xylene as the reaction medium at temperature 137-141° C for about 5 hours. The sub-acute toxicity of MBGC is studied on *wistar rats*. 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 diet control group, vehicle control group and also experimental group of rats. The changes in body weight, haematological and biochemical parameters are statistically insignificant after administration of MBGC in a dose of 300 µg/rat/day intraperitoneally for consecutive 21 days when compared to that of diet control group, vehicle control group and experimental group of rats. Histopathologically no abnormality is found on liver, kidney, heart, lungs and spleen of experimental group of rats after treatment when compared to that of control group of rats. This preliminary study suggests that, MBGC can be safely subjected to clinical trial for specialized application such as control release drug formulation, granular pesticides, fertilizers etc. and other purposes where biodegradable polymers be needed.

Index Terms - Malic acid butane-1, 4-diol-glycerol co-polyester (MBGC), Sub-Acute Toxicity, Wistar Rat, Histopathology, Biochemical Parameter, Haematological Parameter.

1. INTRODUCTION

Toxicology is the aspect of pharmacology that deals with the adverse effect of bioactive substance on living organisms. In order to establish the safety efficiency of a new drug, toxicological studies are very essential experiment in animals like mice, rat, guinea pigs, dog, rabbit, monkey etc. under various condition of drug. No drug is used clinically without its clinical trial as well as toxicity studies. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. Depending on the duration of drug exposure to animal's toxicological studies may be three types, a) acute b) sub-acute and c) chronic toxicological studies. In acute toxicity studies, single dose of drug is given in large quantity to determine immediate toxic effect. Acute toxicity studies are commonly used to determine LD_{50} of drug or chemicals. In sub-acute toxicity studies, repeated doses of drug are given in sub-lethal quantity for a period of 14 to 21 days. [1], [2], [3]

Sub-acute toxicity studies are used to determine effect of drug on biochemical and haematological parameters of blood as well as to determine hispathological changes. In chronic toxicity studies, drug is given in different doses for a period of 90 days to over a year to determine carcinogenic and mutagenic potentiality of drug. [4], [5]

Toxicological studies is useful for the development of tests for the prediction of risks, facilitating search for safer chemicals and for national treatment of manifestations of toxicity. Toxicology [6] is concerned with the deleterious effects of chemical and physical agents on living systems. 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 study is used to

i) Determine or evaluate the efficacy, safety or toxicity of a new drug
ii) Determine the factors that modifies drug safety and efficacy
iii) Get information about drug metabolism
iv) Reveal the toxic effects on different organs
v) Predict therapeutic dose
vi) Establish dose interval time
vii) Determine lethality (LD_{50}) bioassay

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Our research trend was to investigate the sub-acute toxic effect of our synthesized biodegradable polymer so that, it can be further used in human body without any adverse effect.

2. MATERIALS AND METHOD

The sub-acute toxicity studies of the biodegradable polymer were performed on normal adult healthy *wister 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. [7] The following parameters were studied during this course of time.

a) Gross general observations
b) Haematological profiles
c) Biochemical parameters of blood
d) Histopathology of liver, kidney, heart, lungs and spleen.

2.1 Experimental Animal and Their Collection

The experiment was carried out on wister male rats. They were four months old, weighing between 160-200 gm. they were collected from the Animal Research Branch (ARC) of International center for Diarrhoeal Disease Research, Bangladesh (ICDDRB), Mohakhali, Dhaka.

2.2 Maintenance of Rats

The rats were housed in iron cages (considering group) under temperature and light controlled condition. They were fed a balanced diet and tap water. The animals were maintained in this condition for 15 days before experiment to adjust with food and environment. They were given ideal food comprising the following ingredients per gm. of dried mixture.

