



Safety assessment of McB-E60 (extract of a *Momordica* sp.): Subchronic toxicity study in rats



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ABSTRACT

Momordica charantia plant is consumed as a foodstuff in some south Asian curries while its extract preparations have been traditionally used for lowering blood glucose levels in patients with diabetes mellitus. Nutritional Health Institute Laboratories (NHIL), LLC, Florida informed that it patented a new plant McB, as an interhybrid of three plants of *Momordica* genus. The objective of the present study was to investigate potential adverse effects, if any, of McB-E60 (extract of a *Momordica* sp.) in rats following subchronic administration. Sprague-Dawley rats (10/sex/group) were administered via oral gavage 0 (control), 250, 500 and 1000 mg/kg body weight (bw)/day of McB-E60 for 90 days. Additional 28-day recovery groups were maintained at control and high dose levels. No mortality or significant and adverse changes in clinical signs, neurological signs, body weight gain or feed intake were noted. No toxicologically significant changes in hematology, clinical chemistry, urinalysis and organ weights were noted. Gross and microscopic pathology examinations did not reveal treatment-related abnormalities. Any changes noted were incidental and within historical control ranges. Based on the results of this study, the No-Observed-Effect Level (NOEL) for McB-E60 (extract of a *Momordica* sp.) was determined as greater than 1000 mg/kg bw/day, the highest dose tested.

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1. Introduction

Approximately 40% of Americans use complementary and alternative medicine (CAM) for health promotion and the treatment of illness [10]. Of these users, 2 and 3 million specifically use CAM to lower blood sugar levels and to treat various stages of diabetes, despite limited studies of their safety and efficacy [2]. An estimated 79 million adult Americans have pre-diabetes, a condition that is preventable and treatable if recognized early [1]. Individuals with pre-diabetes have glycated hemoglobin (HgA1c) and blood sugar levels that are below the clinical threshold to be classified as type 2 diabetes but are higher than normal [4]. Pre-diabetics, if not treated appropriately, are at risk for becoming diabetic and developing cardiovascular disease [9,13].

Abbreviations: ALKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; FDA, food and drug administration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; NOEL, no-observed-effect level; OECD, organization for economic co-operation and development.

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Momordica charantia, also known as Bitter melon, is a widely used traditional remedy for hyperglycemia. A member of the cucurbitaceae family, Bitter melon is a perennial climber characterized by warty-fruit like gourds or cucumbers. It is a commonly consumed vegetable found throughout the sub-tropical world (China, India, Thailand, East Africa, The Caribbean, Central and South America) and is known by various names, such as balsam pear, bitter gourd, cundeamor, goo-fah, karela, etc [11,6]. The fruit, as well as the whole plant is believed to possess anti-diabetic, anti-viral, anti-bacterial and anticancer properties and has been scientifically evaluated in the recent past [5]. The immature fruits of *M. charantia* can be prepared in many ways for food uses. Fruits, flowers, and young shoots are also used as a flavoring. The young shoots and leaves are sometimes cooked and eaten as leafy vegetables [7]. In recent years, processed bitter gourd in the form of capsules or tablets is commonly marketed as a dietary supplement under the brand names Gourdin, Karela, and Glucobetic in Canada, India, United Kingdom, the United States, and many Asian countries [7].

In several *in vitro* and animal model studies, the plant has been investigated for its mechanism of action as an anti-diabetic. Over 228 different compounds with possible medicinal properties, acting alone or in combination, have been isolated from bitter melon fruit, seeds, leaves, stems, pericaps, endosperm, callus tissues, and cotyledons [3]. Of the various compounds identified,

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charatin, polypeptide-p, vicine, momordin, and similar derivatives have been claimed to improve glycemic control [3]. The available information indicates that bitter melon has been traditionally used to treat high blood sugar and diabetes. For over 70 years, studies have appeared sporadically in the literature indicating the benefit of bitter melon in lowering blood sugar. In human studies, bitter melon juice, fruit, and dried powder have been investigated for hypoglycemic effect. Only a few randomized controlled trials of bitter melon have been conducted. In a prospective, randomized, double-blinded and placebo-controlled trial of bitter melon, a statistically significant decrease in HbA1c levels after 4 months of intervention, compared with a referent group receiving refined soybean oil was noted [14].

In an attempt to further develop improved varieties of *Momordica* species for use in humans to control blood sugar, several investigators have attempted to develop new varieties and strains of *Momordica* by inter species hybridization. NHIL has developed and patented a new plant variety which is a plant grown from seeds derived from a new interhybrid of three plants of *Momordica* genus. This newly derived plant hybrid of genus *Momordica* (McB interhybrid) has been stabilized and asexually reproduced. The McB interhybrid has been described as an interhybrid derived from a cross of *Momordica charantia*, commonly known as bitter melon, *Momordica balsamina*, and a previously unnamed *Momordica* species native to Ecuador. In particular the McB interhybrid is a tetra-cross pollinated hybrid plant of the *Momordica* species. The extract (*McB-E60*) evaluated in the present study is derived exclusively from the leaves, petioles and stem (the fruits, flower and roots are not included) of the new interhybrid plant.

Given the potential use of bitter melon (*M. charantia*) as a dietary supplement in possible support for maintaining blood glucose, the objective of this study was to investigate the long-term repeat dose toxicity of *McB-E60* (extract of a *Momordica* sp.). In the repeat dose subchronic toxicity study, a detailed assessment of the toxic potentials of a standardized extract when administered daily for 90-days via oral gavage to Sprague Dawley rats, was carried out.

2. Materials and methods

2.1. Study design

The 90-day subchronic toxicity study was performed in accordance with Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, No. 408: Repeated Dose 90-day Oral Toxicity Study in Rodents; the U.S. FDA, CDER Guidance for Industry, Botanical Drug Products, June 2004; the ICH Harmonized Tripartite Guideline M3 (R2), Current Step 4 version dated 11 June, 2009; and the WHO Guidelines for Toxicity Investigation of Herbal Medicines, Research guidelines for evaluating the safety and efficacy of Herbal Medicines, 1993. The study was conducted in compliance with the principles of Good Laboratory Practice as set forth in OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, 'OECD Principles on Good Laboratory Practice' ENV/MC/CHEM (98) 17 (as revised in 1997).

