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Comparative Effect of Mass Freak on Biological-Histopathological and Molecular Parameters in Rats after Exercises

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Abstract: Mass FREAK comprehensive formula is being used before sports as it provides body with the precise combination of calories, nutrients, stimulates newmuscle growth, contains anabolic hormone stimulator complex and a nutrient delivery and storage complex. In this study, we aimed to assess Mass FREAK effects on: a) liver and kidney biological functions (GPT, creatinine and urea), b) histopathological changes in testis, liver, &muscles and c) DNA by comet assay. Test male rates were divided into 3 groups according to their age into old group of 6 month age, young of 3 month age and very young of 2 month age. Each age group was subdivided into treated and control. Treated rats were fed mass freak orally in a dose of 0.0075gm/ 100gm three times a week for a month. Control rats received no treatment. Control and treated rats were subjected to exercise (Swimming) prior to mass freak. Blood and tissue samples were collected at the end of the study. The results of this study showed that a- no marked effect of mass freak on kidney and liver biological function. b- No characteristic histopathological changes could be detected in sections of liver, kidney, inter-costal muscles and testes among treated and control groups'- changes in integrity of DNA in young treated rats more than old ones were shown by the comet assay. In conclusion, mass freak did not cause changes in biological functions of kidney and liver which were confirmed by pathological results. On the other hand, it was shown that mass freak should be carefully taken in younger ages due to the possibility of damage at the molecular level.

Key words: Comet Assay · Histopathology · Kidney · Liver · Mass Freak

INTRODUCTION

Mass freak intake is an important component of body building and is highly recommended for regular strength training [1]. Bianco *et al.* [2] reported that 30.1% of male athletes use dietary supplements during training as a "Way to gain muscle and strength".

Mass Freak comprehensive formula is being used before sports as it provides body with the precise combination of calories&nutrients and stimulates new muscle growth. It contains anabolic hormone stimulator complex, a nutrient delivery and storage complex, as amino acids including glutamine, branched chain amino acid (Leucine, valine, isoleucine), complex carbohydrate, fiber, fiber seed oil and whey protein .Whey protein is a popular alternative to soy protein, but it has disadvantages. Massive consumption of whey protein, by bodybuilders for example, leads to a health condition called intestinal toxemia. The end result is a decrease in muscle gains as it severely damages the ability for the body to maintain an anabolic state. Many bodybuilders who use whey protein experience undesirable weight gain, but it's in the form of a toxic sludge in their gut. This blockage reduces the ability for protein to be absorbed by the body [3]. It also contains egg albumin, fatty acid (Medium chain triglyceride, flax seed oil), inulin apple and pectin, (Farm Freak, company Canada).

Swimming was selected as a model for exercise performance, since swimming appears to be natural behavior for rodents and humans [4].

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Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism in response to stress. Swimming has got a number of advantages over other types of exercise such as treadmill running. The amount of work done during the swimming exercise is far greater than that during the treadmill running of identical time duration [5].

Stress is an imbalance between production of reactive oxygen species(ROS) and antioxidant defense. Stress is defined as "A general body response to initially threatening external or internal demands, involving the mobilization of physiological and psychological resources to deal with them" [6].

Intense exercise increases oxygen consumption and may produce an imbalance between ROS and antioxidant levels, inducing oxidative stress as a result. Increased ROS production [7] leads to the destruction of tissue and cell macromolecules, including lipids, proteins and nucleic acids [8].

The aim of this study was to evaluate the effects of mass freak intake on a) liver and kidney biological functions (GPT, creatinine, &urea), b) histopathological changes in testes, liver and muscles and c) DNA damage by comet assay..

MATERIALS AND METHODS

Experimental Section

Mass Freak: As a dietary supplement mix 2 to 4 scoops with (340-680ml) of cold water or milk.

Rats: Male Swiss Webster rats aged 2-6 month (100-300 gm) were obtained from the animal house of National Research centre (NRC). Animals were supplied with standard diet pellets and water that were given *ad. Libitum*. They were kept in plastic cages for 7 days to be accommodated with our laboratory conditions before being treated.

Calculated Doses: Three main groups were used in this study, they were divided according to age into 1) Old of 6 month age. 2) Young of 3 month age.3) Very young of 2 month. (Dose equal 0.0075gm/ 100gm).

Method:

- Oral administration, fast& exercise scheme.

First Two Weeks:

Fast 2 hours--then-----exercise about (15 mint) -----then-----oral administration (Mass freak)---------repeated 3 days per week.

