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CARBARYL INDUCED TESTICULAR TOXICITY IN MALE RATS AND THE PROTECTIVE EFFECT OF *MUCUNA PRURIENS (L)*

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ABSTRACT

Cow-hedge (*Mucuna pruriens* L**.)** is one of the potential tropical legumes having good medicinal properties. The aim of our present study was to investigate the testicular protective activity of methanolic extract of *Mucuna pruriens* (MEMP) seeds against carbaryl induced testicular toxicity in rats. The MEMP at the dose of 300 mg/kg of body weight were administered orally once daily for 28 days to the infertility induced male rats. Sperm count, sperm motility and abnormality were analyzed. Further histopathological examination of the testes section was carried out to support the induction of testicular carbaryl toxicity , and testicular protective efficacy of methanolic extract of seeds of *Mucuna pruriens* plants treated with propiconazole and ABA.. The extract showed potent activities on sperm count, motility and abnormality. The histopathological observation supports the biochemical evidence of testicular protection of MEMP further strengthen the testicular protective observation of MEMP. The results of the present study strongly revealed that MEMP has potent testicular protective activity against carbaryl induced testicular damage in experimental rats.

KEY WORDS: ***Mucuna pruriens.*** **Testicular Toxicity, Histology, Carbaryl.**

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INTRODUCTION

Animals and humans are generally exposed to environmental and occupational pollutants whose contribution to the burden of reproductive system and leading to dysfunction of reproductive function. 1-Naphthyl methyl carbamate also known carbaryl is a widely used insecticide in the carbamate chemical family used in dozens of products and best known brand name is Sevin. Carbaryl in the long term, reduce sperm movement and make changes in spermatogenesis and also carbaryl induced sperm DNA damage by producing the oxidative stress in mammalian sperm[1,2&3]. The frequent exposure of carbaryl induced very low sperm counts as compared to a control group of unexposed workers and sperm samples found that the number of sperm abnormalities was increased in workers who were being exposed to carbaryl[4]. The carbaryl exposure showed distorted shape of seminiferous tubules, disturbed spermatogenesis and accumulation of cellular mass in the lumen of tubules and loss of sperms of varying degrees in testes[5]. The use of plant extracts as fertility enhancer in mammals is now in the increase because of the shifting of attention from synthetic drugs to natural plant products [6]. *Mucuna pruriens* Linn. (Leguminosae), commonly known as "the cowhage" or "velvet" bean; and "atmagupta" in India, is a climbing legume endemic to India and in other parts of the tropics including Central and South America. The plant are rich in alkaloids such as prurienine, prurieninine and prurienidine[7]. Triterpenes and sterols (sitosterol, ursolic acid etc) were found in the root and seeds of *M.pruriens*. The seed also contain proteins, amino acids such as L- DOPA[8] The aim of our present study was to investigate the testicular protective activity of methanolic extract of seeds of *Mucuna pruriens* (MEMP) against carbaryl induced testicular toxicity in rats.

MATERIALS AND METHODS

1.Plant material and extraction

Mucuna pruriens plants treated with Propiconazole and ABA from which fresh seeds were collected from matured pods during the months of August to September, 2012 from the Botanical Garden of Annamalai University, Annamalai Nagar. The seeds were air dried in shade and powdered in a mechanical grinder. The seed powder was extracted with 1000 ml of methanol using Soxhlet extractor for 72 hrs at temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatmann filter paper (No. 1) and then concentrated in vacuum and dried. The extract thus obtained was directly used for bio assay**.**

2. Experimental Design

Male Wister albino rats (average weight 180-200g) were used in the present experiment. Animals were obtained from Faculty of Medicine, Rajah Muthiah Medical College and Hospital, Annamalai Nagar, Chidambaram. The local committee approved the design of the experiment and the protocol conforms to the guidelines of the Animal Ethical Committee of Institute of Rajah Muthiah Medical College and Hospital (IAEC No: 855). Animals were caged into four groups and each group bearing 6 animals were given feed and water *ad libitum*. First group was used as control, while group II (Toxicant) was orally treated with carbaryl alone (Sevin) at 100 mg/ kg of body weight(LD_{50} value 850 mg) and group III with combination of carbaryl and methanolic extract of *Mucuna pruriens* (MEMP) seeds at 300mg/kg of body weight respectively. Group IV animals were received plant extracts alone (MEMP). Rats were orally administrated with their respective dose every day for 88 days.