2.2. Test article

Standardized *McB-E60* (extract of a *Momordica* sp.) used in the present study was provided by Nutritional Health Institute Laboratories (Tallahassee, FL, USA) and was manufactured at Metaugus, Inc., GA30125, USA as production batch number 9012701 of year 2008. The product is a green colored clear liquid.

The product quality was defined by parameters such as ash, pH, protein, dietary fibre, gentisic acid, stigmasterol and total

Table 1

Description and specifications of *McB-E60* (extract of a *Momordica* sp.).

Product Description		
It is a green clear crystalline liquid extract obtained from a new interhybrid of three plants of <i>Momordica</i> genus		
Parameter	Results	Method
pH	4.12	USP 791
Gentisic Acid	<0.01 ppm	Internal (HPLC) Method—KK123
Stigmasterol	0.01% (w/w)	Internal (GC) Method—KK329
Total sterols	0.0115% (w/w)	Internal (GC) Method—KK329
Dry Ashing	<1%	—
Residual ethanol	48,000 mg/kg	USP/NF 467
Total dietary fibre	<0.2% (w/w)	—
Combustion proteins	1.41%	AOAC 990.03, 992.15
Heavy metals		
Total heavy metals	<0.02 ppm	—
Cadmium	<0.002 ppm	AOAC 993.14 Mod.
Lead	<0.007 ppm	AOAC 993.14 Mod.
Mercury	<0.004 ppm	AOAC 993.14 Mod.
Arsenic	<0.007 ppm	AOAC 993.14 Mod.
Microbiological assays		
Yeast and moulds	<10 (est) CFU/g	BAM Chapt. 18
Total Aerobic plate count	<10 (est) CFU/g	AOAC 990.12
Total coliform	<10 (est) CFU/g	AOAC 991.14
Escherichia coli	<10 (est) CFU/g	AOAC 991.14
Staphylococcus aureus	<10 (est) CFU/g	AOAC 2003.07
Salmonella	Not Detected per 25 g	AOAC 2003.09
Other Contaminants		
Apart from above, the sample was analysed for several pesticides—organochlorine, pyrethroids, organonitrogen, organophosphorous, carbamates and for aflatoxins, to ensure that it was free from undesired levels of the same.		

Ref. (except for product description and ash content): Certificate of analysis—AR-15-KK-000614-01 dated January 12, 2015, of Eurofins Scientific, Inc., Supplement Analysis Center, CA for sample # 740-2014-00021037.

sterols. The product had a characteristic odor (a balanced herbal estery lightly ethanolic smell) and bitter taste. The concentrate was checked for heavy metals, microbial load and pesticide residues. The physical characteristics and chemical specifications of the product are presented in Table 1.

2.3. Animals

Healthy male and female Sprague-Dawley rats were procured from Reliance Life Sciences (Navi Mumbai, India) and were acclimatized to experimental room conditions for up to six days. Veterinary examinations were then conducted following which 100 rats (50 males and 50 females) were selected such that the weight variation within each sex of rats did not exceed $\pm 20\%$ of the mean weight for that sex (male rats 196.6 g; female rats 160.7 g). The selected rats were then assigned to control and treatment groups (10/sex/group) using random numbers generated with MS-Excel. The rats were group housed with up to two rats of similar sex per cage in sterilized solid bottom polypropylene cages with bedding of clean and sterilized paddy husk. Cages had facilities for food and water bottle, and were suspended on movable stainless steel racks. HVAC conditions in the experimental animal room were set to maintain 10–15 air changes per hour of 100% fresh and filtered air, conditioned with temperature between 19–25 °C and relative humidity 30–70%. The animal room was illuminated for 12 h light on each day while darkness was maintained for rest of the 12 h. The rats were fed *ad libitum* with Nutrilab rodent feed manufactured by Provimi Animal Nutrition India Pvt. Ltd. (Bangalore, India) and were provided with potable water filtered through 'AquaGuard' water filter having built-in ultra violet irradiation process.

2.4. Treatment

Groups of 10 rats per sex were gavaged daily with *McB-E60* (extract of a *Momordica* sp.) at dose levels of 0 (Group I- control), 250 (Group II- low dose), 500 (Group III- mid dose), and 1000 (Group IV- high dose) mg/kg body weight/day for 90 days. Additional recovery groups of five rats per sex were maintained at control (Group V) and high dose (Group VI) levels, and were observed for a period of 28 days after cessation of the 90 days of treatment. This 90-day study was preceded by a 14-day oral toxicity study on *McB-E60*, serving as a dose range finding study in which rats (5/sex/group) were administered 0, 100, 300 and 1000 mg/kg bw/day for 14 consecutive days. This 14-day study did not reveal any adverse effects of *McB-E60* extract with respect to parameters such as clinical signs, body weight gain, feed intake, necropsy, organ weights and clinical laboratory investigations. The choice of dose levels for the present 90-day study thus was made on the basis of history of usage of *Momordica charantia* as a food by humans, as well as on the basis of findings of above 14-day oral toxicity study. The dose of 1000 mg/kg bw/day has been specified as a 'limit dose' by the OECD test guideline. As described in ensuing paragraphs, the rats in this 90-day subchronic study were subjected to observations during the 90-day treatment period as well as during the 28 days of recovery period, and, at their termination, were further subjected to evaluations for organ weights, laboratory investigations for hematology, urinalysis and blood chemistry, and for gross and microscopic pathology.

2.5. Parameters investigated

2.5.1. Survival, clinical signs, body weight and food consumption

All rats were examined for survival and moribundity twice on each working day. All signs of ill health, and any behavioral changes or reaction to treatment were recorded for individual animals. Cage side clinical examinations were conducted daily on all rats while detailed clinical examinations were conducted weekly during the 90-day treatment period and during the 28-day recovery period. The detailed observations were made outside the home cage to note any changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity such as lacrimation, piloerection, pupil size, and unusual respiratory pattern. Any changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies or bizarre behavior, were also recorded.

Ophthalmoscopic examination was carried out on all rats prior to initiation of the experiment and before termination of the treatment period on day 89, on animals from control and high dose groups. During the 12th week of treatment, all animals were examined for assessment of sensory reactivity, assessment of grip strength and motor activity. These included the functional observational battery [8]. Individual animal body weights were recorded prior to commencement of treatment (day of grouping—day 0), weekly thereafter during the treatment and recovery periods, and at necropsy (overnight fasted body weights). The quantity of feed consumed by rats in each cage was recorded weekly. Feed intake per rat per day was calculated using the amount of food offered to each cage during the period of measurement, and left in each cage at end of this period, and the number of rats surviving in each cage.

