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CHRONIC EFFECTS OF 'MENERGY', A DIETARY SUPPLEMENT CAPSULE (MDSC) FOR MALE SEXUAL LIFE IMPROVEMENT; THREE-MONTH CHRONIC TOXICITY STUDY

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ABSTRACT

Dietary supplements have gained popularity as a means of enhancing nutritional intake and overall well-being. However, ensuring the safety of these supplements is essential to protect consumers from potential harm. Pre-clinical studies, often involving animal models, play a crucial role in assessing the chronic toxicity of dietary supplement capsules. These studies aim to mimic long-term human exposure to the supplement. The dietary supplement capsule (MDSC), 'MENERGY', is formulated with a combination of vitamin B1, B6, Zinc, Selenium, and a blend of dietary herbs including Holy Basil (Tulsi), Tamarindus indicum, Curculigo orchioides, Anacyclus pyrethrum, Withania somnifera, and Panax ginseng extract. Previous studies have consistently shown the importance of the mentioned supplements in various aspects of male health, encompassing overall well-being, sexual desire, orgasm, erection, and mood. The purpose of this study was to evaluate the long-term safety of MENERGY, a dietary supplement capsule using vitamins, minerals, and traditional herbs in combination within specified limits. **Study Methodology:** The study is a non-clinical, experimental, control group to investigate the safety of 'MENERGY' Dietary Supplement Capsules (MDSC) in a 90-day chronic toxicology experiment in male Wistar rats. The total of 36 Wistar (rats) were categorised in three groups in the experiment: a control group (CG), two treatment groups (TG-A and TG-B), and each group was given daily doses of MDSC of 30 mg/kg and 60 mg/kg, respectively. The study measured the rats' body weight, food and water intake, organ weights, tests to determine how effectively their liver and kidneys functioned, as well as their testicular, liver, and kidney histology. **Results:** The results showed that the body weight, food and water consumption, organ body weights, liver and kidney function tests, and histopathology of the liver, kidney, and testicles remained unchanged in all three groups. Moreover, spermatogenesis in the testicles showed a qualitative improvement in the treatment groups compared to the control group. **Conclusion:** The findings of this study indicate that 'MENERGY' Dietary Supplement Capsules are safe and do not cause any gross pathology, liver or kidney damage, or histopathological damage to the liver, kidney, or testicles. Furthermore, the supplement may have a beneficial effect on spermatogenesis.

KEYWORDS: Dietary Supplement, sexual dysfunction, non-clinical, Chronic Toxicology, Safety.

INTRODUCTION

Considering male health and well-being, 'MENERGY' (Dietary Supplement Capsule) is a carefully formulated blend of vitamins, minerals, and herbs that have been used for generations in south Asian countries to improve men's health.[1,3] This formulation has been enhanced with vitamin B1, B6, zinc, and selenium, providing users with the potential benefits of improved immunity, energy, mood, and fertility without any risks.[4,5] In recent years, the use of alternative medicines to treat infertility has seen a rise.[1] However, only a minuscule fraction of available medicinal plants, i.e., 1%, have been studied.[6] 'MENERGY' Dietary Supplement is a natural supplement that contains herbal ingredients of MDSC,

which are based on the Ayurvedic system of medicine.[3] These herbs have been used for centuries to enhance immunity, boost energy, improve mood, and reproductive system, as well as act as powerful antioxidants.[2,7] Additionally, saffron, an herbal antioxidant, has been found to improve sperm parameters in rats exposed to cadmium, making it a promising fertility supplement.[8] It is evident that most chronic disorders can be effectively treated with Ayurveda, and 'MENERGY' Dietary Supplement is a great way to get the benefits of Ayurvedic medicine.[9] 'MENERGY' Dietary Supplement Capsule is a unique blend of vitamins, minerals, and herbs that have been carefully selected from the Scientific Review published on the internet about Benefits and Uses, Volume IV and the Natural Health Products Ingredient Data base (NHPID) Canada.[3] These ingredients are widely referenced in the Ayurvedic Pharmacopoeia of India, United States Pharmacopeia (USP), European Pharmacopeia, Traditional Chinese Medicine (TCM), European Medicines Agency (EMA), BP Monographs, American Botanical Council, United Natural Product Alliance Listed (UNPA), and the American Herbal Product Association.[3] This combination of vitamins, minerals, and herbs is designed to provide the body with the essential nutrients it needs to maintain optimal health.[7,9] Most of the previous studies have established the efficacy and safety of vitamins B1 and B6, as well as the micronutrients zinc and selenium, with maximum allowable limits and NRV limits of 1.3 mg/day and 11.0 mg/day, and11.0 mg/day and 0.55 mcg/day, respectively.[9-12] The safety evaluation of individual herbal ingredients in MDSC considered the minimum and maximum consumption values reported in pre-clinical toxicity, neurotoxicity, genotoxicity, and safety studies conducted on individual dietary herbs. For example, acute toxicity of Holy Basil, Tamarindus indica, sub-acute toxicities of Holy Basil, chronic toxicities of Curculigo orchioides, neurotoxicity of Withania somnifera, acute toxicity of Ana-cyclic pyrethrum, and safety of ginseng didn't cause harmful side effects or loss of life and was not associated with changes in body weight, food intake, blood and biochemical profiles, or the physical appearance / structure of important organs in rats.[13-18] There was also no evidence of genotoxicity in rats and improved HRQL in patients with EOC.[13,15,18] These results indicate that the herbal ingredients extract used in the preparation of MDSC are within pre-clinical evaluated limits and can be taken safely[3]. This study's objective was to assess 'MENERGY' Dietary Supplement's long-term safety capsule using vitamins, minerals, and traditional herbs in combination with specified limits. In this research gross pathology individual animal body weight, organs weight changes, clinical biochemistry, including liver function test, creatinine, urea and histopathology of the Liver, kidney and testis was determined which was considered most relevant.