TABLE 1

DAILY FOOD COMPOSITION OF RATS

Composition	Amount (gm.)
Ata (Flour)	40
Matar dal powder	25
Skimmed milk powder	28
Soyabean oil	05
Salt mixture	01
Vitamin mixture	01

The diet supplied to each rat was about 20 gm. per day, which was approximately isocaloric. They were kept in a clean animal house with an optimal room temperature. [8]

2.3 Grouping of Rats

Individual weight of the rats was taken and they were grouped in three, randomly. The rats of group C (4 rats, average weight 197.95 gm.) used for experiment (received MBGC respectively), group B (4 rats, average weight 162.50 gm.) used for vehicle control and group A (4 rats, average weight 196.50 gm.) were used as diet control. [9], [10]

TABLE 2

DOSAGE REGIMEN ADJUSTMENTS FOR EACH GROUP OF RATS

Group	No. of Rats	Average body weight, gm.	Sex	Average age (Month)	Dose (i.p) µg/rat/day
A	4	196.50	Male	4	Received food only
B	4	162.50	Male	4	300 µl. vehicle only
C	4	197.95	Male	4	300 µg of MBGC

2.4 Administration of Sample

Malic acid butane-1, 4-diol-glycerol co-polyester (MBGC) was dissolved in distilled water with the help of polyoxyethylene 20 sorbitan mono laurate as co-solvent in such a way that 0.3 ml of final preparation contained 300 µg of the co-polyesters.

Each rat of experimental group (Group C) was administered with 0.3 ml sample solution (contain 300 µg compound) daily, for 21 consecutive days and each rat of control group (Group B) was administered with 0.3 ml isotonic vehicle daily, for 21 consecutive days. [11] Intraperitoneal route was used for these administrations.

2.5 Gross General Observation after Drug Administration

The rats were very keenly observed daily to note the following features:

i) Behavior
ii) CNS excitation
iii) CNS depression
iv) Food intake
v) Salivation
vi) Diarrhoea
vii) Muscular weakness.
viii)

2.6 Monitoring the Change of Body Weight

The body weight of each rat of group A, group B and group C were measured before administration of the drugs and after completion of the treatment prior to sacrificing the animals. These weights were then compared.

2.7 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, group B and group C 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 MBGC was administered intraperitoneally regularly to the rats of group C, but group A received food and group B received vehicle only.
iv) The tests were repeated on 7^{th}, 14^{th} and 21^{th} day after the commencement of polymer administration following the same procedure as that done on normal rats.

2.8 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 nercosis, 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, B and C 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

The tests were done by using the procedures and reagents described in Bochriger Mannheim Gmbll Diagnostica.

2.9 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˚C 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˚C.

2.10 Histopathology of Liver, Kidney, Heart and Drugs

Histopathology of liver, kidney, lungs and spleen 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

2.11.1 Collection and Processing of the Tissues

The liver, kidney, heart, lungs and spleen 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.

a) Staining

The section were deparaffinized at two changes of xylene (5 min. each) and hydrated in descending order of alcohol (2-3 min. each). The sections were then cleaned into sets of xylene (5 min. each).

b) Mounting

Glass slides containing the tissues area were wiped, dried and then a drop of Canada balsam was put on the section and cover slip was gently placed on it. On the sections, thin film between the cover slip and the slide with the mounting medium (Canada balsam) was formed to attach them.

c) Histopathological examinations

Histopathological examinations were done under high power microscope and were recorded by photographs.

3. RESULTS AND DISCUSSION

The rats of group A, group B and group C treated with diet food, vehicle and Malic acid butane-1, 4-diol-glycerol co-polyester (MBGC) respectively showed no signs of tremor, convulsions and reflex abnormalities. No muscular weakness, salivation or diarrhoea was observed. The food intake per day was also found normal.

3.1 Monitoring the change in body weight

Average body weights of all rats before and after treatment were presented in table-3 and fig-1. After 21 days treatment group A was gained weight 1.56%, group B gained weight 1.44% & experimental group C gained weight 1.40%. The change in body weight for group A, group B and group C were insignificant.