2.5.2. Clinical pathology

After completion of 90 days of treatment or the 28 days of recovery period, blood samples were collected from all surviving animals from the respective groups in this study. Prior to blood sampling, animals were fasted overnight. Sampling of blood was made after the rats were asphyxiated following inhalation of high (>70%) concentration of carbon dioxide. Blood samples were collected separately in tubes containing anticoagulants EDTA for hematology,

heparin for clinical chemistry and sodium citrate for coagulation parameters. Smears of freshly obtained blood samples were prepared on glass slides and subjected to staining with Leishman's Stain.

2.6. Hematology

The hematological parameters included: Hemoglobin, Packed cell volume, Total red cell count (RBC), Total white cell count (WBC), Differential white cell counts (Neutrophils, Lymphocytes, Eosinophils, Monocytes, Basophils), Platelet count, Reticulocyte count, Absolute RBC indices (Mean corpuscular volume-MCV, Mean corpuscular hemoglobin- MCH, Mean corpuscular hemoglobin concentration—MCHC). Coagulation Parameters included Prothrombin Time and Activated Partial Thromboplastin Time. The hematological estimations were performed using 'Abbott Cell Dyn 3700' Hematology Analyser and 'Semi- Automated Coagulation Analyser Start[®] 4 (Diagnostica Stago, France).

2.7. Clinical chemistry

Clinical chemistry parameters included: Sodium, Potassium, Glucose, Total Cholesterol, Urea nitrogen (BUN), Urea, Creatinine, Protein (total), Albumin, Globulin, Total Protein, Albumin/Globulin (A/G) Ratio, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma glutamyl transpeptidase (GGT), Bilirubin total, Calcium, Phosphorus and Triglycerides. Plasma samples were analysed individually (not pooled) for determination of clinical chemistry parameters using Synchron CX-5 Pro Fully Automatic Random Access Analyser (Beckman Coulter, Inc.), except that the Calcium determination was performed using Dimension X-pand Plus Clinical Chemistry System (Siemens Healthcare Diagnostics Inc.). Sodium and Potassium determinations were made using Easylyte Analyser (Medica Corporation, MA, USA).

2.8. Urinalysis

Urinalysis was performed in last week of the study, on day 88, before termination of the treatment period. Initially urinalysis was performed on animals from control (Group I) and high dose groups (Group IV). Urine samples were collected using a battery of specially designed stainless steel urine collection cages. Each rat was housed in this cage. Urine samples were collected over a period of about 4 h. Urinalysis parameters included Colour, Appearance, Specific gravity, pH, Protein, Glucose, Ketone, Volume (timed), Bilirubin, Urobilinogen, Nitrite, Leucocyte, Occult blood, and Microscopy. Tests were performed using Multistix[®] 10 SG multiple reagent diagnostic strips manufactured by Siemens Healthcare Diagnostics and were read using "Clinitek Status" Urine Analyser' (Siemens Medical Solutions Diagnostics).

2.8.1. Necropsy and pathology

2.8.1.1. Terminal necropsy and tissue collection. On completion of 90 days of treatment or the 28-day recovery periods, all rats were euthanized by exsanguination, preceded by their asphyxiation by inhalation of high (>70%) concentration of carbon dioxide for a brief period. The rats were subjected to a complete gross necropsy which included examination of external surfaces, orifices, cranial, thoracic and abdominal cavities and all organs. At terminal necropsy, organs viz. kidney (right and left), liver, heart, adrenal glands (right and left), spleen, brain, testes (right and left), uterus, thymus, epididymides (right and left), ovaries (right and left), from all surviving animals were carefully dissected and trimmed to remove fat and other contiguous tissue, and then were weighed immediately to minimize the effects of drying on organ weight. Values of these

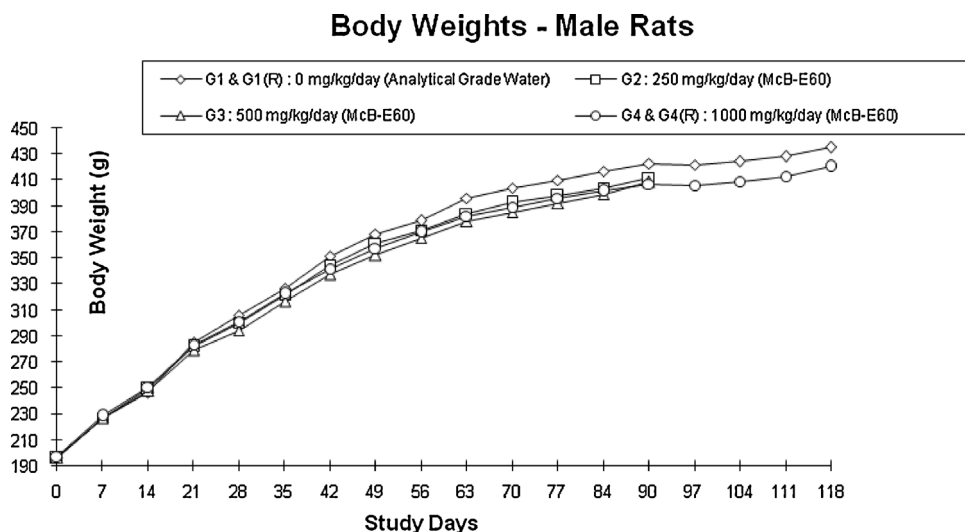


Fig. 1. Body weights of male rats treated with *McB-E60* (extract of a *Momordica* sp.). Mean body weights for male rats during the treatment period of 90 days and the 28-day recovery period. The values are presented as means \pm standard deviation.

