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AMELIORATIVE ACTIVITY OF *ERYTHROXYLUM MONOGYNUM* IN CHROMIUM INDUCED TESTICULAR TOXIC RATS

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ABSTRACT

Pollution, stress, bad food habits, smoking and consuming of alcohol are some of the reasons for various health problems. With these reasons most of the men are unable to have children due to lack of sufficient sperm count and motile sperms. Though the availability of various therapies to solve these problems none of them are reaching the common man. Medicinal plants have major role in treating the diseases. The present work was done to know the effect of *Erythroxylum monogynum* leaf extract in Chromium ($K_2Cr_2O_7$) induced testicular toxic male albino rats. The extract was prepared by the hydroalchol solvent (70% ethanol and 30% water) (Erythroxylum monogynum hydrolacoholic extract) through maceration technique. The fertile male albino rats were divided into 4 groups. Group- I as Control, group- II as Chromium ($K_2Cr_2O_7$) (150ppm in dstl. water), group-III Chromium + EMHE 200mg/kg and group-IV Chromium + EMHE 300 mg/kg. The administration of the Chromium and extract was done through oral for 30 days. On 31st day the rats' blood samples were collected through retro orbital plexus. The blood samples were used to perform serological tests. Then the rats were sacrificed to separate the cauda epididymis and testes. The cauda epididymis was finely teased in saline to study the sperm count and motility. The testes were allowed for the histological process. The decreased sperm count and motility was observed in the group-II. The sperm count and motility were increased in the group-III and group-IV compare to group-II. The SGOT, SGPT and ALP were decreased in the group- III and IV compare to group-II. The increased HDL, total protein and albumin levels were seen in the extract treated groups. The reduced levels of total cholesterol, triglycerides, LDL and VLDL were observed in the group-III and IV rats. The testes sections were also supported the ameliorative activity with reforming germinal cells in seminiferous tubules of the testis of extract treated rats.

INTRODUCTION

Male infertility is often caused either by blocking of sperm ducts or disorder in sperm production. On the basis of the semen quality and sperm count the fertility capacity can be judged. Azoospermia (no sperm in semen), oligospermia (less number of sperms), normospermia (normal number of sperms) in case of male semen and necrosprmia (dead sperm), asthenospermia (slow moving sperm) in female after coitus, are the terms which explain the male infertility. Infertility occurs due to azoospermic conditions while sub fertility occurs due to oligospermic (<20mil/ml of semen) situation. Disturbances in the hormones related to hypothalamic pituitary also cause defects in fertility. Ex. Hyperprolactinemia may cause hypogonadism, erectile dysfunction, decreased libido, gynecomastia and infertility.

Excessive heat on the genital organs causes defects in the functioning of testicles. Exposure to the toxic chemical substances, pollution, living familiar to radiation, prone to oxidative stress (ROS-free radicals cause damage to DNA of sperms by reducing antioxidant levels) all are consider as environmental factors that induce infertility. Smoking also cause oxidative stress in the male by releasing Reactive Oxygen Species and damage the DNA of sperm.

Anabolic androgen steroids, which used by athletes for building muscle mass cause infertility in men. Anabolic steroids also oppose the sperm production similar to the supplemented testosterone. Men using 5-alpha-reductase inhibitors (finasteride, dutaseride, and propecia) as medicine for the prostate enlargement and hair loss may decrease the sperm count in semen. Alpha blockers (Hytrin, Silodosin, Cardura, Tamsulosin, Alfuzosin) generally used for curing the urinary symptoms caused by the prostate enlargement. This medication also decreases the ejaculatory volume.

Drugs like Colchicine, Acrylamide, Chloroprene, Copper, Lead, Alkyl mercury, Anesthetic gases, Cocaine, Heroin etc are also proved for their drastic effect on sperm count and motility and other male reproductive organs. Body exposed to high chromium (VI) can cause respiratory, gastrointestinal, haematological, reproductive and developmental effects. The decreased sperm count, damaged reproductive organs, changes of architecture in the epididymis etc are some of the effects on reproductive system. There are no reports stressing the damage of reproduction in human by chromium. Experimented animals showed a drastic effect on the reproductive system especially in male population. The morphological changes of seminiferous tubules, decreases

sperm count and increased abnormal sperm were observed in the 6 day chromium (VI) administered wistar rats (≥ 5.2 mg/kg/day). The oral treatment of chromium (VI) to monkeys, rats and rabbits observed to be most effective. Decreased sperm count, motility, histopathological changes in epididymis, depleted germinal cells, sertoli cell fibrosis and leydig cell hyperplasia were studied in 2.1mg chromium (VI)/kg per day (180days) treated monkeys[1].

Many plants were screened for their aphrodisiac activity- like *Alium sativum*[2], *Camilia sinensis*[3], *Citrus sinensis*[4], *Sesame radiatinum*[5], *Terminalia catuppa*[6], *Zingiber officinale*[7].

MATERIALS AND METHODS

The medicinal plant *Erythroxylum monogynum* was collected from the village area of Gudur, Warangal District, Telangana. It was identified and authenticated by Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal. The plant was stored in the herbarium of the lab by allocating voucher number.

The plant is generally called as Devadari in Telugu and Bastard sandal in English. It is from the family Erythroxylaceae. It is a small tree and abundant in foot hills shrub jungles. It can be identified with dark brown and rough bark. The wood of the tree is very hard and reddish brown.

The plant is being practiced by the village people for various ailments. They are getting relief from inflammations, malaria[8] and stomach ache. It is proved for antibacterial activity[9].