Third and Fourth Week:

Oral administration (Mass freak) ------then-----fast 2 hours-----then------exercise about(15 mint)----then------oral administration(Mass freak)-----repeated 3 days per week.

Fig. 4: Show Exercise.

After month all RATS were scarified, then Blood and tissue samples were collected at the end of the study.

Blood Sample Analysis: Five millilitres of blood was withdrawn from the rats into each of two sterile vaccutainers; one containing EDTA and the other without additives to separate serum.

Serum samples were assayed, within 2 hours, for urea, GPT and creatinine using the automated clinical chemistry analyzer Olympus AU 400 analyzer, The other tube containing EDTA used immediately to measure DNA damage by the Comet Assay.

Histopathological Analysis: Post mortem examination of studied rat groups was carried out. Tissues specimens of liver, kidney, testes and intercostal muscles were fixed in 10% buffered formalin for pathological examination. Then washed in water and dehydrated through series of alcohol and cleaned by xylene solutions then embedding in paraffin blocks. Thickens of 4 µm sections were prepared and stained with hematoxylin and eosin (HE) and examined under a light microscope (Nikon Eclipse E – 600).

Measurement of Comet Assay: The alkaline (pH >13) comet assay was performed according to the method described by Singh *et al*. [9] with minor modifications

Cell Preparation: Peripheral blood leukocytes were isolated by centrifugation (30 min at 1300g) in Ficoll-Paque density gradient (Pharmacia LKB Biotechnology, Piscataway, NJ, USA). After centrifugation, leukocytes were aspirated and washed twice by phosphate-buffered saline at pH 7.4 (PBS).

Preparation of Cell Microgels on Slides: The comet assay was performed according to Singh and colleagues [9]with modifications according to Blasiak and colleagues [10]. Cellmicrogels were prepared as layers. The first layer of gel was made by applying 100 µl of normal melting point agarose (0.7%) onto a precleaned microscope charged slides and cover slipped gently. The coverslip was removed after the agarose was solidified at 4°C. Low melting-point agarose (0.5%) was prepared in 100mmol/L PBS and kept at 37°C. Approximately 150 µ of peripheral blood leukocytes were mixed with the low melting-point agarose and 100 µl of the mixture was applied to the first gel layer. The slides were then covered with a coverslip and placed at 4°C for solidification. After the second layer solidified, the coverslips were removed from the cell microgels. A final layer of low-melting agarose was added followed by coverslips, left to solidify for 10 minutes then the coverslips were removed.

Lysis of Cells, DNA Unwinding, Gel Electrophoresis, DNA Staining: The slides were covered with 100 ml of ice-cold freshly prepared lysis solution buffer pH 10 (2.5 mol/L NaCl, 100 mmol/L EDTA, 1% sodium hydroxide, 10 mmol/L Tris, 1% Triton X-100, 10% DMSO) for at least 1 h. After draining, microgels slides were treated with DNA unwinding solution (300 mmol/L NaOH, 1 mmol/L EDTA, pH 13) for 30 min at 4°C and placed directly into a horizontal gel electrophoresis chamber filled with DNA-unwinding solution. Gels were run with constant current (300 mA at 4°C) for 30 min. After electrophoresis, the microgels were neutralized with 0.4 M Trizma base at pH 7.5 for 10 min. The slides were stained with 20 µlethidium bromide (10 µg/ml).

Visualization and Analysis of Comet Slides: The slides were examined at 40× magnification using an inverted fluorescence microscope (IX70; Olympus, Tokyo, Japan) equipped with an excitation filter of 549 nm and a barrier filter of 590 nm, attached to a video camera (Olympus). Damaged cells were visualized by the "Comet appearance", with a brightly fluorescent head and a tail to one side formed by the DNA containing strand breaks that were drawn away during electrophoresis. Samples were analyzed by counting the damaged cells out of 100 cells per slide to calculate the percent of damage.

RESULTS

Biochemical and Histpathological Examination: Data were presented as mean ±SD. The compiled data were computerized and analyzed by SPSS PC+, version 14. The following tests of significance were used: t test between means to analyze mean difference. A value of $p \leq 0.05$ was considered significant, $p<0.001$ was considered highly significant and $p>0.05$ was considered insignificant.

The results of this study showed no characteristic effect of mass freak on kidney and liver biological function. Also no characteristic histological changes could be detected in sections of liver, Kidney, inter-costal muscles and testes among treated and control groups where the DNA damage was significant increase.