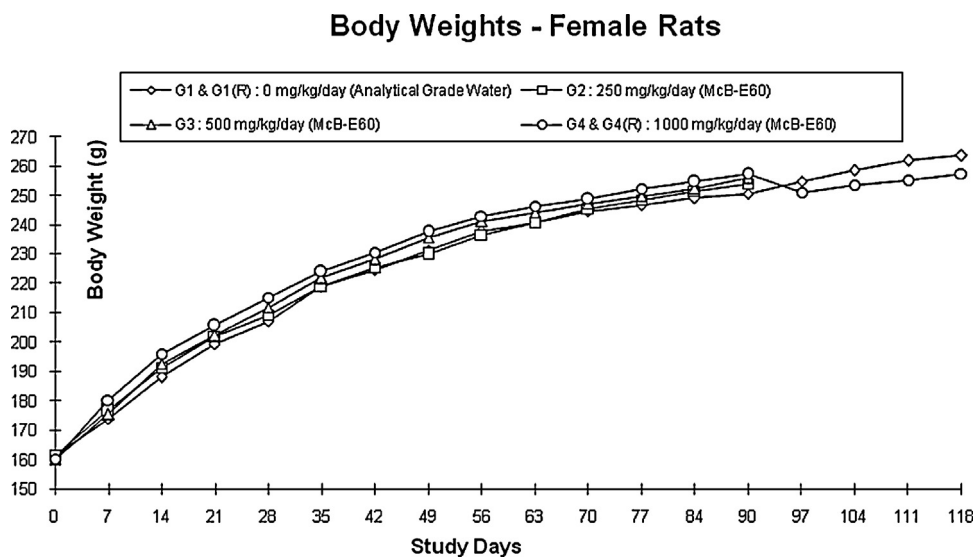


Fig. 2. Body weights of female rats treated with *McB-E60* (extract of a *Momordica* sp.). Mean body weights for female rats during the treatment period of 90 days and the 28-day recovery period. The values are presented as means \pm standard deviation.

organs as percent of necropsy body weights were calculated (relative organ weights). In addition to these organs collected for measurements, all the tissues (total over 35) were collected and preserved in 10% neutral buffered formalin. In addition, samples of any macroscopically abnormal tissues were preserved, along with samples of adjacent normal tissue where appropriate.

2.9. Histopathological examination

Tissues and organs mentioned above and any other tissues with gross lesions were fixed in 10% neutral buffered formalin (lungs were inflated with fixative prior to immersion), embedded in paraffin wax, sectioned at about 5 μ m thickness and stained with hematoxylin and eosin, for microscopic examination. Microscopic examinations were carried out on all listed organs and tissues of all surviving animals of Group I (control) and Group IV (high dose), sacrificed at termination of the treatment period. Since treatment related adverse effects were not observed in any tissues/organs at the high dose level, the investigations were not extended to the low

dose (Group II), mid dose (Group III) and to the recovery groups. The microscopic findings were allocated grades of severity using a convention adopted at the test facility.

2.10. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistical Software (version 23). The body weight, food consumption, hematology, clinical chemistry, organ weight and some neurological examination data, of different groups were subjected to one-way analysis of variance (ANOVA) after assessing their homogeneity by Levene's test. Dunnett's test was employed as a post-hoc test wherever necessary. Comparisons of data from two groups such as those of body weights, food intake, hematology, clinical chemistry and organ weight data of recovery groups, and urinalysis parameters (control and high dose groups) were performed by employing Independent t test. The variance was evaluated at 5% level of significance.

Table 2
Hematological parameters of male rats treated with *McB-E60* (extract of a *Momordica* sp.) (on study day 91).

Parameter	Units	mg/kg/day			
		(G-I)	(G-II)	(G-III)	(G-IV)
		0	100	500	1000
RBC	$\times 10^6 \mu\text{L}$	8.71 \pm 0.30	8.80 \pm 0.52	8.77 \pm 0.46	8.67 \pm 0.63
Hb	g/dL	15.12 \pm 0.53	15.27 \pm 0.80	15.16 \pm 0.70	14.98 \pm 1.01
PCV	%	43.19 \pm 1.63	43.29 \pm 1.91	42.81 \pm 1.76	42.50 \pm 2.62
MCH	pg	17.37 \pm 0.36	17.37 \pm 0.39	17.30 \pm 0.36	17.29 \pm 0.47
MCV	fl	49.62 \pm 1.08	49.27 \pm 1.58	48.87 \pm 1.02	49.09 \pm 1.97
MCHC	g/dL	35.01 \pm 0.53	35.27 \pm 0.74	35.41 \pm 0.42	35.24 \pm 0.59
PLT	$\times 10^3 \mu\text{L}$	939.20 \pm 104.08	924.60 \pm 88.45	892.60 \pm 71.52	901.10 \pm 81.24
WBC	$\times 10^3 \mu\text{L}$	7.21 \pm 1.51	7.14 \pm 1.36	6.47 \pm 0.53	6.70 \pm 1.74
RET	%	2.86 \pm 1.89	2.51 \pm 0.53	2.30 \pm 0.33	2.28 \pm 0.52
NEU	%	25.90 \pm 6.01	23.46 \pm 3.85	28.07 \pm 6.01	25.78 \pm 6.21
LYM	%	71.84 \pm 5.98	74.21 \pm 3.90	68.45 \pm 6.63	71.07 \pm 8.54
MON	%	0.683 \pm 0.432	0.805 \pm 0.495	1.597 \pm 2.175	1.459 \pm 2.833
EOS	%	1.573 \pm 0.336	1.522 \pm 0.529	1.833 \pm 0.599	1.582 \pm 0.393
BAS	%	0.000 \pm 0.000	0.006 \pm 0.014	0.029 \pm 0.070	0.092 \pm 0.274
General blood picture		NAD	NAD	NAD	NAD
Coagulation Values					
PT	sec	17.01 \pm 3.21	16.60 \pm 1.35	15.93 \pm 1.29	16.25 \pm 1.33
APTT	sec	12.03 \pm 1.05	12.69 \pm 1.81	12.89 \pm 1.47	13.19 \pm 2.05

Values are mean \pm SD for 10 rats in each group. RBC = red blood cell count; Hb = hemoglobin; WBC = white blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet count; RET = reticulocyte count; NEU = neutrophil; LYM = lymphocyte; MON = monocyte; EOS = e eosinophil; BAS = basophil; PT = prothrombin time; APTT = activated partial prothrombin time.

Table 3
Hematological parameters of female rats treated with *McB-E60* (extract of a *Momordica* sp.) (on study day 91).