3. Necropsy

The body weight of rats was recorded at the time of initiation and completion of the experiment (prior to necropsy). Prior to the termination of the experiment, rat's over night fasted were weighed and killed by using overdose of anesthetic either on the next day of the last treatment. Testes, epididymis, prostate gland, vas deference and seminal vesicle were quickly removed, cleared of the adhesive tissues and weighed.

4.Analysis of sperm parameters

Sperm analyses

Epididymal sperm count was determined by the method as described by[9]. Briefly a 0.5 mL aliquot of epididymal sperm was diluted with 9.5 mL diluent (50 g sodium bicarbonate, 10 ml 35% formalin and 0.25 g trypan blue were added and made up to a final volume of 1 L with distilled water). Approximately 10 mL of the thoroughly mixed diluted specimen was transferred to each of the counting chambers of the hemocytometer, which was allowed to stand for 5 min in a humid chamber to prevent drying. The cells sedimented during this time and were counted with a light microscope at 200 X magnification. Progressive sperm motility was evaluated by using the fluid obtained from cauda epididymis as described previously [10]. Both cauda epididymides were excised from each animal and minced in 2 ml with Tris buffer. Subsequently, epididymal preparation from each animal was placed in an incubator at 37 $^{\circ}$C in an atmosphere of 5% CO_2 in air for 30 min to allow sperm to swim out of the epididymal tubules. To facilitate the determination of sperm motility, epididymal fragments were removed from the above preparation, leaving mainly sperm suspension in the buffer. An aliquot of this solution was placed on the slide and covered with cover-slip. The slides were examined with a phase contrast microscope at 400 X magnification. The temperature of the microscope stage was maintained at 37 $^{\circ}$C throughout the observation. Number of progressively motile sperm was determined by counting sperm exhibiting forward motion. Motility estimations were performed from 3 different fields in each sample. The mean of the three estimations was used as the final motility score. By using this quotient, the percentage of progressively motile sperm was calculated. Assessment of dead and abnormal sperm was performed using eosin–nigrosin staining as described[11]. The sample was stained with 1% eosin yellow and 5% nigrosin and examined at 400 X Magnification or morphological abnormalities.

5. Hormone measurements

Plasma testosterone was assayed using electrochemiluminescence Immunoassay ‘‘ECLIA” Kit obtained from Roche Diagnostics GmbH. D-68298 Mannheim, USA.

6. Histopathology

The testes tissue was dissected out and fixed in 10% formalin solution. It was then dehydrated in ethanol (50%-100%), cleared in xylene and embedded in paraffin wax. Afterwards thin sections (5-6 mm) were made and then stained with hematoxylin and eosin dye for photomicroscopic observation.

3. Statistical analysis

All values were presented as mean ±SD. Differences were considered to be significant at $P < 0.05$. One-way analysis of variance (ANOVA) and Dunken test were used to determine the differences between the groups. The SPSS/PC program (Version16.0; SPSS, Chicago, IL) was used for the statistical analyses.

RESULTS

1.General Toxicity

The rats were observed for responses with respect to overall appearance, food intake, water intake, animal behavior and co-ordination, urination and respiration in control and treated animals. All the animals were apparently normal and no unusual behavior (viz, Circling, Head flicking, Head searching, Biting, Licking, Self mutilation and Walking back wards) were observed in all the experimental group rats.

And also the feed and water intake were normal in the control and treated group rats.

2. Body and organ weight

Oral administration of carbaryl caused significant decrease in the weight of the animal's body and organ such as testes, epididymis, vas difference, seminal vesicles when compared with control (Tables 1&2) where as the plant extract(MEMP) treated group animals organs weight increased significantly.

Table 1
Body weight of the male rat treated with carbaryl, carbaryl plus MEMP

Groups	Body weight (g/rats) On the Day of initiation of drugs	On the Day of sacrifices
Control (Group I)	140± 2.78	200± 3.12
Carbaryl alone (Group II)	165±3.14	109± 3.87
Carbaryl+ MEMP (Group III)	170±2.98	205±1.93
MEMP ONLY (Group IV)	150±4.90	200±2.63

Values are expressed as means ± SD; n = 6 for each treatment group. Significant difference from the control group at *P < 0.05.

3.Sperm analysis

Treatment of male rats with carbaryl caused significant($p<0.05$) decrease in the sperm count and motility (%) while dead sperm count increased as compared to those of control animals. Co-administration of MEMP caused a significant increase in semen quality and minimized the toxic effect of carbaryl (Table3).