The collected plant material leaves were dried in shade for about 15 days. The leaves were powdered with electrical grinder. The collected coarse powder then passed through No.10 mesh and the fine powder was used for the extraction.

Maceration technique was employed to prepare the extract from leaf powder of the plant. Hydroalcohol solvent (70% ethanol &30% distilled water), 50g of powder was taken in stoppered conical flasks; it was mixed with 250ml of solvent and allowed for 24hrs at room temperature with random shaking. Then the filtrate-I was collected and the marc dissolved in 250mlof solvent for 24 hrs and collected the filtrate-II. Then the filtrates (I&II) were subjected to distillation to get extracts and stored in well closed amber glass containers in refrigerator temperature prior to use. The Extract was given name as EMHE (Erythroxylum monogynum hydroalcoholic extract).

The following experimental procedure was employed to study the ameliorative activity of the EMHE. The fertile male albino rats weighing about 200-250g were selected for the study. The

albino rats were brought from the Mahaveer Enterprizes, Hyderabad. The protocol was approved by Institutional Animal Ethical Committee (IAEC/03/UCPSc/KU/10). The rats were housed in polypropylene cages and acclimatized to the well conditioned house. The house was maintained with the temperature 25 ± 5^0C and relative humidity 50 to 60% and 12:12 hr light and dark cycle. They were fed with standard rat pellet (Hypro Nutrients, Pune), water *ad libitum*. The husk was used as bed to animals. Before keeping the animals, the polypropylene cages were sterilized along with water feeding bottles. The rats were divided into following groups (with 8 rats in each group).

Group –I was considered as control (2.5ml/kg dstl. water was given for 30 days)

Group –II was given potassium dichromate at 150ppm in distl. water (0.424g/L) daily for 30 days

Group –III was given potassium dichromate and 200mg/kg EMHE

Group- IV was administered with potassium dichromate and 300mg/kg EMHE

The treatment was performed for 30 days and administration of both drug and extract was done by oral through gastric gavage[10].

On the 31st day final body weights were recorded and the blood samples were taken from the rats through retro orbital plexus and allowed for centrifugation to get the serum and serum samples were used for the serological tests with the commercially available kits. The rats were dissected and cauda epididymis were separated and teased finely in the 20ml of normal saline to know the sperm count and motility.

$$\% \text{ motility} = \frac{\text{Motile sperm}}{\text{Motile sperm + Non-motile sperms}} \times 100$$

The separated testis and liver were used for the tissue glycogen (modified Anthrone method) and protein content (Lowrey method). The testes were processed for the histological study.

RESULTS

Body weights and reproductive organs

The final body weights of the group –II rats were decreased compare to the body weights of the other groups. The body weights of group- III and group- IV were observed as normal compare to group- II (Table 1).

The weights of reproductive organs such as testis (Table 2), cauda epididymis and seminal vesicles were decreased in the group-II rats than to the group-I, group-III, and also group- IV (Table 3).

The decrease of cauda epididymis weight was observed in the group-II than to the control group. The change in the weight of cauda epididymis was not much more among the group- III and group-IV rats. The weight gain of seminal vesicles was observed in the group-IV compare to group- II and group- III (Table 3).

Sperm count and motility:

Sperm count was decreased in the chromium induced group. Whereas the sperm count was increased in the group-III and group-IV compare to group-II. (Table 4). The sperm motility was also significantly reduced in the group-II compare to the group-I, group-III and group- IV (Table 4).

The sperm count and motility were increased in EMHE treated rats as the dose was increasing.

Serological parameters:

SGOT, SGPT and ALP:

Serological tests like SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase) and ALP (alkaline phosphatase) were increased in the group-II when compare to the group-I. These values were decreased in the group- III and group- IV (Table 5).

Total cholesterol and Triglycerides:

Table 6 shows the total cholesterol and triglycerides values of serum. The total cholesterol levels were increased in the group- II. The cholesterol levels were lowered in the group-III and group-IV.

Serum triglycerides levels were also increased in the group-II compare to group- I. The decreased levels were seen in the group- III and group- IV.

HDL, LDL and VLDL:

High Density Lipoproteins (HDL) values were decreased in the group- II rats than to the group-I. They were restored in the group-III and group- IV (Table 7).

Low Density Lipoproteins (LDL) levels were elevated in the group-II compare to group- I. The values were lowered in the group- III and group- IV. The lowering capacity of EMHE was high in group- IV (Table 6). VLDL (Very Low Density Lipoproteins) levels were also enhanced in the group-II than to the group-I. The levels were significantly lowered in the group- III and group- IV (Table 7).

Total Protein, albumin and glucose:

Table 8 shows the values of total protein, albumin in serum of different groups.

Total protein levels were decreased in the group-II than to the group-I. The increased values were seen in the group-III and group- IV.

Albumin levels were lowered in the group- II rats. The increased values of albumin were noticed in the group- III and group- IV. The glucose values of group-II were reduced compare to the group-I. The glucose levels were increased in the both group- III and group- IV (Table 9).

Biochemical parameters- tissue glycogen and protein:

Glycogen levels of both testis and liver were decreased in the group-II compare to the group-I. The restored glycogen levels were observed in the group-III and group- IV (Table 10). The glycogen content in the testis of group-III and group-IV was increased than to the group-II.

The liver glycogen content was decreased in group- II when compare to control. Whereas, the glycogen levels were increased in group- III and group- IV.

Protein content of testis and liver in group-II rats was decreased significantly than to the control rats. The protein content was restored in the both EMHE treated groups (III, IV) (Table 11). The protein levels in testis of group- II rats were declined and restored levels were noticed in the group-III and group- IV. The protein content in liver of group-IV was more than to the group- III when compare to group- II.