Parameter	Units	mg/kg/day			
		(G-I)	(G-II)	(G-III)	(G-IV)
		0	100	500	1000
RBC	$\times 10^6 \mu\text{L}$	8.10 \pm 0.69	8.51 \pm 0.48	8.46 \pm 0.31	8.36 \pm 0.28
Hb	g/dL	14.81 \pm 1.05	15.32 \pm 0.70	15.39 \pm 0.54	15.22 \pm 0.65
MCH	pg	18.31 \pm 0.59	18.01 \pm 0.38	18.20 \pm 0.55	18.20 \pm 0.40
MCV	fl	51.17 \pm 2.21	50.96 \pm 1.25	51.01 \pm 1.57	51.37 \pm 0.96
MCHC	g/dL	35.80 \pm 0.98	35.34 \pm 0.41	35.68 \pm 0.71	35.44 \pm 0.87
PCV	%	41.37 \pm 2.77	43.34 \pm 1.72	43.13 \pm 0.99	42.94 \pm 1.39
PLT	$\times 10^3 \mu\text{L}$	929.80 \pm 51.59	941.50 \pm 96.09	851.80 \pm 62.63	872.60 \pm 85.06
WBC	$\times 10^3 \mu\text{L}$	6.13 \pm 1.11	5.73 \pm 0.76	6.43 \pm 1.31	6.46 \pm 0.82
RET	%	4.18 \pm 5.49	3.57 \pm 2.10	3.15 \pm 0.74	2.90 \pm 0.60
NEU	%	27.47 \pm 5.46	24.92 \pm 3.11	27.46 \pm 3.86	27.55 \pm 5.26
LYM	%	68.42 \pm 6.18	69.55 \pm 6.56	67.96 \pm 4.03	68.16 \pm 5.89
MON	%	1.553 \pm 1.946	3.040 \pm 4.536	1.261 \pm 2.075	1.563 \pm 2.485
EOS	%	2.549 \pm 0.931	2.488 \pm 0.703	3.284 \pm 1.201	2.685 \pm 0.794
BAS	%	0.097 \pm 0.286	0.021 \pm 0.041	0.026 \pm 0.082	0.034 \pm 0.108
General blood picture		NAD	NAD	NAD	NAD
Coagulation Values					
PT	sec	15.90 \pm 1.19	16.76 \pm 1.26	16.10 \pm 0.88	16.13 \pm 1.18
APTT	sec	13.57 \pm 1.26	15.76 \pm 8.01	12.76 \pm 1.71	13.09 \pm 1.06

Values are mean \pm SD for 10 rats in each group. RBC = red blood cell count; Hb = hemoglobin; WBC = white blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet count; RET = reticulocyte count; NEU = neutrophil; LYM = lymphocyte; MON = monocyte; EOS = e eosinophil; BAS = basophil; PT = prothrombin time; APTT = activated partial prothrombin time.

3. Results

3.1. Survival, clinical observations, ophthalmological examination

The daily oral administration of *McB-E60* (extract of a *Momordica* sp.) for 90 consecutive days at doses up to 1000 mg/kg bw/day did not cause any adverse effect on the survival of the male and female rats in this study. There was no incidence of any mortality amongst the treated male and female rats. The daily general clinical examinations and the weekly detailed clinical examinations of rats conducted during the treatment and recovery periods did not reveal any remarkable and abnormal clinical signs indicative of systemic toxicity (data not shown), in rats of either sex, following administration of *McB-E60* (extract of a *Momordica* sp.) at doses up

to 1000 mg/kg bw/day. The findings from neurological evaluations following treatment of male and female rats with *McB-E60* (extract of a *Momordica* sp.) at doses up to 1000 mg/kg bw/day did not reveal any remarkable and abnormal alterations in qualitative and quantitative parameters of their sensory reactivity, grip strength and motor activity, as was evident during assessment of the 'functional observational battery' carried out during 12th week of the study (data not shown). The values of quantitative parameters (frequencies of urination, defecation and rearing, the landing foot splay and the grip strength) of the treatment group rats of both sex did not differ significantly ($P > 0.05$) from those of the vehicle control group rats during the 12th week of the study. Ophthalmoscopy prior to study initiation and near experimental completion (Day 85) did not reveal any abnormalities.

Table 4
Clinical chemistry parameters of male rats treated with *McB-E60* (extract of a *Momordica* sp.) (on study day 91).

Parameter	Units	mg/kg/day			
		(G-I)	(G-II)	(G-III)	(G-IV)
		0	250	500	1000
AST	U/L	153.20 ± 24.78	120.80 ^a ± 23.50	130.50 ± 12.72	138.20 ± 29.50
ALT	U/L	117.10 ± 33.69	110.10 ± 30.82	109.40 ± 11.86	114.60 ± 26.62
ALP	U/L	112.90 ± 15.81	113.60 ± 12.53	124.30 ± 19.87	111.40 ± 9.99
TBILI	U/L	0.27 ± 0.18	0.22 ± 0.19	0.29 ± 0.20	0.26 ± 0.21
A/G ratio	g/dL	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.02
BUN	mg/dL	17.50 ± 1.78	16.80 ± 2.35	17.20 ± 1.81	17.60 ± 3.57
UREA	mg/dL	25.20 ± 2.56	24.19 ± 3.38	24.77 ± 2.61	25.34 ± 5.13
CHOL	mg/dL	119.30 ± 15.04	118.00 ± 6.43	113.10 ± 16.16	118.60 ± 16.05
TG	mg/dL	36.80 ± 9.96	44.20 ± 13.51	37.10 ± 11.80	46.00 ± 9.46
GLUC	mg/dL	115.10 ± 24.22	112.10 ± 9.93	108.00 ± 11.26	117.00 ± 11.51
TP	g/dL	6.59 ± 0.32	6.67 ± 0.24	6.51 ± 0.32	6.65 ± 0.47
ALB	g/dL	1.11 ± 0.07	1.11 ± 0.07	1.10 ± 0.08	1.09 ± 0.09
PHO	mg/dL	7.51 ± 1.00	7.36 ± 0.60	6.83 ± 0.54	7.10 ± 0.65
CREA	mg/dL	0.55 ± 0.08	0.53 ± 0.07	0.53 ± 0.05	0.53 ± 0.07
GGT	U/L	5.70 ± 1.16	6.00 ± 0.94	5.40 ± 1.26	5.80 ± 0.63
GLOB	g/dL	5.48 ± 0.26	5.56 ± 0.20	5.41 ± 0.26	5.56 ± 0.42
Na	mmol/L	145.48 ± 1.41	146.59 ± 1.51	145.57 ± 1.60	145.35 ± 1.54
K	mmol/L	4.64 ± 0.56	4.48 ± 0.36	4.42 ± 0.29	4.77 ± 0.45
Ca	mmol/L	10.12 ± 0.40	10.10 ± 0.28	9.69 ± 0.63	9.90 ± 0.32

Values are mean ±SD for 10 rats in each group. Aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total bilirubin (TBILI), urea nitrogen (BUN), blood creatinine (CREA), total cholesterol (CHOL), triglycerides (TG), fasting glucose (GLUC), total serum protein (TP), albumin (ALB), globulin (GLOB), calcium (Ca), inorganic phosphorus (PHO), sodium (Na), potassium (K); a = Group mean value significantly lower ($P < 0.05$) than the respective value of the vehicle control group

Table 5
Clinical chemistry parameters of female rats treated with *McB-E60* (extract of a *Momordica* sp.) (on study day 91).

