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Effect of MAK-4, An Herbal Supplement on Some Biochemical Parameters of Serum in Mice

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Abstract - **Ayurveda is a holistic system of natural health care that originated in the ancient Vedic civilization of India which began in the 15th century. The herbal supplement, Amrit Kalash was developed thousands of years ago by the great Ayurveda sages as the one formula for total health. We studied the effect of MAK- 4, a herbal supplement on some biochemical parameters in mice serum. We found that the serum MDA level decreases in MAK-4 fed mice. The total serum protein level was increased in MAK-4 fed mice. Body weight and organ weight ratio in MAK-4 fed mice was more. Cholesterol level in serum was found to be low in MAK-4 fed mice.**

Keywords: MAK- 4, Total Serum Protein, MDA Level, Serum Cholesterol, Mice

I. Introduction

Ayurveda is the oldest medical system that originated in India at about 6,000 B.C. (Urmila *et al.,* 1986). The concept of this medical system consisted of physical exercise and special herbal food supplements called Rasayana. Rasayana is believed to enhance resistance to infection and diseases, and give longevity (Glaser *et al.,* 1988). Rasayanas are a group of herbal formulations and are used to improve health of the body.

It is well known that dietary factors play an important role in enhancement of health status and physical strength in human. Epidemiological data suggest that ingestion of some constituents from vegetables and fruits may contribute to a reduction in cancer incidence in humans (Steinmertz *et al.,* 1991; Bertram *et al.,* 1987). Maharishi Amrit Kalash 4 (MAK 4) and 5 (MAK 5) are two versions of Rasayana prepared according to the ancient Ayurvedic recipe (Sharma *et al.,* 1996). Maharshi Amrit Kalash (herein after will be referred to as MAK-4) is one of the few Ayurvedic formulations studied for pharmacological actions extensively.

The chemical composition of this herbal mixture includes alpha tocopherol, beta-carotene, ascorbate, bioflavonoid, catechin, polyphenols, trans resveratrol, riboflavin, tannic acid, and other low molecular weight substances (Hanna *et al.,* 1996).

The free radicals produced in the body by normal bodily processes and especially by mental and physical stress, environmental pollution and pesticides in foods can cause extensive damage in all cells, speed up of the ageing processes and contribute to a wide range of diseases. A diet rich in fresh fruits and vegetables supplies a good amount of antioxidants that mop up the damaging free radicals. Comprehensive scientific research has shown that Amrit Kalash is an effective known food supplement to ensure you have an adequate anti-oxidant supply.

Mice are selected for the present study as they have physiological systems and responses similar to the man. Their small size ease of handling, housing maintenance, early puberty and high position in the evolutionary scale also entice us to select them as a test animal to study.

We studied the effect of MAK-4 on total serum protein, serum cholesterol and MAD level. These characteristics were selected because they largely characterized the changes in the main types of metabolism.

II. Methodology

Only the healthy pairs of mice were housed in the separate cages. The temperature of house was maintained in the range of 20º to 25ºC.

A. Experimental Animal

Mice were obtained from IBVP Aundh Pune weighing 25-30 g of age 10 weeks approximately. Mice were kept on specific diet and water ad libitum in an animal room under a 12 hours light- dark cycle at a temperature of 22± 1°C and a humidity of 60 ± 5%. After week acclimatization, they were used for the experiments.

B. Preparation of MAK-4 Extract

MAK-4 suspended in distilled water was given to the mice at 100 and 200 mg/kg per day for 2-month. Healthy mice were grouped into three groups.

Group I: control (water 0.1 ml/10 g of body weight) (Ryoichi *et al* 2005).

Group II: MAK-4 100 mg/kg

Group III: MAK-4 200 mg/kg

Animals were sacrificed after the last administration for the following experiments. After the set up of the experiment, following parameters were studied.