Histological observation:

Histological sections of testis also showed the damage of seminiferous tubules in the group-II [Fig. II (a), (b)]. Well architected seminiferous tubules were observed in the testis section of group-III [Fig. III (a), (b)] and group- IV [Fig. IV (a) & (b)].

Table-1-Body Weights

GROUP	NAME	FINAL BODY WEIGHTS (g)
I	CONTROL	239.62±7.17
II	CHROMIUM INDUCED	202.87±7.19[a]
III	CHROMIUM +EMHE 200 mg/kg	233.62±9.45[c,b]
IV	CHROMIUM +EMHE 300 mg/kg	239.00±7.69[c,b]

Table- 2- Testes Weights

GROUP	NAME	TESTIS WEIGHT (g)
I	CONTROL	1.43±0.11
II	CHROMIUM INDUCED	1.23±0.08[a]
III	CHROMIUM +EMHE 200 mg/kg	1.34±0.11[c,d]
IV	CHROMIUM +EMHE 300 mg/kg	1.34±0.04[c,f]

Table-3- Cauda epididymis and Seminal vesicles Weights

GROUP	NAME	Cauda epididymis (mg)	Seminal vesicles (mg)
I	CONTROL	232.75±9.08	854.62±7.26
II	CHROMIUM INDUCED	216.75±8.66[a]	754.37±10.41[a]
III	CHROMIUM +EMHE 200 mg/kg	224.25±8.22[c,d]	763.12±8.39[a,d]
IV	CHROMIUM +EMHE 300 mg/kg	225.87±8.40[c,d]	827.37±8.56[a,b]

Table-4-Sperm Count and Sperm Motility

GROUP	NAME	SPERM COUNT (mil/ml)	SPERM MOTILITY (%)
I	CONTROL	56.53±4.62	77.98±5.11
II	CHROMIUM INDUCED	29.74±4.33[a]	37.97±5.00 [a]
III	CHROMIUM +EMHE 200 mg/kg	38.63±6.12[a,b]	46.77±4.66[a,b]
IV	CHROMIUM +EMHE 300 mg/kg	46.25±4.89[a,b]	54.19±3.36[a,b]

Table-5- Serological tests- SGOT, SGPT and ALP

GROUP	NAME	SGOT (U/L)	SGPT(U/L)	ALP (IU/L)
I	CONTROL	25.85±3.43	38.80±5.22	132.27±5.50
II	CHROMIUM INDUCED	112.27±6.31[a]	122.25±6.93 [a]	202.67±4.12[a]
III	CHROMIUM +EMHE 200 mg/kg	68.72±4.53[a,b]	72.18±5.96[a,b]	172.14±4.81[a,b]
IV	CHROMIUM +EMHE 300 mg/kg	49.41±5.37[a,b]	57.02±4.97[a,b]	161.17±4.17[a,b]

Table-6- Serological tests- Total Cholesterol, Triglycerides and LDL

GROUP	NAME	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)
I	CONTROL	127.12±6.79	77.73±5.41	66.07±6.94
II	CHROMIUM INDUCED	182.37±5.34[a]	155.76±5.77[a]	123.97±6.95 [a]
III	CHROMIUM +EMHE 200 mg/kg	158.43±3.04[a,b]	114.66±4.31[a,b]	104.46±5.54[a,b]
IV	CHROMIUM +EMHE 300 mg/kg	141.19±4.51[a,b]	96.02±5.12[a,b]	81.88±4.16[a,b]

Table- 7-Serological tests- HDL and VLDL

GROUP	NAME	HDL (mg/dL)	VLDL (mg/dL)
I	CONTROL	45.51±4.21	15.54±1.08
II	CHROMIUM INDUCED	27.05±3.11[a]	29.30±4.52[a]
III	CHROMIUM +EMHE 200 mg/kg	31.00±4.81[a,d]	22.97±0.82[a,b]
IV	CHROMIUM +EMHE 300 mg/kg	39.77±4.46[e,b]	19.20±1.02[e,b]

Table-8- Serological tests- Total protein and albumin

GROUP	NAME	Total Protein (g/dL)	Albumin (g/dL)
I	CONTROL	6.24±0.55	3.17±0.31
II	CHROMIUM INDUCED	4.29±0.76[a]	1.80±0.37[a]
III	CHROMIUM +EMHE 200 mg/kg	5.04±0.71[a,d]	2.21±0.35[a,d]
IV	CHROMIUM +EMHE 300 mg/kg	5.97±0.67[c,b]	2.94±0.43[c,b]

Table- 9-Serological tests- Glucose levels

GROUP	NAME	GLUCOSE (mg/dL)
I	CONTROL	85.77±3.67
II	CHROMIUM INDUCED	66.52±3.70[a]
III	CHROMIUM +EMHE 200 mg/kg	71.87±3.02[a,f]
IV	CHROMIUM +EMHE 300 mg/kg	76.51±4.36[a,b]

Table-10- Biochemical tests- Glycogen

GROUP	NAME	TESTIS (µg/100mg)	LIVER (µg/100mg)
I	CONTROL	381.62±8.42	746.39±9.56
II	CHROMIUM INDUCED	282.63±9.07[a]	462.62±8.70[a]
III	CHROMIUM +EMHE 200 mg/kg	292.60±8.59[a,,d]	595.78±9.66[a,b]
IV	CHROMIUM +EMHE 300 mg/kg	363.21±8.76[a,b]	691.28±9.08[a,b]

Table- 11- Biochemical tests- Protein

GROUP	NAME	TESTIS (mg/g)	LIVER (mg/g)
I	CONTROL	220.65±9.20	183.41±6.10
II	CHROMIUM INDUCED	185.91±6.67[a]	99.71±6.00[a]
III	CHROMIUM +EMHE 200 mg/kg	190.62±4.74[a,d]	125.83±8.16[a,b]
IV	CHROMIUM +EMHE 300 mg/kg	211.70±9.35[c,b]	148.97±9.47[a,b]

All values were expressed in mean ± SD, the values were analyzed with one way ANOVA followed by Dunnett Test. a=p<0.01compare to Group-I and b=p<0.01 compare to Group- II. c= not significant when compare to Group-I, d=not significant when compare to Group- II. e=p<0.05 compare to Group-I, f=p<0.05 compare to Group-II, n=8.