MATERIALS AND METHODS

Animal

As per study inclusion and exclusion criteria, three months male Wistar rats aged 70 to 85 days were obtained from the International Centre for Chemical and Biological Sciences (ICCBS) Animal Facility at the University of Karachi, Pakistan. For a week, the rats were kept in plastic cages with stainless-steel tops to help them acclimate to the laboratory environment. The temperature and humidity were maintained at 24 2 °C and 50-60%, respectively, with a 12-hour light/dark cycle. Standard laboratory food and tap water were provided to the rats. All protocols for animal handling, treatment, and sacrifice were approved by the ICCBS Department.

Experimental Design

Thirty-six adult male Chinese Wistar rats were divided into three groups of twelve animals each. The first group served as the control group (CG) and was given the normal standard via a feeding tube and excess water. The remaining two treatment groups (TG) were treated with MDSC powder at 30 mg/kg/day (TG-A) and 60 mg/kg/day (TG-B), respectively. The two doses of MDSC powder were selected based on the minimum and maximum daily doses of MDSC.

Experimental Duration (Animals Trials)

The total number of animals used for the study was 36 (dividing them into 3 groups: a control, a low dose of 30 mg, and a high dose of 60 mg, each containing 12 animals). The chronic toxicology experiment was based on the Journal of Pharmacology and Pharmacotherapeutics, April–June 2011, vol. 2, issue 2al. The animals were dosed for 90 days. Only male rats, not less than 150 g in weight, were used for the study. The animal dose was prepared in mg/kg, and the sample was dissolved in water (suspension). During the animal's trial, the weight of the animals was checked every seven days until the end of 90 days. The gain and loss of weight were observed. The animals were dissected, blood was collected for biochemical analysis, and the liver, kidneys, testicles, heart, spleen, stomach, and lungs were removed for histopathology.

The Gross Pathology of Animals Body Weight

During the animal's trial, the weight of the animals was checked every seven days until the end of 90 days.

The Gross Pathology of Relative Body Organs Weight

After dissection of animals, the relative weight of the liver, kidney, testicles, heart, brain, spleen, and stomach was calculated by dividing the animal organ weight (mg) by the animal weight (g). Relative weight of organs (mg/g) = organ weight (mg)/animal weight (g), whereas the total weight gain or loss was calculated by subtracting the final weight from the initial weight. Weight gain/loss (g) = final weight (g) minus initial weight (g).

Liver Function Test

Biochemical analyses were carried out to determine the serum concentrations of total protein, albumin, conjugated and total bilirubin, and the activities of liver enzymes such as AST, ALT and ALP using diagnostic kits (Quimica, Clinica, Applicada, S. A. Spain). Total protein was determined by the Biuret method (Peters, 1968), albumin by the bromocresol green method (Doumas et al., 1971), bilirubin was estimated by the method described by Jendrassik and Grof (1938). Alanine and aspartate aminotransferases were determined based on the colorimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (Reitman and Frankel, 1957), alkaline phosphatase by the phenolphthalein monophosphate method (Babson, 1965).

Kidney Function Test, Blood Creatinine Determination

Creatinine was determined by the Jaffe reaction using a photometric colorimetric test for kinetic determination of creatinine at 25 °C and 37 °C without deproteinization. Creatinine forms in an alkaline solution as an orange-red complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample. The absorbance of sample and reagent was measured with a spectrophotometer (Microlab.300, Leitch Group, Dieren, Netherlands) at 492 nm, 30 min, and 2 min after sample and reagent were mixed.

Kidney Function Test, Blood Urea Determination

Blood urea was determined by the Berthelot method using an enzymatic colorimetric test. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. In a modified Berthelot reaction, the ammonium ions react with hypochlorite and salicylate to form a green dye. The absorbance increase at 578 nm is proportional to the urea concentration in the sample.

Testosterone Level Determination

An enzyme-based immunoassay (EIA) system was used to measure testosterone levels in serum samples collected. The EIA kit was obtained from immunometric (London, UK) and contained a testosterone EIA enzyme label, testosterone EIA substrate reagent, and an EIA quality control sample. A quality control was carried out at the beginning and at the end of the assay to ascertain the acceptability with respect to bias and within batch variation. The EIA kit used had a sensitivity of approximately 0.3 nmol/mL (0.1 g/mL) of testosterone. The intra- and inter-assay variations were 10.02% and 10.12%, respectively.