TABLE 3

EFFECT OF MBGC ON BODY WEIGHT OF RATS AFTER INTRAPERITONEAL ADMINISTRATION

Group	Dose level µgm/rat/day	Body weight (in gm) before drug treatment M_1 ±SD, n=4	Body weight (in gm) after drug treatment M_1 ±SD, n=4	% Change	Calculated 't' value	't' value at 5% level of significant	Remarks
A	Received	198.60	202.30				
Food	food	193.42	197.05				
Control	only	196.56	199.45	+ 1.56	2.353	2.447	NS
		197.83	199.80				
		196.60 ± 1.98	199.60 ± 1.86				
B	300 µl of	162.44	163.13				
Vehicle	Vehicle	160.71	164.34				
Control		165.28	168.25	+1.44	2.026	2.447	NS
		163.10	165.17				
		162.88 ± 1.64	165.22 ± 1.89				
C	300 µg of	200.64	204.27				
PPGC	Polymer	197.38	200.85				
(Polymer)		198.00	200.49	+1.40	2.022	2.447	NS
		195.91	197.41				
		197.98 ± 1.71	200.76 ± 2.43				

M_1 and M_2 = Sample mean value, SD_1 & SD_2 = Standard deviation of control and experimental group respectively, n = Number of rats, + = Increase, NS = Non-significant.

3.2 Monitoring the Hematological Profiles

The haematological profiles of the experimental, food control and vehicle control group rats were determined before treatment and after 7th, 14th and 21th days of treatment and compared to check the hematological disorders after intraperitoneal administration of MBGC.

No mentionable change in the values of RBC count, WBC count, platelet count, differential WBC count, hemoglobin and ESR of experimental rats were observed when compared to that of food control group A and vehicle control group B rats (Table-4, 5 and 6)

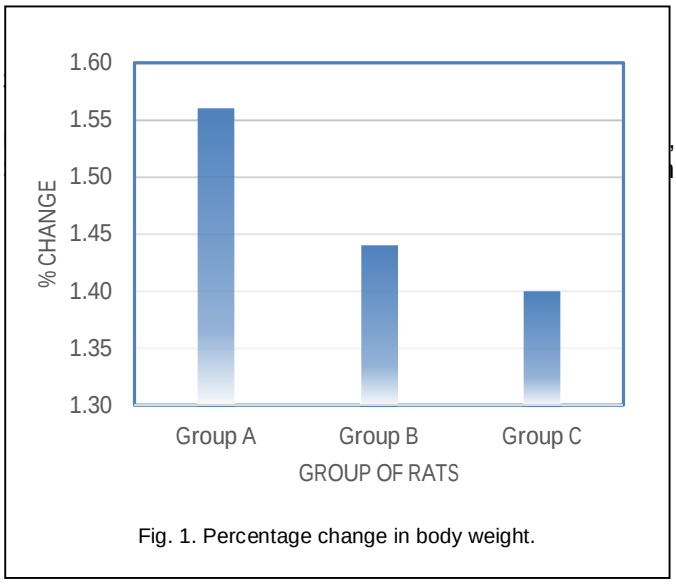

Fig. 1. Percentage change in body weight.

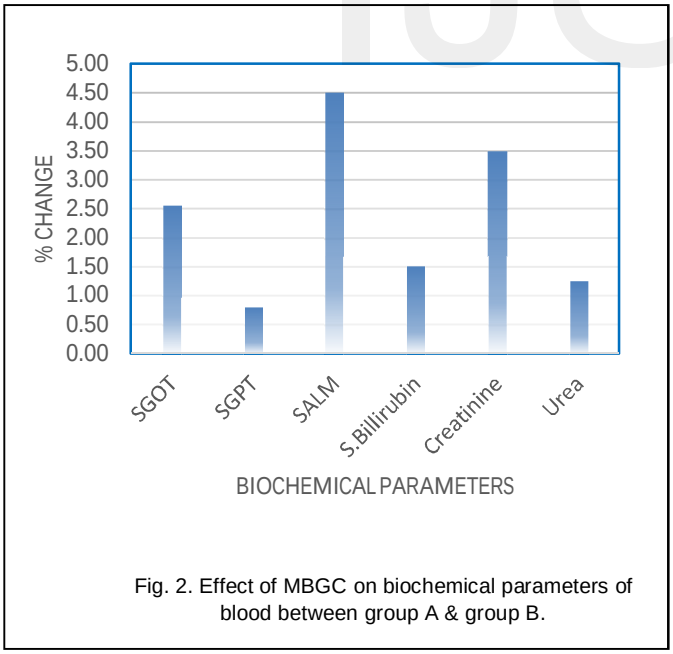

Fig. 2. Effect of MBGC on biochemical parameters of blood between group A & group B.

were determined to check any change of these parameters due to the administration of MBGC, at a dose of 300 µg/rat/day with respect to food control group A and vehicle control group B for 21 consecutive days.