Table 2
Relative weight of sex organ (g/100 g of body weight) and testosterone level (ng/ml) male rat treated with carbaryl and co administration of MEMP.

Organ/Parameter	Experimental Groups			
	Control (Group I)	**Carbaryl** (Group II)	**Carbaryl+ MEMP** (Group III)	**MEMP ONLY** (Group IV)
Testes	0.72±0.093	0.58±0.061	0.79±0.021	0.66±0.07
Epididymis	0.027±0.01	0.19±0.03	0.30±0.08	0.24±0.04
Seminal vesicle	0.35±0.20	0.16±0.067	0.39±0.034	0.24±0.031
Testosterone	2.12± 0.39	1.25±0.39	3.09 ±0.98	3.28 ±0.11

Values are expressed as means ± SD; n = 6 for each treatment group. Significant difference from the control group at *P < 0.05.

Table 3
Changes in sperm count (No. of sp. count× 10^6 /mL), motility(%) and dead sperm(%) of male rats treated with carbaryl and carbaryl + MEMP

parameters	Experimental Groups			
	Control (Group I)	**Carbaryl** (Group II)	**Carbaryl+ MEMP** (Group III)	**MEMP ONLY** (Group IV)
Sperm count	212±15.5	148±8.1	233±12.1	199±7.5
Sperm Motility	72.4±1.89	50.9±2.64	79.7±1.64	70.5±1.43
Sperm abnormality	14.6±1.96	21.3±2.31	11.7±1.27	16.7±1.3

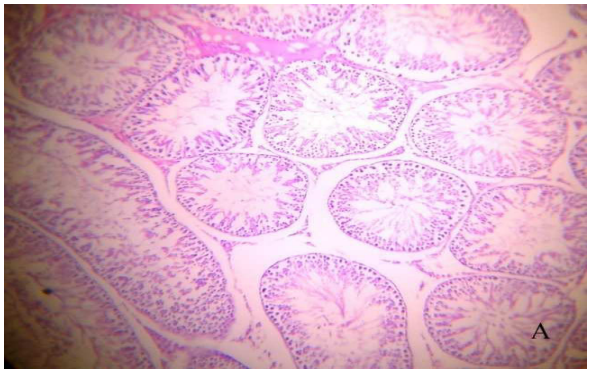
Values are expressed as means ± SD; n = 6 for each treatment group.
Significant difference from the control group at *P < 0.05.

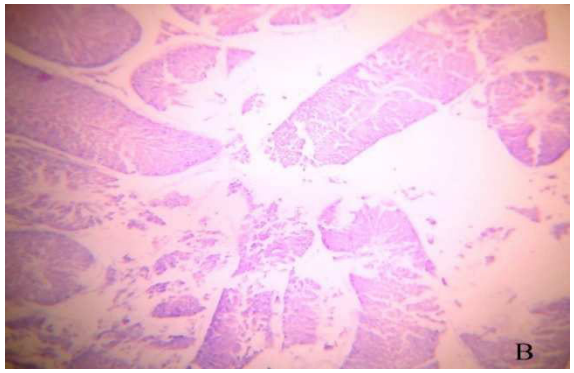
4.Hormone analysis

The significant increase in plasma testosterone concentration ($p<0.05$) in the rat co-administrated MEMP with carbaryl was found in the Group III rats while carbaryl alone treated rats showed (Group II) decreased the testosterone level. MEMP alone treated rats (Group IV) does not showed much significant difference as compared with control (Table2).

5. Histology

Histological appearance of testicular architecture of seminiferous tubules, sertoli cells with spermatogenesis of the control groups was normal (GI). The histological changes such as normal disturbances of the architecture of testicular tissues, necrotic spermatogonoium, loss of sperm bundles, pycnotic spermatogonia, scanty spermatocytes, disintegrated cyst wall, vacuoles in sertoli cells, giant cell formation were observed in carbaryl alone treated group (GII). On the other hand co-administration of MEMP to carbaryl treated rat provided a significant improvement of histological architecture with normal seminiferous and sertoli cells when compared to carbaryl alone group (GIII). MEMP alone treated rats showed the normal histological architecture pattern of seminiferous tubules with normal spermatogenesis (GIV). (Fig.1)