Histopathology

All organs were fixed with 10% formaldehyde and embedded in paraffin wax for slide preparation. In brief, 5 micrometer-thick sections were cut from the paraffin blocks using a microtome. These sections were affixed to slides on a slide warmer and deparaffinized prior to staining with hematoxylin and eosin (H&E) stain. The slides were examined under an Olympic microscope (Model IX2-ILL100) equipped with a micro-photographic system.

Histopathology of Liver

For the histopathological analysis of the liver, five parameters were selected (i.e., hepatocytes, lobular inflammation, portal tract, sinusoids, and central vein).

Histopathology of Kidney

Histopathological analysis of the kidney was based on three parameters selected (i.e., glomeruli, tubules, and interstitial tissues).

Histopathology of Testicles

Five parameters were selected for the histopathology of the liver (i.e., seminiferous tubules, spermatogenesis, Leydig cells, and atypical cells).

Statistics

Data were examined using one-way analysis of variance with the following data formats: mean standard deviation (SD) (ANOVA).

RESULTS

Acute Toxicity on 500mg/kg and 1000mg/kg

This test is to detect any unexpected, unacceptable, or acute toxicity of a substance used in the preparation of MDSC. The test was designed for the safety assessment of the given herbal product in combination with vitamins and herbs (Table 1). During the study, all the Wistar (rats) survived; none of the animals showed weight loss or any toxic signs at the end of the observation period.

Table-1: Product details.

Product (MENERGY)	Dosage	**Test Animal**	Rat (SD)
Batch No. (003)	500mg	**Date (Day 0)**	13.06.2019
Batch No. (003)	1000mg	**Date (Day 0)**	13.06.2019

Three Months Chronic Toxicity

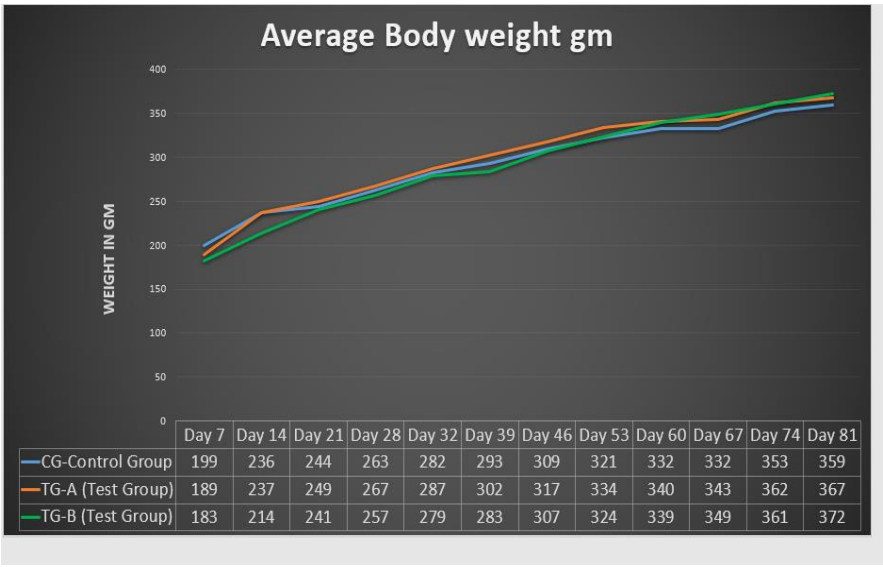
All 36 animals in the CG, TG-A, and TG-B groups were kept on a controlled, normal diet and water quantity (Table 2). In the current experiment, animals' eight were checked every 7 days up to 90 days. The average 7-day data for each group (CG, TG-A, and TG-B) showed no significant weight gain or loss. (Table:02).

Table 2: Gross Pathology of individual Body Weight.

Groups (Male Rats) Body weight	Before treatment (Per day average)	After 45 days Rx (Per day average)	After 90 days of Rx (Per day average)
Control	199	309	362
30mg/kg	189	317	322
60 mg/kg	183	307	347
Food consumption			
Control	20 g	20 g	20 g
30mg/kg	20 g	20g	20 g
60 mg/kg	20 g	20 g	20 g
Water consumption			
Control	70 mL	120 mL	131 mL
30mg/kg	65 mL	130 mL	142 mL
60mg/kg	75 mL	135mL	155 mL

Gross Pathology of Body Weight

The weight of the CG, TG-A, and TG-B groups of animals was measured every seven days, and the average mean value of weight was plotted to see a typical gain or loss of weight. The increase in weight was linear; there was no significant increase or decrease in body weight between the CG, TG-A, and TG-B groups (Graph: 01). No histopathology was evident.



Graph 01: Weight plotted weight gain

Gross Pathology of Organ Body Weights

After 90 days, the six animals from each group (CG, TG-A, and TG-B) were sacrificed in a carbon dioxide chamber, and blood was collected for biochemical analysis (Figure 1). For the individual organs, the results did not indicate any gross pathological impact on the individual organs: heart, kidney, liver, spleen, lungs, testicles, and stomach. the individual body organs collected from the CG, TG-A, and TG-B groups**Figure-2, Figure-3 and Figure-4**

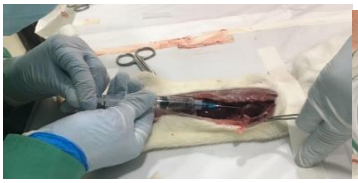


Figure 1: Blood Collection.