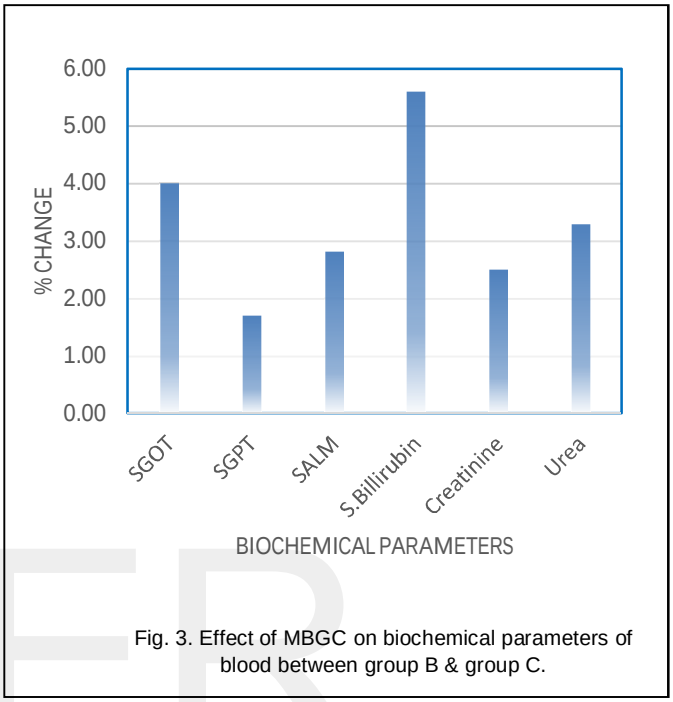

Fig. 3. Effect of MBGC on biochemical parameters of blood between group B & group C.

The results are presented in table-7 and fig-2 & 3. It was found that, most of the parameters were slightly changed with respect to group A and group B within the normal range. These results indicated that, the compound MBGC has no adverse effects on liver and kidney function.

TABLE 4

HAEMATOLOGICAL PROFILE OF GROUP – A (RECEIVED FOOD ONLY)