FIGURE-I (a) – CROSS SECTION OF TESTIS (CONTROL) (10X)

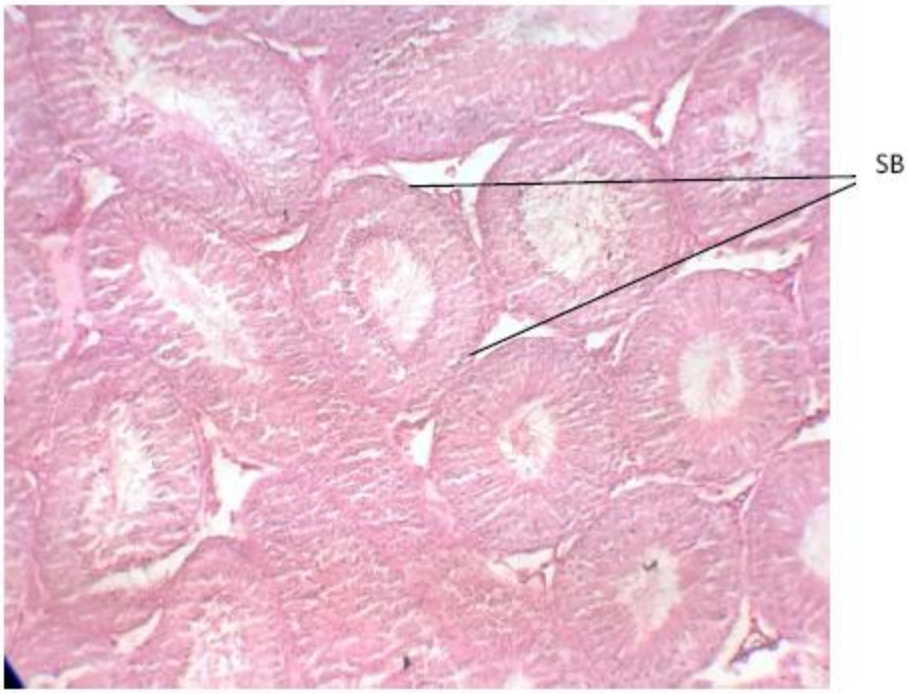
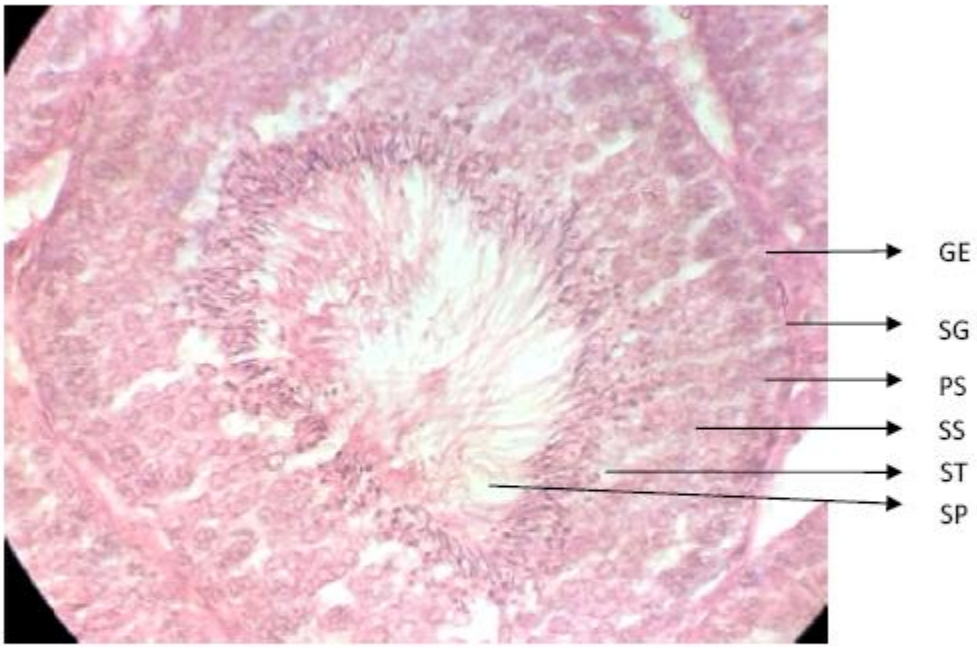


FIGURE-I (b) - CROSS SECTION OF TESTIS (CONTROL) (20X)



Cross section of testis of control rats showing normal architecture of the seminiferous tubule (SB) with GE (germinal epithelium), SG (spermatogonia), PS (primary spermatocyte), SS (secondery spermatocyte), ST (spermatids), sp (sperms)

FIGURE-II (a) – CROSS SECTION OF TESTIS (CHROMIUM INDUCED) (10X)