Figure 2: CG Group.

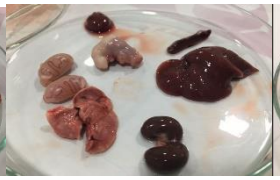


Figure 3: TG-A Group.

Figure 4: TG-B Group.

The average and standard error mean of the absolute organ weights of the heart, liver, kidney, spleen, lungs, testicles, and stomach of CG, TG-A, and TG-B were insignificant, and the standard error mean relative to body organ weight Table-2. The values of SEM obtained for absolute organ weight and relative to body organ weights in Table 4 for the CG, TGG-A, and TG-B groups were insignificant, and no gross pathology was evident. Table-2.

Table 2: Average and standard error mean of absolute organ weights of heart, liver, kidney, spleen, lungs, testicles, and stomach of CG, TG-A, and TG-B.

GROSS PATHALOGY

	Control ( ) Absolute Organ Weight								Control ( ) Relative to body Organ Weight							
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Average	SEM	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Average	SEM
Heart	1.11	1.34	1.23	1.17	1.31	1.21	**1.23**	**0.035**	0.30	0.32	0.35	0.33	0.37	0.33	**0.14**	**0.010**
Liver	12.39	14.84	12.08	10.66	12.12	11.75	**12.31**	**0.564**	3.35	3.52	3.39	3.05	3.44	3.16	**1.35**	**0.073**
kidney	1.79	2.44	2.06	1.81	1.71	2.02	**1.97**	**0.109**	0.48	0.58	0.58	0.52	0.49	0.54	**0.22**	**0.017**
Spleen	0.84	1.02	0.7	0.76	0.89	0.81	**0.84**	**0.045**	0.23	0.24	0.20	0.22	0.25	0.22	**0.09**	**0.008**
Lungs	2.06	1.94	1.74	1.76	2.59	1.77	**1.98**	**0.133**	0.56	0.46	0.49	0.50	0.74	0.48	**0.22**	**0.042**
Testicles	3.45	3.04	3.07	2.85	2.51	3.18	**3.02**	**0.129**	0.93	0.72	0.86	0.81	0.71	0.85	**0.33**	**0.035**
Stomach	7.25	6.244	6.07	4.58	7.84	9.16	**6.86**	**0.648**	1.96	1.48	1.71	1.31	2.23	2.46	**0.76**	**0.181**
Body Weigh	370	422	356	350	352	372	370.33	10.996								

	30 mg/kg (Menergy) Absolute Organ Weight								ng/kg (Menergy) Relative to body Organ Wei							
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Average	SEM	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Average	SEM
Heart	1.28	1.28	1.61	1.31	1.48	1.18	**1.36**	**0.064**	0.37	0.37	0.44	0.41	0.39	0.33	**0.38**	**0.016**
Liver	7.87	8.79	9.07	7.94	9.37	8.04	**8.51**	**0.264**	2.25	2.51	2.47	2.48	2.47	2.28	**2.41**	**0.051**
kidney	1.64	2.09	2.08	1.9	2.27	2.04	**2.00**	**0.087**	0.47	0.60	0.57	0.59	0.60	0.58	**0.57**	**0.022**
Spleen	0.49	0.51	0.68	0.52	0.64	0.55	**0.57**	**0.031**	0.14	0.15	0.19	0.16	0.17	0.16	**0.16**	**0.007**
Lungs	1.69	1.89	1.86	1.62	1.61	1.97	**1.77**	**0.062**	0.48	0.54	0.51	0.51	0.42	0.56	**0.50**	**0.021**
Testicles	3.4	3.07	3.87	2.99	3.39	3.65	**3.40**	**0.137**	0.97	0.88	1.05	0.93	0.89	1.03	**0.96**	**0.033**
Stomach	2.28	2.49	2.22	2.7	2.52	1.43	**2.27**	**0.183**	0.65	0.71	0.60	0.84	0.66	0.41	**0.65**	**0.064**
Body Weigh	350	350	367	320	380	353	353.33	8.229								

	60 mg/kg (Menergy) Absolute Organ Weight								ng/kg (Menergy) Relative to body Organ Wei							
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Average	SEM	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Average	SEM
Heart	1.11	1.34	1.23	1.17	1.31	1.21	**1.23**	**0.035**	0.30	0.32	0.35	0.33	0.37	0.33	**0.33**	**0.011**
Liver	12.39	14.84	12.08	1.66	12.12	11.75	**10.81**	**1.886**	3.35	3.52	3.39	0.47	3.44	3.16	**2.89**	**0.532**
kidney	1.79	2.44	2.06	1.81	1.71	2.02	**1.97**	**0.109**	0.48	0.58	0.58	0.52	0.49	0.54	**0.53**	**0.019**
Spleen	0.84	1.02	0.7	0.76	0.89	0.81	**0.84**	**0.045**	0.23	0.24	0.20	0.22	0.25	0.22	**0.23**	**0.009**
Lungs	2.06	1.94	1.74	1.76	2.59	1.77	**1.98**	**0.133**	0.56	0.46	0.49	0.50	0.74	0.48	**0.54**	**0.046**
Testicles	3.45	3.04	3.07	2.85	2.51	3.18	**3.02**	**0.129**	0.93	0.72	0.86	0.81	0.71	0.85	**0.82**	**0.038**
Stomach	7.25	6.24	6.07	4.58	7.84	9.16	**6.86**	**0.648**	1.96	1.48	1.71	1.31	2.23	2.46	**1.86**	**0.198**
Body Weigh	370	422	356	350	352	372	370.33	10.996								