Parameter	Units	mg/kg/day			
		(G-I)	(G-II)	(G-III)	(G-IV)
		0	250	500	1000
AST	U/L	118.30 ± 26.41	105.20 ± 11.85	113.40 ± 14.70	112.20 ± 10.35
ALT	U/L	90.10 ± 26.39	76.50 ± 12.35	85.40 ± 14.03	92.10 ± 20.42
ALP	U/L	83.60 ± 18.11	94.60 ± 28.00	108.80 ± 22.74	107.90 ± 25.81
TBILI	U/L	0.10 ± 0.00	0.11 ± 0.03	0.10 ± 0.00	0.10 ± 0.00
A/G ratio	g/dL	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
BUN	mg/dL	19.80 ± 2.78	18.10 ± 1.52	18.50 ± 2.95	20.50 ± 2.95
UREA	mg/dL	28.51 ± 4.00	26.06 ± 2.19	26.64 ± 4.25	29.52 ± 4.25
CHOL	mg/dL	146.50 ± 20.78	142.70 ± 14.37	145.10 ± 17.67	157.60 ± 15.49
TG	mg/dL	31.70 ± 6.25	37.50 ± 7.98	34.40 ± 11.20	29.70 ± 3.13
GLUC	mg/dL	105.40 ± 14.49	105.00 ± 8.83	101.90 ± 4.68	106.70 ± 7.42
TP	g/dL	6.58 ± 0.34	6.56 ± 0.35	6.58 ± 0.21	6.54 ± 0.08
ALB	g/dL	1.11 ± 0.06	1.10 ± 0.07	1.10 ± 0.08	1.08 ± 0.04
CREA	mg/dL	0.57 ± 0.09	0.55 ± 0.07	0.57 ± 0.07	0.62 ± 0.08
GGT	U/dL	5.10 ± 1.52	5.20 ± 1.23	5.30 ± 1.06	5.50 ± 1.51
PHO	mg/dL	5.62 ± 0.71	6.05 ± 0.55	5.97 ± 1.03	5.59 ± 0.84
GLOB	g/dL	5.47 ± 0.31	5.46 ± 0.30	5.48 ± 0.17	5.46 ± 0.10
Na	mmol/L	144.61 ± 0.96	144.18 ± 1.26	143.76 ± 1.37	144.38 ± 1.00
K	mmol/L	4.33 ± 0.29	4.34 ± 0.31	4.29 ± 0.26	4.35 ± 0.39
Ca	mmol/L	10.08 ± 0.34	10.18 ± 0.34	10.05 ± 0.34	10.12 ± 0.16

Values are mean ±SD for 10 rats in each group. Aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total bilirubin (TBILI), urea nitrogen (BUN), blood creatinine (CREA), total cholesterol (CHOL), triglycerides (TG), fasting glucose (GLUC), total serum protein (TP), albumin (ALB), globulin (GLOB), calcium (Ca), inorganic phosphorus (PHO), sodium (Na), potassium (K); a = Group mean value significantly lower ($P < 0.05$) than the respective value of the vehicle control group

3.2. Body weights and feed consumption

Daily oral administration of *McB-E60* (extract of a *Momordica* sp.) at doses up to 1000 mg/kg bw/day did not induce any adverse effects on the body weight gain by the male and female rats, during the treatment period of 90 days and the 28-day recovery period (Figs. 1 and 2). The values of body weights of treated male and female rats did not differ significantly ($P > 0.05$) from those of the vehicle control group rats during the treatment and recovery period. The daily oral administration of *McB-E60* (extract of a *Momordica* sp.) at dose levels up to 1000 mg/kg bw/day did not induce any adverse effects on the average daily feed intake by the male and female rats, during the treatment and recovery periods. The values of daily feed consumption by the rats, determined

weekly, were found to be comparable to those of the vehicle control group rats during the treatment and recovery period. Very small, biologically insignificant but statistically significant ($P < 0.05$) differences, between the values of feed intake were noted during recovery period (week 17—male rats; week 14—female rats). These were considered to be incidental.

3.3. Clinical pathology

3.3.1. Hematology

Oral administration of *McB-E60* (extract of a *Momordica* sp.) to male and female rats for 90 days at dose levels up to 1000 mg/kg bw/day did not induce any alterations in their hematological parameters determined at termination of the treatment period

Table 6Terminal organ weight in grams of male and female rats treated with *McB-E60* (extract of a *Momordica* sp.) (on study day 91).

Organ	Sex	(G-I)	(G-II)	(G-III)	(G-IV)
		0 mg/kg/day	250 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
Organ weights in grams					
Body weight (fasted)	M	399.70 ± 21.68	392.90 ± 33.23	385.90 ± 19.52	388.10 ± 21.53
Adrenal	M	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Brain	M	2.10 ± 0.12	2.16 ± 0.13	2.16 ± 0.17	2.09 ± 0.12
Epididymides	M	1.09 ± 0.14	1.11 ± 0.08	1.11 ± 0.06	1.09 ± 0.06
Heart	M	1.50 ± 0.14	1.52 ± 0.22	1.37 ± 0.09	1.43 ± 0.08
Kidney	M	3.15 ± 0.30	3.09 ± 0.39	3.05 ± 0.39	3.19 ± 0.35
Liver	M	12.75 ± 1.48	12.05 ± 1.30	12.04 ± 1.31	12.09 ± 1.10
Spleen	M	0.63 ± 0.08	0.65 ± 0.11	0.59 ± 0.09	0.62 ± 0.07
Testes	M	3.01 ± 0.43	3.09 ± 0.14	3.08 ± 0.19	3.35 ± 0.54
Thymus	M	0.45 ± 0.10	0.39 ± 0.09	0.41 ± 0.13	0.42 ± 0.08
Body weight (fasted)	F	231.30 ± 11.99	237.70 ± 20.70	241.10 ± 11.07	244.30 ± 16.61
Adrenal	F	0.046 ± 0.005	0.045 ± 0.004	0.048 ± 0.009	0.045 ± 0.007
Brain	F	2.00 ± 0.13	2.01 ± 0.13	2.01 ± 0.05	2.00 ± 0.12
Heart	F	0.98 ± 0.06	1.01 ± 0.08	1.07 ± 0.05	1.03 ± 0.10
Kidney	F	2.16 ± 0.28	2.21 ± 0.27	2.23 ± 0.17	1.98 ± 0.23
Liver	F	7.92 ± 0.74	7.78 ± 0.88	8.28 ± 0.35	7.76 ± 0.91
Ovaries	F	0.102 ± 0.023	0.101 ± 0.014	0.102 ± 0.021	0.096 ± 0.022
Spleen	F	0.48 ± 0.10	0.45 ± 0.07	0.46 ± 0.04	0.45 ± 0.05
Thymus	F	0.30 ± 0.03	0.33 ± 0.06	0.35 ± 0.06	0.35 ± 0.05
Uterus and oviducts	F	0.47 ± 0.07	0.86 ± 0.60	0.58 ± 0.17	0.64 ± 0.32