Haematological Parameters		Normal rats	Rats received food only		
		1st day	7th day	14th day	21th day
		$M_1 \pm SD_1$	$M_1 \pm SD_1$	$M_1 \pm SD_1$	$M_1 \pm SD_1$
i. Total RBC count (Million)		3.40	3.70	4.00	4.10
		4.20	4.60	4.70	4.50
		3.30	3.40	4.60	5.00
		4.10	4.60	4.90	4.80
		3.75 ± 0.4031	4.075 ± 0.5356	4.55 ± 0.3354	4.60 ± 0.3391
ii. Total WBC count (Thousand/cc)		8.40	8.60	8.90	9.00
		9.10	8.90	9.10	9.30
		9.40	9.40	9.30	9.40
		8.60	8.90	9.20	9.50
		8.85 ± 0.51	8.95 ± 0.28	9.13 ± 0.15	9.30 ± 0.22
iii. Differential count of WBC	a. Neutrophil	46.00	47.00	51.00	50.00
		42.00	46.00	49.00	52.00
		48.00	51.00	50.00	48.00
		50.00	47.00	48.00	51.00
		46.50 ± 2.96	47.75 ± 1.92	49.50 ± 1.07	50.25 ± 1.48
	b. Lymphocyte	40.00	39.00	37.00	40.00
		46.00	45.00	43.00	43.00
		48.00	47.00	44.00	41.00
		44.00	43.00	41.00	40.00
		44.50 ± 2.95	42.25 ± 2.86	41.25 ± 2.68	41.00 ± 1.22
	c. Monocyte	2.00	2.00	2.00	3.00
		1.00	2.00	2.00	2.00
		2.00	3.00	3.00	4.00
		2.00	1.00	2.00	2.00
		1.75 ± 0.433	2.0 ± 0.7071	2.25 ± 0.433	2.75 ± 0.8281
	d. Eosinophil	1.00	2.00	2.00	2.00
		2.00	2.00	3.00	3.00
		1.00	1.00	2.00	2.00
		2.00	2.00	1.00	2.00
		1.5 ± 0.5	1.75 ± 0.433	2.0 ± 0.7071	2.25 ± 0.433
iv. Platelet count (Lack/cc)		2.20	2.40	2.70	2.90
		2.00	2.20	2.50	2.60
		2.30	2.25	2.40	2.80
		2.45	2.50	2.80	2.60
		2.24 ± 0.16	2.34 ± 0.12	2.60 ± 0.16	2.73 ± 0.16
v. Hemoglobin (gm./DL)		8.00	8.20	8.60	9.00
		8.50	8.40	8.50	9.10
		8.25	8.50	8.40	9.30
		8.40	8.60	8.80	8.60
		8.29 ± 0.19	8.43 ± 0.15	8.58 ± 0.15	9.00 ± 0.25
vi. ESR (mm/1st hour)		11.00	13.00	11.00	13.00
		10.00	11.00	12.00	14.00
		12.00	12.00	11.00	12.00
		10.00	10.00	11.00	13.00
		10.75 ± 0.96	11.5 ± 1.29	11.25 ± 0.5	13.0 ± 0.82

TABLE 5

HAEMATOLOGICAL PROFILE OF GROUP – B (RAT TREATED WITH VEHICLE)

Haematological Parameters		Normal rats	Rats Treated with Vehicle only		
		1st day	7th day	14th day	21th day
		$M_1 \pm SD_1$	$M_1 \pm SD_1$	$M_1 \pm SD_1$	$M_1 \pm SD_1$
i. Total RBC count (Million)		3.20	3.70	4.00	4.00
		3.70	3.90	4.10	4.40
		4.10	4.20	4.10	4.20
		3.90	4.10	4.30	4.10
		3.725 ± 0.3345	3.975 ± 0.192	4.125 ± 0.1089	4.175 ± 0.1497
ii. Total WBC count (Thousand/cc)		7.90	8.30	8.70	9.00
		8.30	8.70	8.90	8.80
		8.70	8.60	9.00	9.20
		9.10	9.60	9.40	9.50
		8.50 ± 0.44	8.80 ± 0.41	9.00 ± 0.25	9.13 ± 0.22
iii. Differential count of WBC	a. Neutrophil	47.00	46.00	47.00	50.00
		46.00	48.00	51.00	49.00
		51.00	53.00	50.00	52.00
		49.00	47.00	51.00	53.00
		48.25 ± 1.92	48.50 ± 2.69	49.75 ± 1.64	51.00 ± 1.58
	b. Lymphocyte	41.00	36.00	39.00	38.00
		43.00	40.00	38.00	36.00
		38.00	47.00	36.00	37.00
		39.00	40.00	37.00	35.00
		40.25 ± 1.92	38.25 ± 1.78	37.50 ± 1.72	36.50 ± 1.72
	c. Monocyte	2.00	3.00	2.00	2.00
		1.00	2.00	2.00	4.00
		2.00	2.00	3.00	2.00
		2.00	1.00	2.00	2.00
		1.75 ± 0.433	2.0 ± 0.7071	2.25 ± 0.433	2.50 ± 0.866
	d. Eosinophil	2.00	2.00	2.00	4.00
		2.00	2.00	3.00	3.00
		1.00	1.00	2.00	2.00
		2.00	3.00	2.00	2.00
		1.75 ± 0.433	2.0 ± 0.7071	2.25 ± 0.433	2.75 ± 0.8281
iv. Platelet count (Lack/cc)		2.30	2.25	2.50	2.40
		2.15	2.30	2.35	2.70
		2.05	2.20	2.55	3.00
		2.40	2.50	2.30	2.65
		2.22 ± 0.13	2.31 ± 0.11	2.43 ± 0.20	2.69 ± 0.21
v. Hemoglobin (gm./DL)		8.50	8.80	8.70	9.10
		8.25	8.40	8.65	8.90
		8.75	9.00	9.10	9.00
		8.40	8.60	9.00	9.30
		8.48 ± 0.18	8.70 ± 0.50	8.86 ± 0.19	9.08 ± 0.15
vi. ESR (mm/1st hour)		10.00	11.00	13.00	13.00
		13.00	12.00	14.00	12.00
		11.00	12.00	14.00	13.00
		12.00	11.00	12.00	14.00
		11.50 ± 1.29	11.5 ± 0.58	13.25 ± 0.96	13.0 ± 0.82