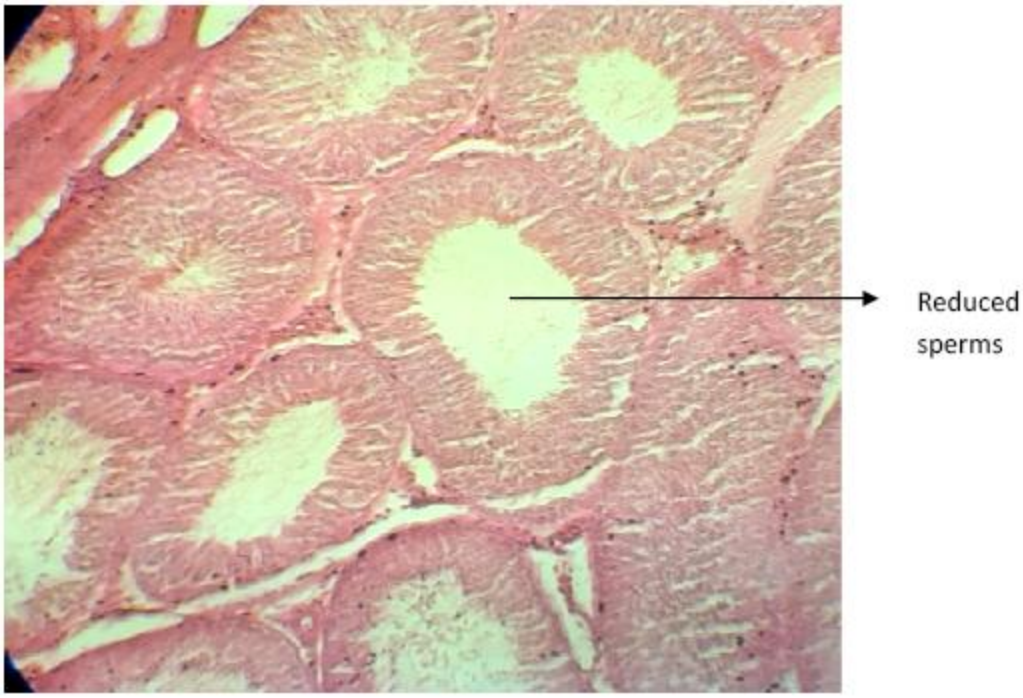
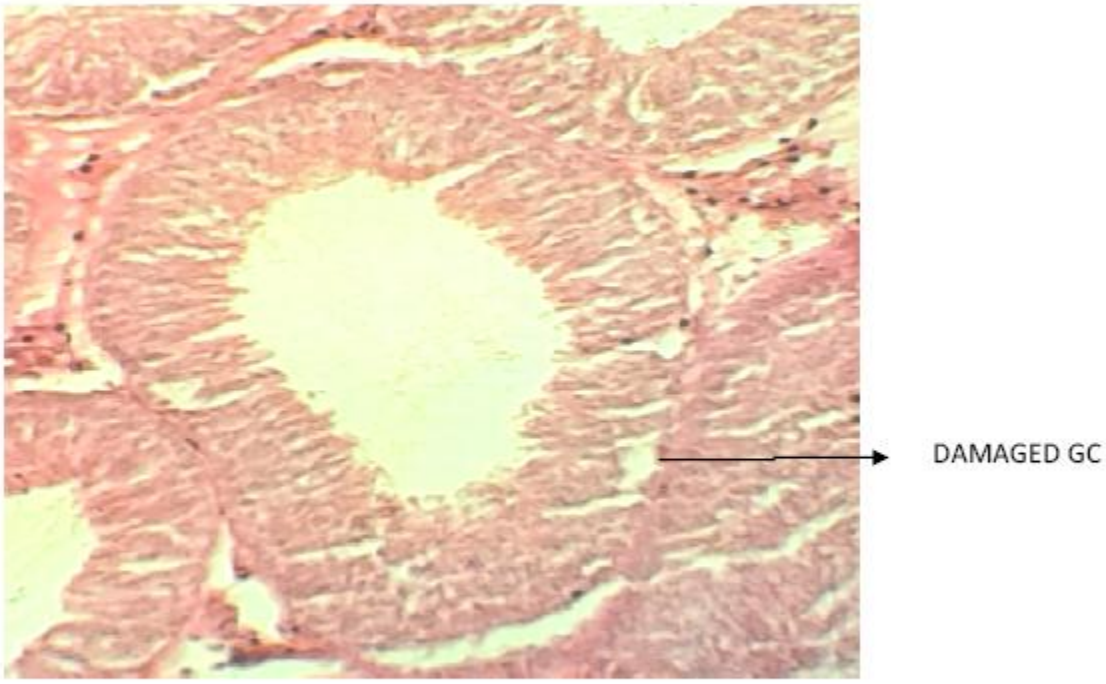


FIGURE-II (b) - CROSS SECTION OF TESTIS (CHROMIUM INDUCED) (20X)



The damaged germinal cells (GC), loss of sperms in the lumen were seen in the seminiferous tubules of the group-II rats

FIGURE- III (a) - CROSS SECTION OF TESTIS (CHROMIUM+EMHE200mg/kg) (10X)