1. ***Body Weight:*** Weight of mice were taken before and after the administration of the MAK -4,
2. ***Organ Weight:*** Mice were dissected after the experimental period, and liver was taken out and weighed. Organ body ratio was calculated as following formula: Organ weight % = organ weight in gm *100] / Body wt in gm
3. ***Food Consumption:*** The food intake of the mice was calculated by the formula:

   Food consumption/ day /g= Wt of food offered to mice – Wt of food remain after 24 hrs.
4. ***Study of Biochemical Parameters in Serum:*** All animals were killed by decapitation at the end of the experiment. Blood samples were collected in tubes, allowed to clot and the serum was removed by centrifugation at 2000g for 10 min. All serum samples were sterile, hemolysis-free, and were kept at 40^0C before determination of the biochemical parameters.
5. ***Total Protein in Serum:*** Total protein in serum was estimated by the method of Folin Phenol (Lowery *et al* 1951).
6. ***Cholesterol Assay in Serum:*** Cholesterol assay in serum was carried out by the method of Liebermann. (Plummer 1990).
7. ***Plasma TBARS Assay:*** The MDA content of the plasma was determined spectrophotometrically by measuring the presence of thiobarbituric acid-reactive substances (Uchiyama and Mihara, 1978).

III. Statistical Analysis

The data was analyzed by using the standard bio-statistical method i.e. standard error of mean, students T. test, etc.

A. Standard Error of Mean: Standard error of mean was applied to all readings of MDA levels found was used for further interpretation by using standard method in bio-statistics.

S.E = SD/

Where S.D. =Standard deviation

N = number of observations

B. Students T Test

Students T test was applied to interpret whether MAK – 4 can affect the different parameters or not, by using standard Bio-statistical methods.

t = x1- x2 /S.E

Where, x1= mean of one variable

x2 = mean of second variable

IV. Results And Observation

A. Body Weight

Effect of MAK-4 on mean body weight of mice during the study is shown in table I.

It is found that there is increase in the weight of MAK-4 treated mice than that of the mice of the control group. Wt gain is more in group III i.e. mice on the diet 200mg/kg body wt.

Table I Effect of Mak-4 on Body Weight of Mice

Group	N	Body Weight in gm		Gain of wt in gm ±SE
		Initial weight ±SE	After 20 days ±SE	
Control	2	21.06 ±0.13	23.08 ± 0.14	2.02 ±0.02
MAK-4 - 100 mg/kg	2	22.10 ±0.4	30.14* ±0.07	8.03 ±0.24*
MAK-4 - 200 mg/kg	2	20.12 ±0.34	34.28* ±0.7	13.16±0.06*

Values are means Â±SE for mice sampled from three groups Means were compared by Student's t test; statistically significant differences: *P < 0.05. N=Number of mice in each group.

B. Organ Weight Ratio

It is found that the weight of the organ increases in MAK-4 treated mice.

Table II Effect of Mak-4 on Organ wt Ratio % of Mice.

Group	Liver
	Wt ±SE
Control	1.89± 0.12
MAK-4 100 mg/kg	2.10±0.17
MAK-4 200 mg/kg	2.23±0.19

Values are means Â±SE for mice sampled from three groups Means were compared by Student's t test; statistically significant differences: *P < 0.05.

C. Food Consumption by Mice in gm /Day

We found that the there was no significant difference in the food consumption in three groups of mice.

Table III Effect of Mak -5 on Food Consumption in Mice.

Group	N	food weight in gm		Food intake ±SE
		Initial weight ±SE	After 24 hours ±SE	
Control	2	9.05 ±0.17	4.6 ±0.12	4.4 ±0.21
MAK-4- 100 mg/kg	2	10.10 ±0.21	6.24 ±0.17	4.04 ±0.28
MAK-4- 200 mg/kg	2	10.67± 0.2	6.56* ±0.23	. 4.10* ±0.15

Values are means Â±SE for mice sampled from three groups means were compared by Student's t test; statistically significant differences: *P < 0.05.

D. Effect of MAK-4 on Total Serum Protein in Mice

It is found that the there is increase in the total protein in MAK-4 administered mice.

Table IV Effect of Mak-4 on Total Serum Protein in Mice.

Group	Total protein g/l ± SEM
Control	0.31±0.12
MAK-4- 100 mg/kg	1.00±0.03
MAK-4- 200 mg/kg	1.3±0.05*

Values are means Â±SE for mice sampled from three groups means were compared by Student's t test; statistically significant differences: *P < 0.05.

E. Effect of MAK-4 on Serum Cholesterol level in of Mice

In our experiments , we found significant decrease in the serum cholesterol level of MAK-4 treated mice. Cholesterol level is lowest in group III (200mg MAK-4/kg body wt.