Values are mean ±SD for 10 rats in each group.

Table 7Incidence of histopathological findings from male rats treated with *McB-E60* (extract of a *Momordica* sp.) (Terminally sacrificed—day 91).

Organs	Lesion	mg/kg/day	
		(G-I)	(G-IV)
		0	1000
Stomach (non-glandular)	Cyst, keratinized, solitary	1	–
Terminal ileum	Hyperplasia, lymphoid, sub-mucosal, minimal	1	–
Heart	Lymphocytic infiltration, minimal	1	–
Trachea	Infiltration, lymphocytic, sub-mucosal, minimal	3	3
Colon	Hyperplasia, lymphoid, sub-mucosal, minimal	2	–
Kidney	Dilatation, tubular, minimal	7	6
	Cyst, hyaline	1	1
	Regeneration, tubular, minimal	1	–
Lungs	Infiltration, lymphocytic, minimal	4	3
	Perivascular aggregation, lymphocytic, minimal to mild	4	2
	Peribronchial lymphoid tissue hyperplasia, mild	4	3
	Alveolar macrophages, minimal	2	–
Rectum	Hyperplasia, lymphoid, sub-mucosal, minimal	–	1
Pituitary Gland	Cysts, pars distalis	1	–
Prostate	Infiltration, neutrophilic, mild	–	1

(day-91) and at end of the 28-day recovery period (day-119) (Tables 2 and 3). The values of all hematological parameters evaluated in this study of rats treated with *McB-E60* (extract of a *Momordica* sp.) did not differ significantly from those of the respective vehicle control group values ($P > 0.05$) on days 90 and 119. Although the group mean values of % monocytes and % basophils in male rats exhibited a dose dependent increase, the values were well within the historical control ranges relative to the sex, strain and species of the animals tested. The differences were also statistically insignificant, and hence considered as incidental or of no toxicological significance. The microscopic examination of stained blood smears did not reveal any abnormal and immature cells.

3.3.2. Clinical chemistry

Oral administration of *McB-E60* (extract of a *Momordica* sp.) to male and female rats at dose levels of 250, 500, and 1000 mg/kg bw/day (Groups II, III and IV, respectively) for 90 days did not induce any alterations in their clinical chemistry parameters (Tables 4 and 5) at termination of the treatment period (day 91), and on day 119, at end of the 28-day recovery period.

Although, a slight but statistically significant ($P < 0.05$) decrease in the levels of AST in Group II (low dose) male rats as compared to control group (Group I) was noted, it was considered to be an incidental finding with no toxicological significance. This was since this observed value of AST (120.8 IU/L) was well within the historical control range relative to the sex, strain and species of the animals tested, and also since there was an absence of any alterations in gross pathology, organ weights and other clinical chemistry parameters associated with liver in this group. Similarly a slight but statistically significant 'lower than control' group mean value of Calcium in Group IV(R) female rats observed at end of the 28-day recovery period (9.76 ± 0.26 mg/dL) was found to be of no biological significance due to its comparability to the historical control range of 9.8–11.7 mg/dL relative to the sex, strain and species of the animals tested.

3.3.3. Urinalysis

There were no treatment-related adverse effects in urinalysis parameters, including altered microscopic appearance of the centrifuged deposits, when examined prior to end of the treatment

Table 8
Incidence of histopathological findings from female rats treated with *McB-E60* (extract of a *Momordica* sp.) (Terminally sacrificed—day 91).

Organs	Lesion	mg/kg/day	
		(G-I) (G-IV)	
		0	1000
Trachea	Infiltration, lymphocytic, sub-mucosal, minimal	3	2
Colon	Hyperplasia, lymphoid, sub-mucosal, minimal	2	1
Kidney	Dilatation, tubular, minimal	4	3
	Infiltration, lymphocytic, minimal	1	–
Lungs	Perivascular aggregation, lymphocytic, minimal to mild	2	3
	Peribronchial lymphoid tissue hyperplasia, mild	3	2
	Alveolar macrophages, minimal	1	–
Rectum	Hyperplasia, lymphoid, sub-mucosal, minimal	–	1
Esophagus	Acanthosis and hyperkeratosis, minimal	–	1

period, on day 88, in male and female rats (data not shown) following administration of *McB-E60* (extract of a *Momordica* sp.) at dose level of 1000 mg/kg bw/day. The observations recorded for qualitative urine parameters viz. color, appearance, and graded quantities of analytes such as protein, glucose, ketones, occult blood and bilirubin were found to be comparable between the high dose group rats (Group IV) treated with *McB-E60* (extract of a *Momordica* sp.) and the vehicle control group (Group I) of rats. Group mean values of specific gravity, urobilinogen and pH of urine of male and female rats treated with the extract did not differ significantly ($P > 0.05$) from those of the vehicle control group rats as evident on the 88 day of treatment.

3.4. Organ weights

The absolute organ weights of different groups following treatment with *McB-E60* (extract of a *Momordica* sp.) at dose levels of 250, 500, and 1000 mg/kg bw/day for 90-days are summarized in Table 6. No toxicologically or statistically significant changes in organ weights were observed in absolute (Table 6) and relative organ weights (data not shown) when compared to the vehicle control group.