Liver Function Test Results

In the current study, the impact of MENERGY on TG-A and TG-B was evaluated after three months of consumption. No significant change was observed in parameters attributed to a healthy liver. The results of TG-A and TG-B were comparable to CG, and fatty liver symptoms were evident in all three groups, the details in Table-3

Table-3: Liver Function Test (LFT) CG, TG-A and TG-B.

Animal Group/Sample		Total bilirubin	Direct bilirubin	In Direct bilirubin	SGPT	Alkaline Phosphate	Gamma GT
	Reference Range(mg/dL)	< 1.3	< 0.3	< 0.20	Upto 41	Upto 483	10 to 70
CG Result (mg/dL)	S1	0.23	0.08	0.15	65	498	2
	S2	0.23	0.07	0.16	90	601	4
	S3	0.18	0.06	0.12	65	575	4
TG-A Result (mg/dL)	S1	0.24	0.07	0.17	38	315	3
	S2	0.18	0.06	0.12	39	220	2
	S3	0.22	0.08	0.14	32	142	2
TG-B Result (mg/dL)	S1	0.18	0.08	0.1	70	299	3
	S2	0.24	0.07	0.17	67	292	4
	S3	0.22	0.07	0.15	65	259	3

Kidney Function Results (Creatinine and Urea)

The level of creatinine in all groups of rats was found to meet the reference range limits of creatinine 0.4–0.8 mg/dl and urea 10–50 mg/dl, respectively. From the results, it is evident that dosing the quantity of MDSC at 30 mg/kg and 60 mg/kg of body weight is safe and did not lead to any biochemical pathology in the kidney. The details summarized in Table: 04.

Table-04: Creatinine and Urea Test CG, TG-A and TG-B.

Animal Group/Sample		Urea	Creatinine
	Reference Range(mg/dL)	10 to 50	0.4-0.8
CG Result (mg/dL)	S1	31	0.6
	S2	28	0.62
	S3	26	0.57
TG-A Result (mg/dL)	S1	43	0.67
	S2	38	0.67
	S3	35	0.67
TG-B Result (mg/dL)	S1	28	0.72
	S2	26	0.75
	S3	26	0.73

Histopathology of Liver

In the CG, TG-A, and TG-B groups, liver histopathology showed normal, Hydropic changes not seen, Steatosis: not seen, Necrosis is not seen, and fibrosis is not seen. Nuclear changes: unremarkable. Lobular inflammation: sparse chronic inflammation scattered in the parenchyma, Portal tract: unremarkable, Central vein: unremarkable; no necrosis noted. The lobular inflammation in the parenchyma was scattered in the parenchyma. Sinusoids: Mostly dilated haemorrhage and congestion were consistent in the CG, TG-A, and TG-B groups.

Figure 05: CG Liver.

Figure 06: TG-A Liver.

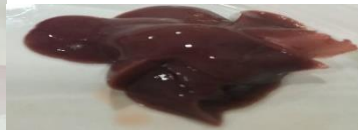
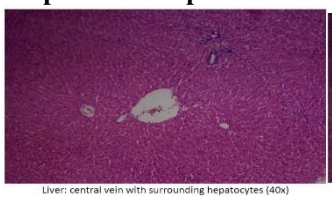
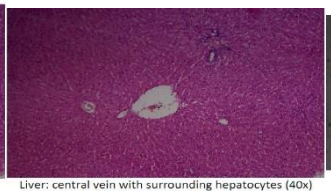


Figure 07: TG-B Liver.

The microscopic films of specimens of CG, TG-A and TG-B group (Figure 8-16)



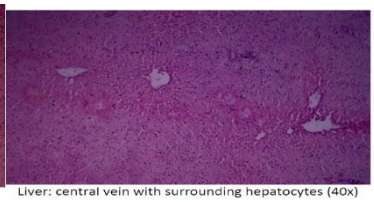
Normal Food
Fig. 08: CG, S1, Liver.



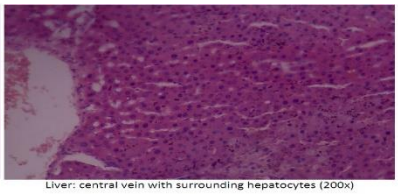
Normal Food
Figure-09. CG, S2, Liver.

Normal Food
Figure-10. CG, S3 Liver.

Normal Food 30mg/kg
Figure-11: TG-A, S1.



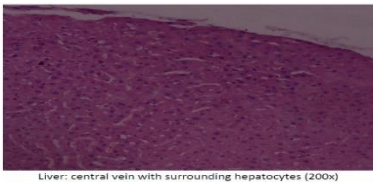
Normal Food 30mg/kg
Figure-12: TG-A, S2.