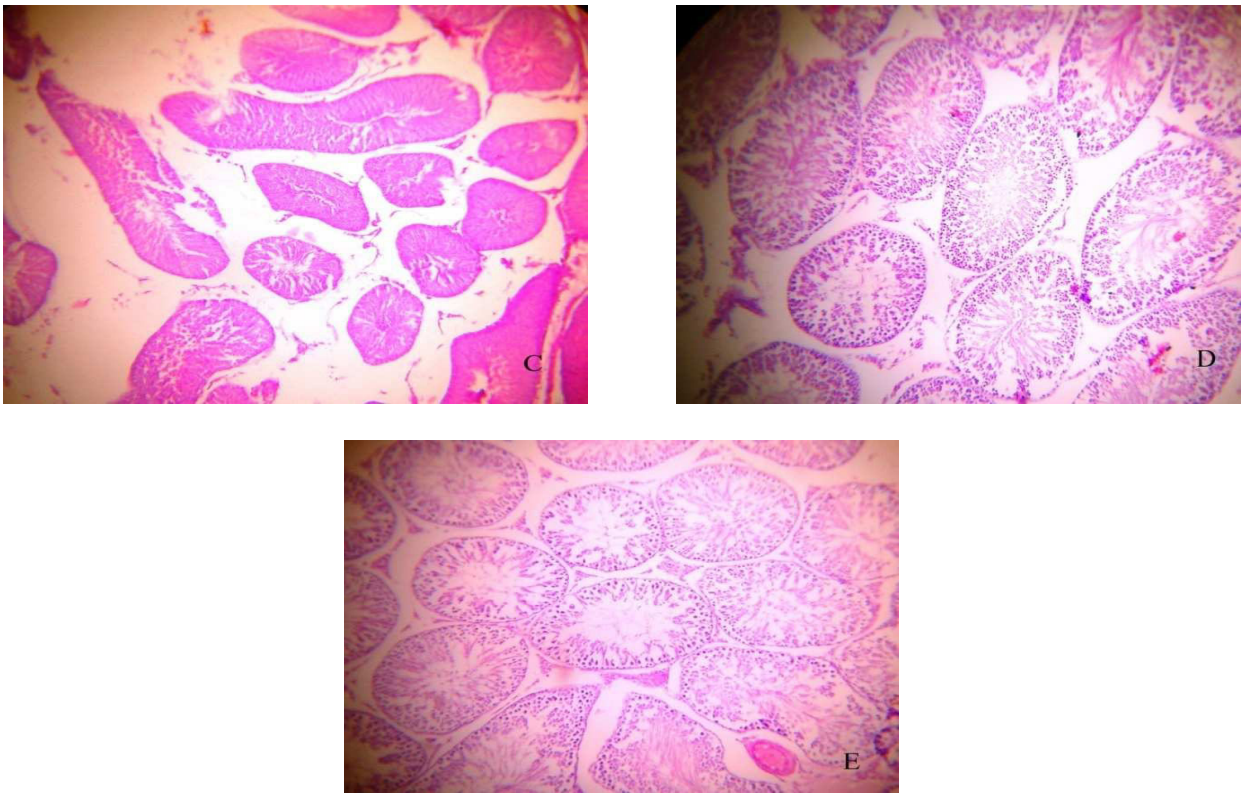


Figure 1

Figure 1 (A) Testis section of a control rat displaying normal histological architecture of seminiferous tubules, sertoli cells with spermatogenesis (H&E; 40 X). (B)and (C) section of carbaryl treated rat exhibits normal disturbances of the normal architecture of testicular tissues, necrotic spermatogonoium, loss of sperm bundles, pycnotic spermatogonia, scanty spermatocytes, disintegrated cyst wall, vacuoles in sertoli cells, giant cell formation(H & E, 40 X). (D) Testis section of carbaryl with co-administration of MEMP treated rat showing significant improvement of histological architecture with normal seminiferous and sertoli cells (H & E 40 X). (E) Testis section of MEMP alone treated rats showing the normal histological architecture pattern of seminiferous tubules with normal spermatogenesis (H& E. 40 X).