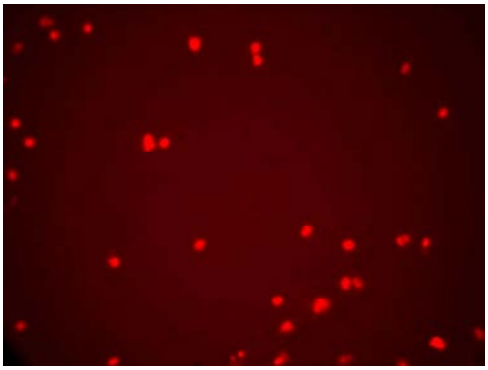

Fig. 1: Age 6 month DNA damage

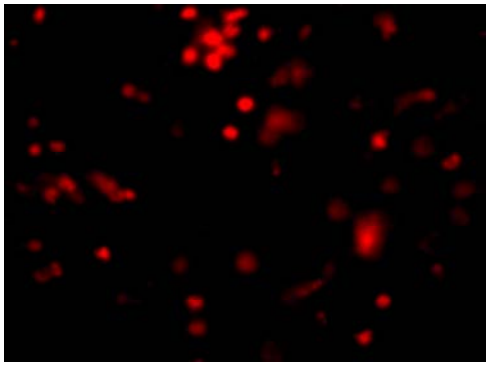

Fig. 2: Age3 month DNA damage

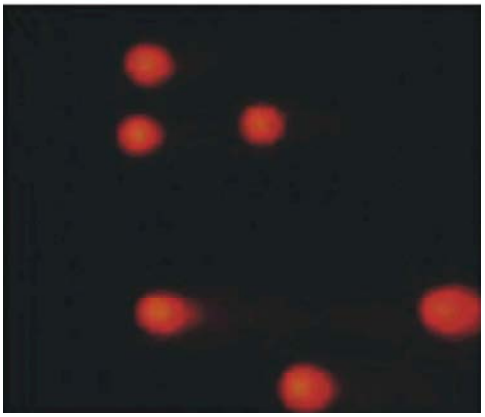

Fig. 3: Age2 month DNA damage

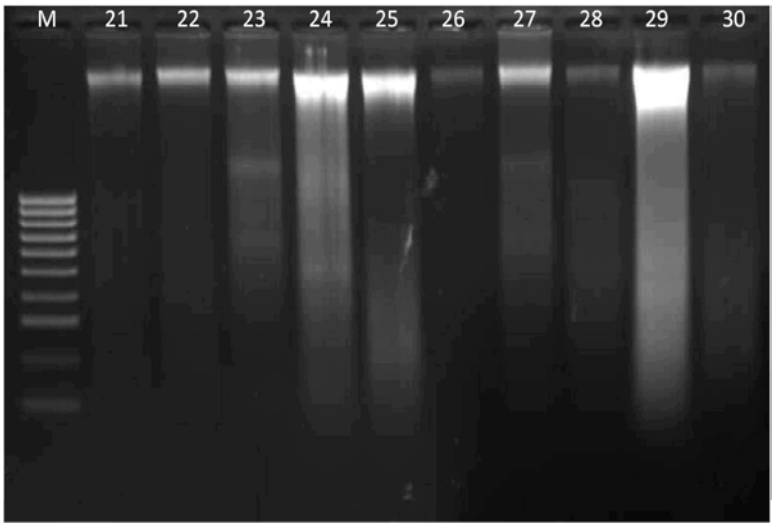

Fig. 4: DNA fragmentation induced by (Mass freak)

Table 1: The laboratory tests applied to experimental animals

Age\month	GPT	Creatinine	Urea	DNA%
6 treatment	49.000±2.164	0.56333±0.0183	36.000±1.948	14.3333±3.61478
6 control	50.66±2.164	0.5300±0.0183	33.00±1.948	6.0000±1.15470
3 treatment	37.66±2.164	0.546±0.0183	34.666±1.948	24.3333±5.39135*
3 control	53.00±2.650*	0.60333±0.0183	39.33±1.948	5.0000±1.15470
2 treatment	62.500±2.650	0.5166±0.0183	38.666±1.948	21.6667±2.58199*
2 Control	53.66±2.164	0.5366±0.0183	31.666±1.948	6.0000±1.15470

*p ≤0.05 was considered significant

Table 2: Shows the weight different of rats 6 month old(during one month)of treatment of the 6 month old rats