Normal Food 30mg/kg
Figure-13: TG-A, S3.

Normal Food 60mg/kg
Figure-14: TG-B, S1.

ormal Food 60mg/kg
Figure-15: TG-B, S2.



Normal Food 60mg/
Figure-16: TG-B, S3.

Lobular inflammation and dilated sinusoids with haemorrhage and congestion are considered preconceived attributes in all groups. No significant change was attributed to the usage of MDSC powder in TG-A and TG-B. Histopathology of the Kidney In the CG, TG-A, and TG-B groups, kidney histopathology

showed normal. The microscopic details are as follows: Glomeruli: Enlargement: unremarkable, Fibrosis not seen; inflammation of mesangium not seen; Basement membrane: unremarkable Gross examination: tubular atrophy or degeneration not seen, cast: Not seen, Substance in tubules: unremarkable, Hydropic changes: could not be appreciated, Inflammation could not be appreciated, and pigmentation could not be appreciated. Interstitial tissue: haemorrhage and congestion, focally present, Inflammation: could not be appreciated due to fixation artefacts and the similarity between the samples of CG, TG-A, and TG attributed to pre-existing pathology. Necrosis: unremarkable or not appreciated, Kidney: Glomeruli with tubules showing unremarkable features (40X).

Figure-17: CG Kidney.

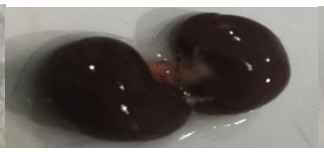
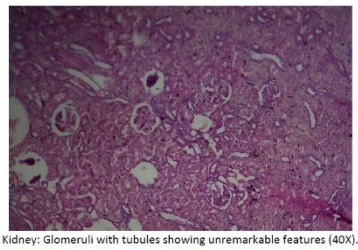


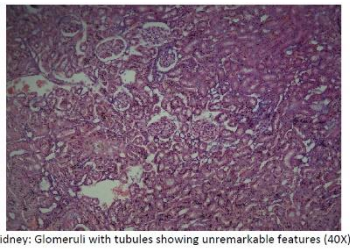
Figure-18: TG-A Kidney.

Figure-19: TG-B Kidney.



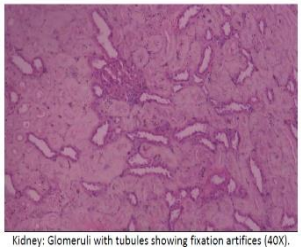
Kidney: Glomeruli with tubules showing unremarkable features (40X).

Normal Food
Figure-23: CG, S1.



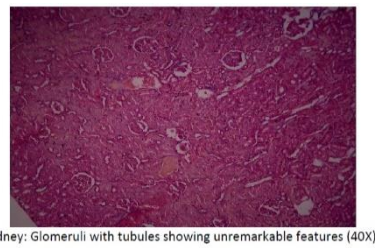
Kidney: Glomeruli with tubules showing unremarkable features (40X).

Normal Food
Figure-24: CG, S2.



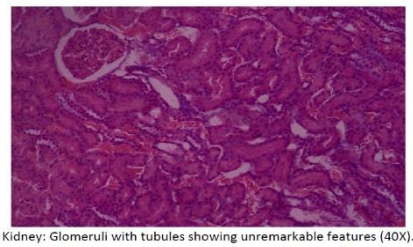
Kidney: Glomeruli with tubules showing fixation artifices (40X).

Normal Food
Figure-25: CG, S3.



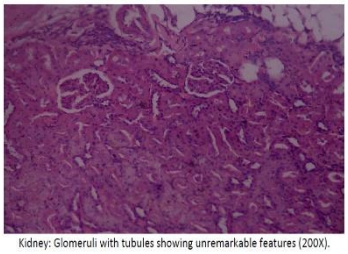
Kidney: Glomeruli with tubules showing unremarkable features (40X).

MDSC 30mg/kg
Figure-26: TG-A, TG-B S1.



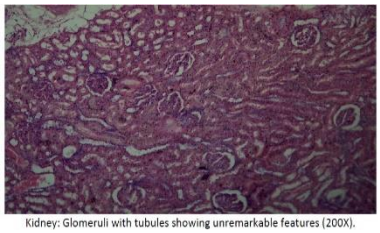
Kidney: Glomeruli with tubules showing unremarkable features (40X).

MDSC 30mg/kg
Figure-27: TG-A, TG-B, S2.



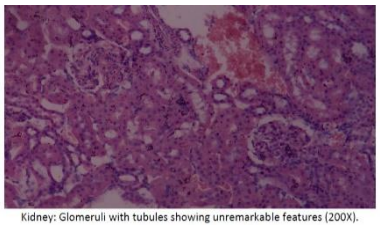
Kidney: Glomeruli with tubules showing unremarkable features (200X).

MDSC 30mg/Kg
Figure-28, TG-B, S3:



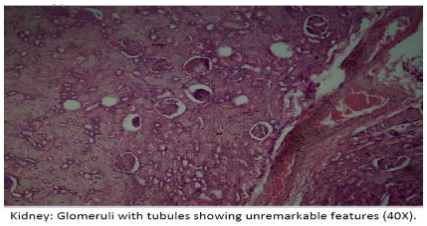
Kidney: Glomeruli with tubules showing unremarkable features (200X).