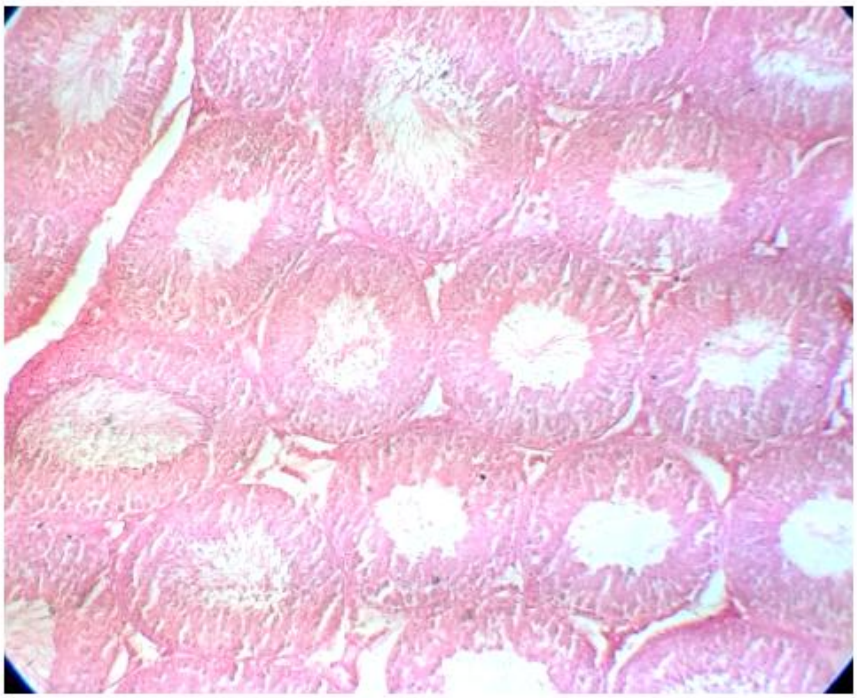
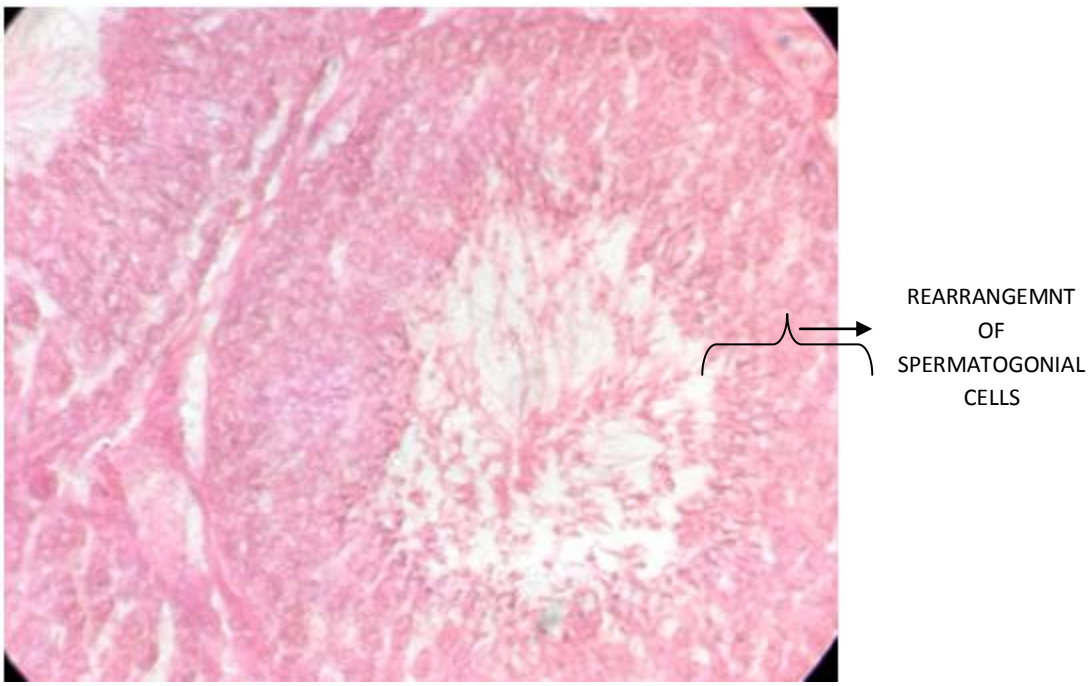


FIGURE- III (b) - CROSS SECTION OF TESTIS (CHROMIUM+EMHE200mg/kg) (20X)



Rearranged germinal cells in the seminiferous tubules and interstitial cells were seen of group-III rats

FIGURE- IV (a) - CROSS SECTION OF TESTIS (CHROMIUM + EMHE 300 mg/ kg) (10X)