	Weight (gm)									
Dose	1st.wt.	2nd.wt.	3rdwt	*4th.wt.	5thwt	6th wt.	7thwt	8thwt	9th.wt.	10th.wt.
Control I	300	256	264	260	259	290	321	300	310	301
Control II	300	245	245	240	230	254	266	255	266	270
Control III	300	260	269	250	243	259	268	260	288	272
Old treated 1	220	221	215	240	213	233	300	300	325	304
Old treated 2	230	223	222	250	265	298	236	234	250	257
Old treated 3	200	170	157	200	208	230	228	221	238	245

• *wt Mean weight after two weeks

Table 3: Shows the weight different of rats of 3 month age (during one month)

	Weight (gm)									
Dose	1st.wt.	2nd.wt.	3rdwt	*4thwt	5thwt	6th wt.	7thwt	8thwt	9th.wt.	10th.wt.
Control I	150	130	120	120	110	100	110	120	120	123
Control 2	125	110	106	90	95	90	90	100	100	105
Treated 1	140	144	123	140	140	159	159	156	154	185
Treated 2	140	145	140	150	148	160	162	175	175	197
Treated 3	150	146	144	150	165	182	180	187	189	200
Treated 4	130	122	107	140	140	140	138	135	144	152

• *wt Mean weight after two weeks

Table 4: Shows the weight different of rats young of 2 month age

	Weight (gm)									
Dose	1st.wt	2ndwt	3rdwt	*4thwt	5thwt.	6thwt	7thwt	8thwt	9thwt	10thwt
Control I	90	87	88	90	80	88	90	90	111	113
Control 2	100	90	90	100	110	100	110	110	130	126
Treated 1	130	113	110	140	157	134	135	144	184	155
Treated 2	130	112	108	120	156	129	129	140	180	147
Treated 3	120	111	108	120	149	112	113	125	171	144
Treated 4	100	100	90	110	140	110	111	122	160	140

• *wt Mean weight after two weeks

Effect on Weight: Different weight, were weighed to rats through month At first two weeks as shown in Tables 2, 3 & 4 decrease in weight e.g. Table 2 weight decrease from 220 gm at first weight to215gm at third weight for treated 1, also treated 2 decrease weight from 230 to 222gm, treated 3decrease weight from200gm to157gm.

Through third and fourth weeks of month increase in weight was shown. In Tables 2, 3&4 increase in weight, e.g. Table 2 weight increase to 240 gm at fourth weight for treated 1, also treated 2 increase weight to 250 gm, treated 3increase weight to200 gm.

DNA isolation by (Tissue Kit – QIAGEN), Collected from different tissues of treated rats confirmed result of comet assay.

DISCUSSION

Some studies showed that the exercise increases the oxygen consumption rate by 10–20 times, resulting in an enhanced production of reactive oxygen species(ROS) which can increase the rates of cellular death [11]. On the other hand, the overproduction of ROS stimulates DNA fragmentation that can cause harmful damage to the mitochondria and plasma membrane [12]. This agree with our studies

The objective of this study wasto investigate the effect of mass freak supplementation on changes in serum urea, creatinine and GPT. Our findings showed that daily supplementation of mass freak has not effect on them. Other observations suggested that a small increase in the energy intakes during (Month) periods was related to increased liver and DNA damage[13]. Examined the effects of whey protein intake during a 14-day period on protein levels and muscle force recovery after eccentrically-induced muscle damage in healthy individuals and reported that whey protein supplementation attenuated muscle force impairment that occurs during recovery from exercise-induced muscle injury.

Kesteloot and Joossens [14] studied the relationship between dietary protein intake and serum creatinine, urea and uric acid concentrations and reported significant correlations in both sexes for total protein and animal and vegetable protein intake.

We find the urea and creatinine did not change, this disagrees with chenys *et al*. [15] who found significant increase after swimming and reduction after treatment.

Exercise training seems to be able to exert modifying effects on oxidative stress depending on the training load, training specificity and the basal level of training [16].

The increase of DNA damage might be due to swimming stress which decreases antioxidant and elevated DNA fragmentation in elite athletes [17].

Vijayprasad *et al.* [18] reported that the stress doesn't show any significant effect on testicular index, this agrees with our study. In case of effect of weight noticed, decrease in weight at first two week occurred due to those animal don't take mass freak before fasting. In case of control, high temperature decrease weight All time not increase(naturally) caused by increase temperature in summer Season.

CONCLUSIONS

Mass freak did not cause changes in biological functions of kidney and liver which were confirmed by pathological results. On the other hand, it was shown that mass freak should be carefully taken in younger ages as it causes damage at the molecular level. Future studies are needed to further support our conclusions.

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