MDSC 60mg/kg
Figure-29: CG, S1.



Kidney: Glomeruli with tubules showing unremarkable features (200X).

MDSC 60mg/kg
Figure-30: CG, S2.



Kidney: Glomeruli with tubules showing unremarkable features (40X).

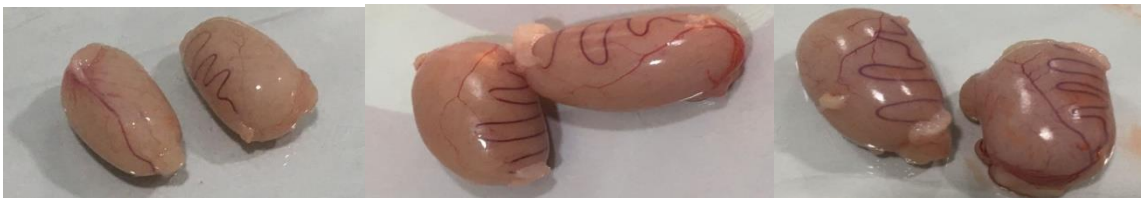
MDSC 60mg/Kg
Figure-31: CG, S3.

Overall histopathology of kidney in **CG, TG-A and TG-B** groups was found normal without any adverse impact of MDSC in **TG-A and TG-B groups with 30m/kg and 60mg/kg treatment with MDSC**

Histopathology of Testicles

Specimen received in formalin and consisted of pair of testes collectively measuring 3 x1.5 cms. The cut Surface is homogenous. Representative sections taken and processed.

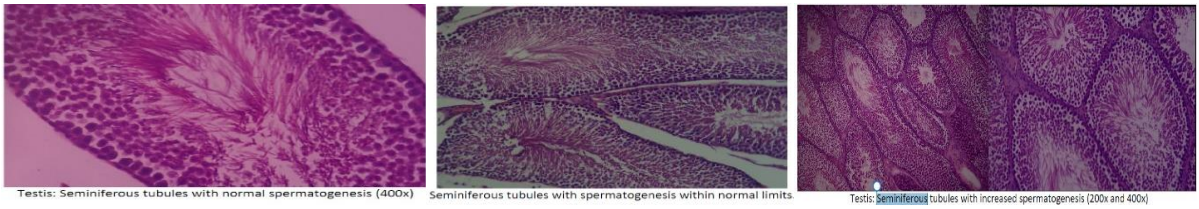
The testis were **formed by seminiferous tubules surrounded by tunica albuginea**. There are interstitial connective tissues between the tubules. The seminiferous tubules are uniform in size and shape and lined by regularly arranged rows of spermatogenic cells of different stages of maturation.



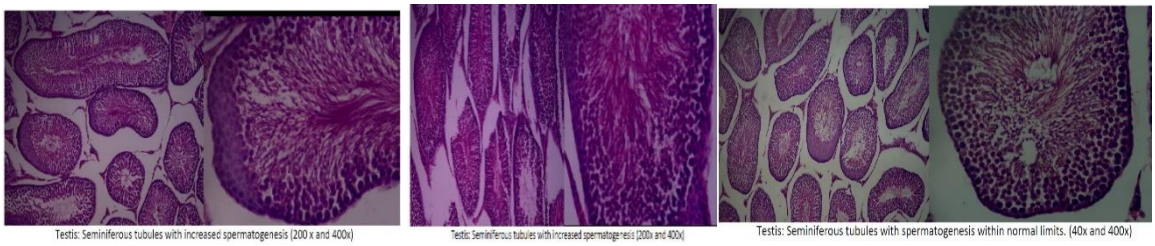
CG Testis Figure-32: **TG-A Testis Figure-33:** **TG-B Testis Figure-34:**

Histopathology of kidneys in the CG, TG-A, and TG-B groups was found to be normal without any adverse impact of MDSC in the TG-A and TG-B groups with 30 mg/kg and 60 mg/kg treatment with MDSC. Histopathology of Testicles The specimen was received in formalin and consisted of a pair of testes collectively measuring 3 x 1.5 cm. The cut surface is homogenous. Representative sections were taken and processed. The testis was formed by seminiferous tubules surrounded by tunica albuginea. There are interstitial connective tissues between the tubules. The seminiferous tubules are uniform in size and shape and lined by regularly arranged rows of spermatogenic cells at different stages of maturation.