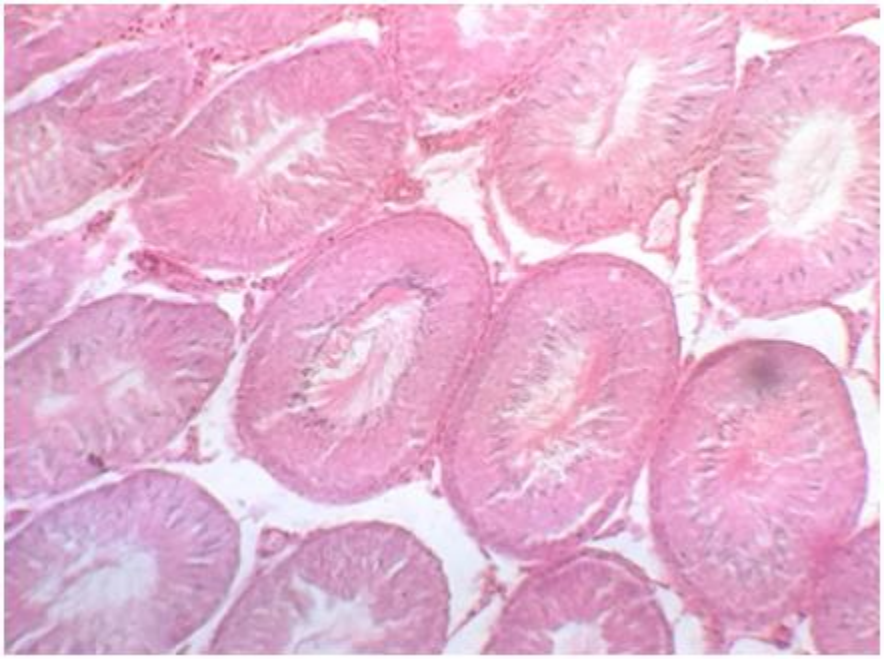
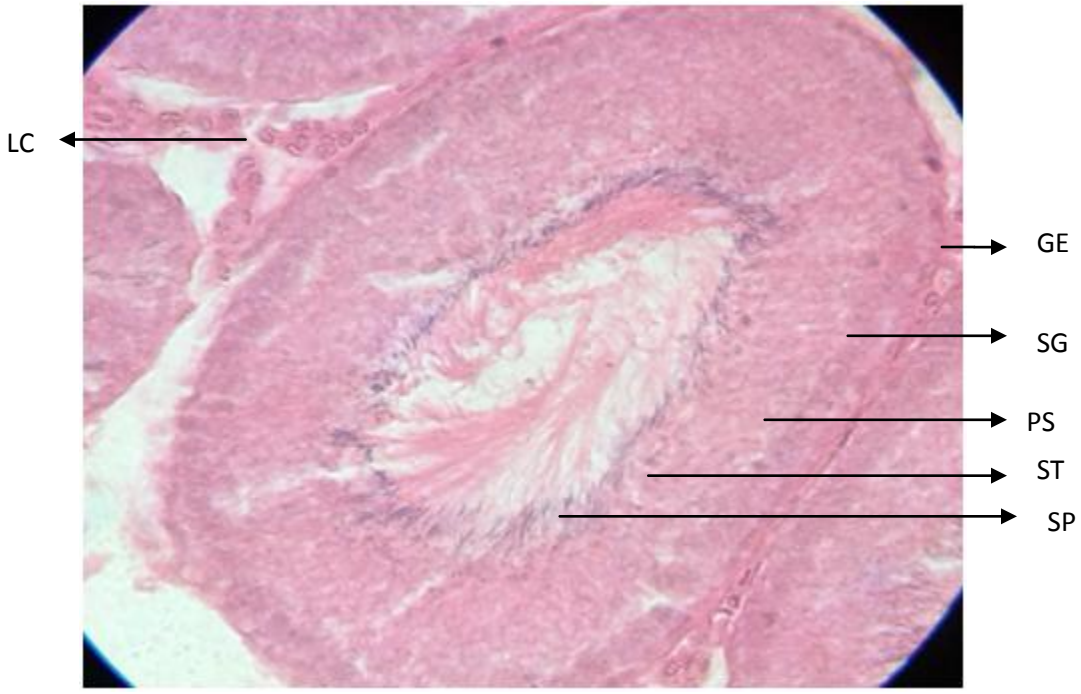


FIGURE- IV (b) - CROSS SECTION OF TESTIS (CHROMIUM + EMHE 300 mg/ kg) (20X)



The proper arranged germinal cells in the seminiferous tubules and interstitium were seen in the testis cross section of group-IV rats. Seminiferous tubule (SB) with GE (germinal epithelium), SG (spermatogonia), PS (primary spermatocyte), SS (secondary spermatocyte), ST (spermatids), SP (sperms), LC (Leydig cells)

DISCUSSION

The body weights and testes weights were decreased in chromium induced rats. This may be because of the toxic nature of the chromium. It has been reported that toxic nature of the compound may cause decrease or increase of the body weight or organ weight [11]. The reduction in the weights of cauda epididymis, seminal vesicles were observed in the group II compare to the group- I. Similar results were found in the *Cyproterone acetate* treated rats [12]. The decrease in the cauda epididymal weight was noticed in the group- II. The effect of sulfasalazine and its analogs were also shown similar findings in male rats [13]. It was also proved that chromium intoxicate the testis by generating its reactive radicals. These radicals cause damage lipid bilayer and DNA of the cells [14].

It is well known that free radicals may cause the oxidative stress which cause harm to several organs of the body. The antioxidant properties can counter act to control the free radicals not to prone to diseases. The EMHE treated rats were seen not losing their body weight compare to group-II rats. The toxic nature of the chromium might be controlled by EMHE and protected the testis against the oxidative stress. The weight of the testis increased in group- III and IV compare to the group-II. Similar results were seen in the rats treated with the *Zingiber officinale* extract on male rats [15].

The sperm count was increased in the group-III and IV compare to group-II. The decrease of sperm count in group- II may be because of the altered gonadotrophins (LH, FSH) which are required for normal sperm production, development and maturation [16]. The decline of sperm count and motility was also seen in sulfasalazine administered male albino rats [17]. The reduced sperm count may be because of the insufficient testosterone. The reduced sperm count results were also seen in sodium chromate induced rats[18]. The increased sperm count was observed in the EMHE treated rats of group- III and IV. The increase in sperm count may be activated by the testosterone synthesized from the leydig cells of the rats in the group –III and group- IV.

The deformation of leydig cells caused the inefficiency to produce testeosterone in the group –II rats. The reduced motility may be because of the alteration in the enzymatic activities of oxidative phosphorylation required for ATP production. The ATP helps in the forward movement of the spermatozoa. The EMHE treated (group-III and IV) rats showed more sperm count than to the chromium induced group. The antioxidant property of the EMHE might have

helped in increasing the spermatozoa. The effect of extracts of *Sesame radiatum* leaves also noticed similar results by having rich antioxidants and enhancing the sperm count in rats [19].