Table V. Effect Of Mak-4 On Serum Cholesterol Level In Of Mice.

Group	Total Cholesterol (mg/dl) ± SE
	mean
Control	112±1.51
MAK-4- 100 mg/kg	90±0.8*
MAK-4- 200 mg/kg	79.0±1.07*

Values are means Â±SE for mice sampled from three groups means were compared by Student's t test; statistically significant differences: *P < 0.05.

F. Effect of MAK-4 on Plasma TBARS assay of Mice

It is found that the plasma TBARS assay is greatly decreased in MAK-4 treated mice. MDA level is lowest in group III and the lowest in control group. This shows that MAK-4 could decrease lipid peroxidation.

Table VI Effect of Mak-4 on Plasma Tbars Assay of Mice

Group	Plasma MDA (nmoles/ml) ± SE
Control	11.62 ±003
MAK-4- 100 mg/kg	7.36 ±0.3
MAK-4- 200 mg/kg	6.30 ±0.20*

Values are means Â±SE for mice sampled from three groups means were compared by Student's t test; statistically significant differences: *P < 0.05.

V. Discussion

This study reports that the MAK - 4 exhibit a significant effect on the biological and physiological parameter of mice.

In present study it is found that the body weight of mice after administration of MAK - 4 was increased. We also found significant increase in the organ weight ratio of the organ liver.

It is found that the food consumption in MAK-4 treated groups was not significantly different than that of control. We found the similar results to that of Ryoichi who stated that MAK-5 administration scarcely affected the food intake in mice. (Ryoichi *et al* 2005).

In this study, it is found that serum cholesterol level in MAK – 4 treated mice decreases. We further observed that the decrease in cholesterol level in MAK – 4 treated mice is dose dependent. Thus we support the reports stated by Rao *et al* (1991). According to Roa *et al*, the MAK-4 supplementation reduced total cholesterol in blood in rabbit. Thus this findings resembles the finding of Dongra and Bhargava (2000).

The study conducted with watanable Heritable Hyperlipidemic (WHHL) rabbits suggests that MAK-4 reduces atheroma formation through its antioxidant activity. (Lee et al 1996).

We report that total protein in MAK – 4 treated mice is high than the control one we could not find any research work regarding this parameter due to limited number of published researches on MAK.

Hypercholesteromic rabbits supplemented with MAK-4 for 7 weeks showed a reduction in plasma and hepatic lipid peroxidation. (Rao and Sharma 1991). We found that MAK-4 may increase antioxidant protection in plasma. We report that plasma TABRS level significantly goes down in MAK-4 administered mice.

According to Sharma *et. al,* research with Watanable Heritable Hyperlipidemic rabbits suggests that MAK-4 may yield increased anti-oxidant protection in the brain, and may therefore be useful in preventing or treating free radical induced neurological disorders.(Sharma *et. al* 1996). Thus MAK-4 may have potential benefits in reducing oxidation stress. (William *et.al* 1997).

Treating guinea pigs with MAK at a dose of 500 mg/kg body weight daily for two months reduced lipid peroxidation in brain regions and helped restore normal oxygen consumption in older animal. This indicates anti-oxidant properties of MAK. (Vohra *et. al* 2001).

As studied in Watanabe Heritable Hyperlipidemic rabbit, MAK-4 may yield increased anti-oxidant protection in the brain and may therefore be useful in preventing or treating free radical-induced neurological disorders. (Sharma *et. al* 1996).

Ethanol and aqueous extract of MAK-4 and MAK-5 were able to quench generation of reactive oxygen species in vitro within an isolated cerebrospinal fraction enriched in mitochondria and nerve endings. Thus these herbally - derived mixtures possess distinctive qualities,which may be of utility in the design of preventive or therapeutic approaches related to the mitigation of excess oxidative events. (Stephen *et. al*, 1994).

VI. Conclusion

Thus we conclude that phytochemicals of MAK-4 may provide protective effect to the biological system. It could also has a nutritive role and it keeps the metabolic status of the body in pace. But in our views this study is not sufficient to apply the finding of this research on human. But further research is required to correlate this study to human being.

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