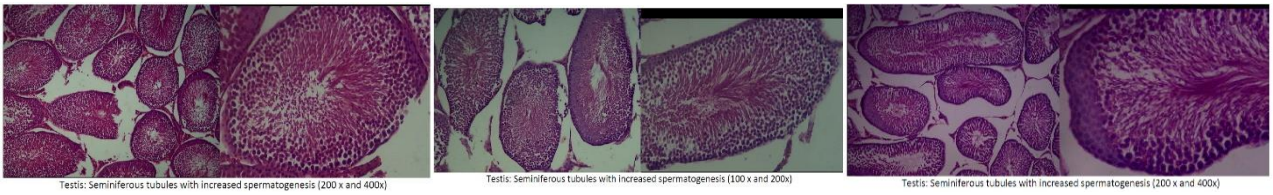
Microscopy Description: The histopathology of Testicles in CG, Table-7, TG-A, Table-8 and TG-B, Table-9 was found normal. The sections showing testicular tissue with intact seminiferous tubules. The basement membrane is intact and unremarkable. spermatogenesis is within normal limits interstitial tissue is showing Leydig cells, no evidence of atypical cells, inflammation of any other pathology seen, except Figure-8, and Figure-9 of control TG-A and TG-B groups where the spermatogenesis was more than the Figiue-7 of CG further safety and efficacy of MDSC.



Normal Food Figure-35: CG, S1 Testicles. **Normal Food Figure-36: CG, S2 Testicles.** **Normal Food Figure-37: CG, S3, Testicles.**



MDSC 30mg/kg Figure-38: TG-A, S1. **MDSC 30mg/kg Figure-39: TG-A, S2.** **MDSC 30mg/kg Figure-40: TG-A, S3.**



MDSC 60mg/kg Figure-41: TG-B, S1. **MDSC 60mg/kg Figure-42: TG-B, S2.** **MDSC 60mg/kg Figure-43: TG-B, S3.**

DISCUSSION

Dietary supplements have gained popularity as a means of enhancing nutritional intake and overall well-being.[19] However, ensuring the safety of these supplements is essential to protecting consumers from potential harm.[20] Pre-clinical studies, often involving animal models, play a crucial role in assessing the chronic toxicity of dietary supplement capsules.[21] These studies aim to mimic long-term human exposure to the supplement. The results of pre-clinical studies assessing the chronic toxicity of dietary supplement capsules can reveal several important findings, including dose-dependent

effects, organ specific effects, cumulative effects, gender and species differences, and changes in biochemical markers, such as alterations in enzyme levels or metabolic pathways, that can provide insights into the mechanisms of toxicity. In the past multiple pre-studies were conducted on the major components of MENERGY. Acute toxicity study (14 days) and sub-acute toxicity study (28 days) on Holy Basil (Tulsi) using n=6 group/sex of mice on 50% ethanolic extract was conducted on 200mg, 600mg and 2000mg/kg of body weight, biochemical, hematological, and histopathological changes in tissues (liver, kidney, spleen, heart, and testis/ovary) did not produce any hazardous symptoms or death and CNS and ANS toxicities.[21] Similarly, a chronic toxicity (6- Months) study on Tamarindus indica using 75 mg/kg to 1000 mg/kg on Wistar rats for 6 months on 120 males and 120 females did not lead to abnormalities in haematology and blood biochemistry parameters caused by long-term use.[22] Another component of MENERGY, Curculigo orchioides assessed the neurotoxicity study using 200 mg/kg to 400 mg/kg on 5 mice for 14 days using 100%. methanol extract (Root)[23] The phytochemical showed no neuroprotective effect.[23] The results of the Curculigo orchioides indicate that the hydroethanolic extracts of different parts of two varieties of Anacyclus. pyrethrum was not toxic in mice at low concentrations, whereas some toxic effects were detected in mice treated at 2000 mg/kg.[24] Withania somnifera, another important ingredient of "MENERGY" in past conducted an acute toxicity (14 days) on 18 human volunteers using 750mg/day to 1250 mg/day Aqueous Extract (Root)[25] The results revealed that in 14 days, it was found to be safe on haematological and biochemical organ function tests.[25] Another vital component of MENERGY, Panax ginseng was tested in randomized, double-blind placebo, controlled study to assess the safety on 30 human volunteers with 500 mg/day dried root of Panax ginseng (20) for 12 weeks.[26] The 30 human volunteers showed that red ginseng may be effective in reducing genotoxicity and improving HRQL in patients with EOC who received chemotherapy after surgery.[26] Moreover, red ginseng can be taken safely.[26] Like in the case of dose-dependent effects, in the current study, the suggested doses of 500 mg and 1000 mg showed no weight loss or any toxic signs at the end of the observation period. In the current study, as per the study results, dietary supplement capsule did not cause any gross pathology in any of the mentioned organs. In the dietary supplement, it is also important to assess the cumulative effects, the chronic exposure to certain dietary supplements may lead to cumulative toxicity over time, even at doses that appear safe in the short term. In current study, the 3-month toxicity was evaluated in a controlled group, with normal diet and taking water, which showed no toxicity with no weight loss. For the safety evaluation of dietary supplements, the changes in biochemical markers, such as alterations in enzyme levels or metabolic pathways, can provide insights into the mechanisms of toxicity. In current study, the liver function test was evaluated after three months consumption showed no significant changes in the alterations in enzyme levels. Overall, the results of study evaluated a comprehensive understanding of the potential implications of MENERGY including risk-benefits assessment, consumer awareness and regulatory implications.

CONCLUSION

The findings of this study indicate that 'MENERGY' Dietary Supplement Capsules are safe and do not cause any gross pathology, liver or kidney damage, or histopathological damage to the liver, kidney, or testicles. Furthermore, the supplement may have a beneficial effect on spermatogenesis.

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