TABLE 6

HAEMATOLOGICAL PROFILE OF GROUP – C (RAT TREATED WITH MBGC)

Haematological Parameters		Normal rats	Rats Treated with MBGC		
		1st day	7th day	14th day	21th day
		$M_1 \pm SD_1$	$M_1 \pm SD_1$	$M_1 \pm SD_1$	$M_1 \pm SD_1$
i. Total RBC count (Million)		3.50	3.90	3.80	4.00
		3.10	3.40	3.70	4.10
		3.30	3.50	3.60	3.90
		3.90	4.10	4.30	4.20
		3.45 ± 0.2958	3.725 ± 0.2861	3.85 ± 0.2692	4.05 ± 0.1118
ii. Total WBC count (Thousand/cc)		8.20	8.70	9.10	9.00
		8.50	9.10	8.90	9.20
		9.00	8.80	9.20	9.40
		9.20	9.50	9.60	10.00
		8.73 ± 0.40	9.03 ± 0.31	9.20 ± 0.25	9.40 ± 0.37
iii. Differential count of WBC	a. Neutrophil	45.00	47.00	50.00	55.00
		47.00	51.00	49.00	52.00
		44.00	47.00	52.00	50.00
		49.00	46.00	53.00	56.00
		46.25 ± 1.92	47.50 ± 1.92	51.00 ± 1.58	53.25 ± 2.38
	b. Lymphocyte	47.00	46.00	44.00	45.00
		52.00	50.00	47.00	44.00
		49.00	46.00	47.00	43.00
		46.00	49.00	46.00	42.00
		48.50 ± 2.29	47.75 ± 1.79	46.00 ± 1.22	43.50 ± 1.12
	c. Monocyte	1.00	2.00	2.00	1.00
		2.00	3.00	3.00	3.00
		2.00	2.00	3.00	4.00
		2.00	2.00	2.00	2.00
		1.75 ± 0.433	2.25 ± 0.433	2.5 ± 0.5	2.50 ± 1.118
	d. Eosinophil	2.00	2.00	3.00	2.00
		0.00	1.00	1.00	2.00
		2.00	2.00	2.00	3.00
		1.00	1.00	2.00	2.00
		1.25 ± 0.8291	1.5 ± 0.5	2.0 ± 0.7071	2.25 ± 0.433
iv. Platelet count (Lack/cc)		2.50	2.40	2.70	2.90
		2.15	2.30	2.50	2.80
		2.35	2.35	2.50	3.00
		2.00	2.20	2.30	2.85
		2.25 ± 0.19	2.31 ± 0.07	2.50 ± 0.14	2.89 ± 0.074
v. Hemoglobin (gm./DL)		8.10	8.40	8.90	9.40
		8.75	8.90	8.70	9.10
		8.50	8.70	9.00	10.00
		9.00	9.30	9.40	9.30
		8.59 ± 0.33	8.83 ± 0.33	9.00 ± 0.25	9.45 ± 0.34
vi. ESR (mm/1st hour)		11.00	14.00	13.00	12.00
		12.00	10.00	14.00	14.00
		13.00	15.00	14.00	13.00
		10.00	13.00	12.00	15.00
		11.50 ± 1.29	13.0 ± 2.16	13.25 ± 0.96	13.50 ± 1.29