DISCUSSION

The present study was to investigate the protective effect of MEMP against the damage induced by the pesticide carbaryl. Many animal studies support the fact that carbaryl reaches the mammalian testes, seminal vesicle and prostate gland[12]. Previous report shows that carbaryl induces testicular changes in the seminiferous epithelium and semen evaluation also showed diminished sperm count and motility. Atrophy of the seminiferous tubule, cellular degradation also reported. Carbaryl exposure has also been shown to produce abnormal sperm[13]. Carbaryl adversely affects sperms and germinal cells by inhibiting growth and inducing cell death[14]. Many reports suggested that sperm DNA damage was related to semen quality ,fertilization and pregnancy[15-17]. Sperm chromosome aberrations were also reported to be associated with infertility, pregnancy loss, spontaneous abortions, and birth defects[18]. Accordingly, detecting whether carbaryl could induce spermatotoxic effects in exposed male workers, especially increase sperm abnormality and DNA damage, is necessary and helpful to illustrate the possible cause of carbaryl-induced adverse reproductive outcomes. Many clinical and experimental studies showed that pesticides impair male reproductive function by reducing semen quality, sperm concentration and motility [19-21].

The results obtained from the present study showed that the MEMP (GIII) gained significant body weight, when compared to the carbaryl alone group (GII). The increase in body weight of rats given methanolic extract can be attributed to androgenic activity of *Mucuna pruriens*. According to Clement *et* al[22] increasing bodies of evidence are developing for some plants, the plant extract can cause changes in general metabolic status, body and organ weight of the animals. Further the morphological study revealed increased testicular, seminal vesicle and epidermal weight along with increase in sperm count and motility. It was clear that the administration of MEMP enhances the spermatogenic potential, and the action of MEMP may be at hormonal level.

The present investigation also investigated the histological changes such as normal disturbances of the normal architecture of testicular tissues, necrotic spermatogonium, loss of sperm bundles, pycnotic spermatogonia, scanty spermatocytes, disintegrated cyst wall, vacuoles in sertoli cells, giant cell formation in carbaryl alone treated group (GII). These findings are in agreement with the previous reports [23]. The damages observed in the histological architecture of testis in this work may be elucidated with the direct or indirect effect of carbaryl; the latter induces lipid peroxidation that is a chemical mechanism capable of disrupting the structure and function of testis. On the other hand, co-administration of MEMP to carbaryl treated rat provided a significant improvement of histological architecture with normal seminiferous and sertoli cells when compared to Carbaryl alone group (GIII). MEMP alone treated rats showed the normal histological architecture pattern of seminiferous tubules with normal spermatogenesis (GIV). The histological reports strongly suggested that MEMP had the ability to regenerate the testicular architecture that damaged by the carbaryl.

Testosterone synthesis in Leydig cells and spermatogenesis in seminiferous tubules are the two energy requiring processes in testis. Testosterone secreted from Leydig cells acts on Sertoli cells in seminiferous tubules to create an environment with nutritional and hormonal factors in which normal progression of germ cells happens through spermatogenic cycle. Spermatogenesis in mammals is used as an important indicator of the chemical-induced toxicity on male reproduction [24].

In the correlation with previous result [25], in the present experiment also rat treated with MEMP (GIII) showed higher testosterone level. Perhaps *M. pruriens* increased the production of human growth hormone (HGH) and testosterone level. The increasing body weight can be attributed to the androgenic effect of the extract; with the correlation of one more finding suggested that the increased testosterone at adolescence can shift of the balance of sex steroid signalling to favour androgenic response[26]. In an earlier study, seeds of *M. pruriens* have been showed to significantly improve mating behaviour, libido potency and daily sperm production in streptozotocin induced diabetic male rats[27]. Phytochemically, seeds of *M. pruriens* possessed tannin, alkaloids, glycosides, saponine, steroid and L-Dihydroxy phenyl alanine (L-DOPA)[28-30&31]. Besides number of bioactive alkaloids (Mucunine, Mucunadine, Mucuadinine, Pruriendine) also reported in *M.pruriens*. L-Dopa is a remarkable precursor for the needed and beneficial neurotransmitter of dopamine which is present in pituitary. Dopaminergic neurons and dopaminergic pathway controlling sexual activities[32] and also stimulating the hypothalamus and pituitary to increase the level of Human Growth Hormone (HGH) in the body. This mechanism plays an important regulatory and modulator role in brain hypothalamic control of pituitary luteinizing hormone (LH) releases. These correlations strongly suggested when the plant contains rich in L-Dopa which helps to increase the testosterone level and helps to improve the spermatogenic and steroidogenic activity.

CONCLUSION

It is concluded that the results clearly showed that MEMP treatment improved the

spermatogenesis, thereby results in increased quality of spermatozoa production under carbaryl toxicity. The histological observation done in this study also suggested that the seeds of *Mucuna pruriens* have the ability to quench the testicular toxicity induced by carbaryl.

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