In addition to sperm count and motility the significant changes were also observed in the chromium induced group. The serological parameters such as SGOT, SGPT and ALP were elevated in the group- II. The elevated levels of these markers can indicate the toxic effect of chromium on the liver. The lipid peroxidation caused by the free radicals release these enzymes into circulation [20]. The increased values of these enzymes indicate the toxic nature of the chromium. The reduced levels of these enzymes were noticed in the group-III and group- IV. The lowered SGOT, SGPT and ALP values explains the free radical scavenging activity of the extract. The reduced SGOT, SGPT and ALP levels are helpful for the normal function of the liver in group- III and group- IV.

It was noticed that the serum cholesterol and triglycerides levels were increased in the chromium induced group. The increased cholesterol levels may cause coronary diseases. These values were decreased in the EMHE treated group- III and IV. The reduced cholesterol levels in the group-III and IV indicates the hypolipidemic effect of the EMHE. The decreased cholesterol and triglycerides were seen in the rats treated with *Costus pictus* methanol extract[21]. It was also found that hypercholesterolaemia has detrimental effect on leydig cell function, spermatogenesis and sperm maturity[22]. Increased triglycerides in serum may also cause drastic effect on spermatogenesis[23]. Might be the normal levels of cholesterol and triglycerides supported the normal function of Leydig cell to produce testosterone for spermatogenesis. Cholesterol also plays a major key role in the steroidal hormone synthesis[24]. The reduced levels of serum cholesterol levels indicate the use of the cholesterol for the synthesis of steroidal hormones.

Serum HDL levels were decreased in the chromium induced rats. The LDL and VLDL were increased in the chromium induced group. The reduced HDL also leads to heart problems. The increase of VLDL and triglycerides also has the correlation in decreasing sperm count [25]. The investigation of Mohammad AK, in infertile men revealed that the increase in triglycerides and cholesterol levels related to abnormal sperm morphology and motility[26].

Serum total protein and albumin levels were decreased in the group-II rats. The decrease of these two levels may be because of the damaged liver. The decreased serum albumin may be because of the kidney and liver diseases. The restored levels were seen in the group-III and IV rats. The

normalized values of protein and albumin are helpful in the normal function of the liver. The hepatoprotective activity of EMHE may be recovered the albumin and protein levels in the group-III and IV. The EMHE was observed with the presence of phytochemicals like tannins, phenols, glycosides, flavonoids with antioxidant activity[27].

The reduced glucose levels were seen in the chromium induced group. The decrease in the glucose levels may be because of the enhanced glucose oxidation or disturbances in the liver enzymatic mechanisms. Normal levels of glucose were seen in the EMHE treated rats. These normal glucose levels explain the reformed hepatocytes and its function.

The depletion of glycogen levels in the testis of the group- II rats were observed. The decrease of the glycogen in testis was because of the utilization of glycogen during stress. It is well known that constant supply of glucose is necessary for the proper functioning of the testis and maturation of germinal cells [28]. The restored glycogen levels supported the functional ability of the germinal cells to synthesize the spermatozoa in the EMHE treated rats.

The fall in the glycogen levels of the liver in chromium induced group was because of the decreased enzymatic activity of hexokinase[29]. The normalized levels of glycogen were observed in the group-III and IV rats.

The testis protein levels also decreased in the chromium induced group compare to the control. This was because of the androgen deprivation effect. The toxic effect of the chromium also may be the reason for the reduction of protein content. The protein levels were increased in the EMHE treated rats. The decrease of the liver proteins in chromium induced group also because of the toxic nature of the chromium to the liver. The recovery of the liver proteins was seen in the EMHE treated rats.

The phytochemicals tannins, phenols, flavonoids and glycosides in the EMHE may be the reason for fighting against the free radicals and counteract malondialdehyde, the resultant of lipidperoxidation. The reduced malondialdehyde in the group-III and IV might have helped in lowering the oxidative stress caused by the chromium. Enhance of glutathione peroxidase (GPx), catalase and superoxide dismutase (SOD) may be encouraged by the EMHE in the treated rats. The present results were correlated with the findings of ameliorative effect of *Moringa oleifera* leaf extracts on chromium induced testicular toxicity in rat testis[10].

The antioxidant activity of the EMHE extract also helped for the ameliorative activity. It was proved that the antioxidants protect DNA damage and can improve sperm quality and fertility in human beings [30]. The increased sperm count was also observed in the rats treated with plant *Citrus sinensis* fruit extract rich in flavonoids[3]. The presence of flavonoids in the EMHE also caused enhance in the spermatogenesis by its antioxidative effect.

The testis of chromium induced rats showed degenerated seminiferous tubules and leydig cells, severely damaged spermatocytes and spermatids. Different stages of germinal cells of spermatogenesis were clearly visible in the group-III and group- IV. The reformation of germinal cells was seen in the seminiferous tubules of group –II rat testis. The linear arranged spermatids and different stages of spermatogenesis were observed in the group- IV. The development of the seminiferous tubules, interstitium and leydig cells was increased in the group-IV rat's testis compare to group- III.

CONCLUSION

The increased sperm count, sperm motility, increased serum HDL, normal levels of SGOT, SGPT and other serological parameters in the EMHE treated chromium induced rats show the ameliorating activity of the extract. The presence of the tannins, flavonoids, glycosides, phenols in the extract may be the reason for the effective antioxidant property which protected the testes not to prone to oxidative stress.

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