3.4 Monitoring the Histopathological Studies

The histopathological studies of liver, kidney, heart, lung and spleen of group A, group B and group C (Experimental rats) were performed after intraperitoneal administration of the drugs for consecutive 21 days at a dose of 300 µg/rat/day. No detectable abnormalities were found when these slides of the tissues of food control, vehicle control and drug treated rats were examined under microscope (Table- 7). It appears that, the tested MBGC has no effect on cellular studies, i.e. it does not cause degeneration of the cells of these organs.

TABLE 7

EFFECT OF MBGC ON HISTOPATHOLOGY OF RAT'S KIDNEY, HEART, LUNG, LIVER TISSUE AFTER I.P. ADMINISTRATION OF 300 µG/RAT/DAY FOR 21 CONSECUTIVE DAYS.

Group	Dose (i.p.)	Histopathological Changes				
	µg/rat/day	Kidney	Heart	Lung	Liver	Spleen
A-Control	300 µg gm of vehicle	NAD	NAD	NAD	NAD	NAD
B- MBGC	300 µg gm of polymer	NAD	NAD	NAD	NAD	NAD

NAD indicates no abnormalities detected.

4. 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 MBGC and its present toxicological studies suggest that, MBGC can be safely subjected to clinical trial for specialized application such as control release drug formulation, granular pesticides, fertilizers etc. and other purposes where biodegradable polymers be needed.

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REFERENCES

[1] B. Jeong, M. R. Kibbey, J. C. Birnbaun, Y. Y. Won, and A. Gutowska, 'Thermogelling Biodegradable Polymers with Hydrophilic Backbones: PEG-g-PLGA', Macromolecules, 33 (2000) 8317-8322.

[2] E. W. P. Damen, P. H. G. Wiegerinck, L. Braamer, D. Sperling, D.de Vos, and H. W. Scheeren, 'Paclitaxel Esters of Malic Acid as Prodrugs With Improved Water Solubility' Bioorganic and Medicinal Chemistry 8 (2000) 427-432.

[3] M. A. Bakr, M. A. Islam, M. A. Karim, G. Sadik, M. H. U. Biswas; J. Polym. Mater. 19 (2002) 87-92.

[4] M. A. Bakr, S. Khatun, M. A. Islam and G. Sadik; J. Polym. Mater. (2003), P-20.

[5] M. A. Bakr et all, 'In-vitro Drug Release Patern of Maleic Acid-Succinic Acid- Propane-1, 2-diol co-polyester' J. Polym. Mater.23 (2006) 217-222.

[6] M. A. Bakr., S. Khatun and M. A. Islam, Drug Release Profile of Malic acid-Phthalic acid-Butane 1,4-diol Copolyester, Pakistan Journal of Scientific and Industrial Research, 47(4), (2004),251-255.

[7] M. A. Bakr, K. Hasan, M. A. Islam, S. Khatun, M.A. Manan and K. S. Ara, In-vitro Drug Release Pattern of Maleic Acid-Succinic Acid-Propane 1,2-diol Copolyester, *J. Polym. Mater.* 23, (2006), 217-222.

[8] M. Gruvegard, T. Lindberg and A. C. Albertsson, J. Macromol. Sci. Pure Appl. Chem. A35: (1998), 885.

[9] T. Ouchi, H. Miyazaki, F. Tasaka, A. Hamada and Y. Ohya. *J. Polymer Preprints,* 41(2), (2000), 1637.

[10] D. Pramanick and T. T. Roy, Synthesis and Biodegradation of Copolymers from Citric Acid and Glycerol, Polymer Bulletin, 19, (1980), 365-370.

[11] M. A. Islam, S. Khatun, M. A. Bakr, Toxicological Study of Malic acid-Phthalic acid-Propane- 1,2-diol Copolyester, J Agric Rural Dev 5(1&2), (2007),150-156.