3.5. Necropsy and microscopic findings

There were no treatment-related and remarkable gross abnormalities in the organs/tissues of rats in any of the groups as evidenced during the gross necropsy performed at termination of the 90 days of treatment or of the 28 days of recovery period. The incidence and severity of histopathological findings from vehicle control group (Group I) and the high dose group (Group IV) following treatment with *McB-E60* (extract of a *Momordica* sp.) at dose levels of 0 and 1000 mg/kg bw/day, respectively for male and female rats are summarized in Tables 7 and 8. However, as described below few instances of microscopic findings (incidence pooled for sexes) noted were considered to be unrelated to treatment with *McB-E60* (extract of a *Momordica* sp.) due to their small incidence with minimal severity, and to the comparability of their incidence within the vehicle control and the *McB-E60* treated group, for both sexes.

Such isolated histological instances included: in kidneys a minimal tubular dilatation (11/20 in Group I and 9/20 in Group IV), minimal infiltration with lymphocytes (5/20 in Group I and 3/20 in Group IV), isolated incidence of hyaline cyst in males only (1/10 in Group I and 1/10 in Group IV) and an isolated observation of minimal focal tubular regeneration in male rats only; in lungs a minimal to mild perivascular aggregation of lymphocytes (6/20 in Group I and 5/20 in Group IV), mild peribronchial lymphoid tissue hyperplasia (7/20 in Group I and 5/20 in Group IV), and minimal presence of alveolar macrophages (3/20 in Group I); in trachea a

minimal sub mucosal infiltration of lymphocytes (6/20 in Group I and 5/20 in Group IV); in intestines isolated instances of minimal sub-mucosal lymphoid hyperplasia in terminal ileum (1/20 in Group I), colon (4/20 in Group I and 1/20 in Group IV) and in rectum (2/20 in Group IV).

In addition to above described findings, there were isolated findings of minimal acanthosis and hyperkeratosis in oesophagus (Group IV—female); cysts in pars distalis of pituitary (Group I—male); mild neutrophilic infiltration in prostate (Group IV); keratinized cyst in non-glandular stomach (Group I—male) and minimal lymphocytic infiltration in heart (Group I—male).

All the above listed findings are expected background lesions commonly observed in conventionally housed rats of this age. These limited findings were considered incidental and toxicologically irrelevant as there were no convincing incidence patterns/trends to suggest a relationship to administration of test article *McB-E60* (extract of a *Momordica* sp.).

4. Discussion

The results of present repeat-dose animal toxicity study show that oral (gavage) administration of *McB-E60* (extract of a *Momordica* sp.) to male and female rats, daily for 90 consecutive days by oral gavage, and at the dose levels of 250, 500 and 1000 mg/kg/day, resulted in no incidence of mortality, no incidence of any abnormal clinical signs (including neurotoxicity) in the male and female rats, no effects on the body weights/body weight gain, no effect on the feed consumption, no effects on the hematological parameters, no effects on the blood chemistry and urinalysis parameters, no alterations in the absolute and relative organ weights, and no remarkable gross pathological and histopathological alterations suggestive of systemic toxicity. For all measured parameters the values for both the controls and the unaffected treatment groups were within the range of historical control values relative to the sex, strain and species of the animals tested.

In general, treatment of the animals with *McB-E60* (extract of a *Momordica* sp.) at doses up to 1000 mg/kg bw/day was well tolerated.

In the present subchronic study, a significant decrease in the levels of AST in low dose group male rats treated *McB-E60* (extract of a *Momordica* sp.) was noted as compared with respective controls group. The biologically insignificant decrease in AST value (120.8 IU/L) was well within the historical control range and was not considered to be treatment related, also since there was an absence of any adverse alterations in gross and microscopic appearance, organ weights and other clinical chemistry parameters associated with liver in this group. Additionally, some limited histopathological findings were noted in kidney, lungs, trachea and intestine (Tables 7 and 8). However, there were no convincing incidence patterns/trends to suggest a relationship to treatment with *McB-E60* (extract of a *Momordica* sp.). These findings are known to be expected background lesions commonly observed in conventionally housed rats of this age, and hence were considered to be incidental and toxicologically irrelevant.

None of the above described changes following administration of the *McB-E60* (extract of a *Momordica* sp.) were considered adverse as the changes were minor (within historical control values of the test facility), occurred in only one sex, were not dose-related, non-adverse and/or inconsistent, were not supported by any other changes in related clinical parameters or histopathological observations.

M. charantia or bitter melon is the plant that has received the most attention for its anti-diabetic properties. The available information from published studies suggests blood-glucose-lowering effect of bitter melon when fed orally [7]. The juice formulations

of bitter gourd have proven to be more effective in lowering blood sugar and HbA1c levels than its dried fruit products. In addition to fresh fruit extracts, the glucose lowering effects were also found in ethanolic plant extracts. Biochemical and animal model experiments have produced abundant data and hypotheses accounting for the anti-diabetic effects of *M. charantia*. In recent years, several studies have focused on the glucose-lowering effects of bitter gourd components. In these studies, hypoglycemic activity could not be demonstrated in normoglycemic animals [12]. The findings from the present study also suggest that *M. charantia* is unlikely to cause hypoglycemic activity in normoglycemic animals as treatment of the male and female rats with *McB-E60* (extract of a *Momordica* sp.) at doses up to 1000 mg/kg bw/day for 90 consecutive days by oral gavage did not affect blood glucose levels.

The findings from this study, designed as per OECD guidelines, suggest that oral administration of *McB-E60* (extract of a *Momordica* sp.) at levels up to 1000 mg/kg bw/day does not cause any adverse or otherwise effects in male and female rats. Based on the results of this study, the no-observed effect level (NOEL) of *McB-E60* (extract of a *Momordica* sp.) was found to be greater than 1000 mg/kg bw/day, the highest dose tested. A standardized, encapsulated extract dosage of *McB-E60* (extract of a *Momordica* sp.) ranges from 100 to 200 mg three times daily (up to 600 mg/day). The NOEL for *McB-E60* (extract of a *Momordica* sp.) from the present study provides approximately 100-fold safety factor over the recommended dose of 10 mg/kg/day. Therefore, from the results of the study presented herein, it may be concluded that the use of appropriate levels of the *McB-E60* (extract of a *Momordica* sp.) as a dietary supplement is considered safe.

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