EFFICACY OF ANONNA MURICATA LEAF EXTRACT IN ALLEVIATION OF CYPERMETHRIN INDUCED TESTICULAR TOXICITY IN ALBINO RATS

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ABSTRACT: The chief object of this study was to determine the efficacy of *Anonna muricata* leaf extract in alleviation of cypermethrin induced testicular histology in albino rats. Wistar strain albino rats of 130-250 gms body weight (BW) were divided into six groups of five rats. Group I served as normal control. Group II given cypermethrin (CYP) (0.05 ml/100g BW/day) for 60 days and Group III given aqueous *Anonna muricata* leaf extract (AMLE) 0.05 ml/100g BW/day) for 60 days orally, while Group IVwas treated with Methotrexate (MTX) (1.0mg/100g BW/day) once a week intraperitoneally. Group V and Group IV given cypermethrin (0.05 ml), with supplementations of *A. Muricata* leaf (0.05 ml) and methotrexate (1.0 mg /100 gm BW/week) resp. for 60 days. At the end of experimental period all the animals were sacrificed and analysed for testis histology. On comparison with control group, there was no significant increase in BW of any of the treatment groups, except for a significant decrease in (MTX) alone given group. When compared to CYP treatment, no significant decrease in BW can be seen in both the AMLE as well as MTX supplemented groups. When compared to control, a significant decrease in weight of testis can be observed in all the treatment groups with the decrease being less in the CYP treated MTX supplemented group, On comparison with CYP treatment no significant change in the weight of testis is observed on the treatment with either AMLE of MTX alone or in AMLE supplemented group, but MTX supplementation seems to be effective in increasing the testis weight to a certain extent. CYP treated testis showed degenerative changes and vacuolation in germ cell layers with basement membrane damage and interstitial edema with atrophy of Leydig cells. AMLE treated testis showed seminiferous tubules with spermatogonia on basement membrane and Leydig cells in the interstitial region. MTX treated testis showed regular tubules with degenerating cells separating from basement membrane and rupture in certain regions along with lymphocyte infiltration. AMLE and CYP treated testis showed testicular damage being rectified by appearance of seminiferous tubules enclosing most of the stages of germ cells, and spermatids in the lumen, with slight vacuolations. CYP treated MTX supplemented testis showed

rectification of damage to seminiferous tubules part of the germinal stages, with disruption basement membrane and lymphocyte infiltration in interstitial spaces.

INDEX TERMS: Albino Rats, *Anonna Muricata* Leaf extract, Cypermethrin, Methotrexate, and testicular histology.

I. INTRODUCTION

Cancer is a major public health burden in both developed and developing countries. This is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. (WHO) Cancer cells usually invade and destroy normal cells. There are over 200 different known cancers that afflict humans. Many sources are known to increase the risk of cancer, including tobacco use, certain infections, radiation, lack of physical activity, obesity and environmental pollutions (Anand *et al.*, 2008). These can directly damage genes or combine with existing genetic faults within cells to cause the disease (Kinzler *et al.*, 2002). Approximately five to ten percent of cancers are entirely hereditary.

Nowadays herbal medicines are products from plants that have been widely used as complementary and alternative medicines for health promotion. Graviola is known by its scientific name, *Annona muricata* and belongs to the family Anonnaceae. It is called as "Soursop". It is used for antioxidant, anti-mutagenic agents, diabetic control, and anticancer management.

In the present study, male albino rats were chosen as the experimental model to ascertain the anticarcinogenic effect of AMLE in the current dose and duration. Another new finding that can be elicited from this study is the level of protection of testis obtained by treatment with the herb *Annona muricata* and also comparison with the effect of MTX, the known anticancer drug, from the destructive role of CYP as a model of cancer treatment.

II. MATERIALS AND METHODS

2.1 SELECTION OF THE ANIMAL MODEL

Wister strain albino rats weighing about 130-250 grams, which had comparable absorption, tissue absorption, metabolism and excretion of test comparable to that of human beings, were selected for the present study. The animals were housed in a well

ventilated, temperature and humidity controlled animal house, with a light schedule of fourteen hours and ten hours darkness and were fed with standard diet and drinking water made available at *libitum.*

2.2 PREPARATION OF CYPERMETHRIN

Cypermethrin were purchased from the M.Ramasamy Mudaliar and Sons Chemicals Co. CYP was dissolved in corn oil (6 unit cypermethrin and 6 unit groundnut oil) and dosage used is 0.05ml / 100 gm BW once in two days / rat)

2.2.1 PREPARATION OF *ANNONA MURICATA*LEAF EXTRACT

Hereby 60 gms of ground *ANNONA MURICATA* leaf powder was soaked in 400 ml of hot water (88°c) in water bath for 6 hours, then filtered by carbon silica cloth (150 micron) and the filtrate were stored in dark bottles in the refrigerator at (45°c). These procedures were repeated each week.

2.3 EXPERIMENTAL DESIGN

To achieve the ultimate goal of this study, healthy male albino rats were divided into 6 groups of 5 animals and received the following regimen of treatments.

GROUP I (C) - Animals received normal saline 1ml/100gm BW/week for 60 days and used as control.

GROUP II (CYP) – Animals were given CYP 0.05ml/100gm BW/week for 60 days orally.

GROUP III (AMLE) – Animals received AMLE 0.05ml/100gm BW/week for 60 days

GROUP IV (ME) – Animals injected MTX 1.0mg/100gm BW/day once a week intraperitoneally.

GROUP V (CYP+AMLE) – Animals received both cypermethrin (0.05ml/100gm BW/week) along with aqueous *Annona mricata* leaf extract (0.05ml/100gm BW/week) orally for 60 days.

GROUP VI (CYP+ME) – Animals received both CYP (0.05ml/100gm BW/week) orally along with MTX 1.0mg/100gm BW/day once a week injected intraperitoneally.

All the treatments were given between 9.30 to 10.30 am in the morning. At the end of the treatment protocol, animals were anesthetized with ether and sacrificed by decapitation. Blood was collected in both EDTA coated and uncoated tubes and stored properly. All animals were dissected and their testis were rapidly excised, washed with the saline, blotted with a piece of filter paper and weighed. A bit of tissue from the region of liver were fixed in 10% formalin and used for histological studies.

2.4 STATISTICAL ANALYSIS

Results obtained were tabulated. Statistical analysis was carried out using Dunnetts "t" test. Any significant variation between the control and treated groups were recorded.

III. RESULTS AND DISCUSSION

3.1 EFFECT ON BODY WEIGHT (TABLE 1)

The changes in body weight serve as a sensitive indicator of general health status of animals. Ghaffaire *et al.*, (2014) have reported about the significant decrease in body weight on cadmium treatment. Co administration of ethanolic extract of Tribulus terrestries or vitamin E with cadmium showed significant increase in body weight when compared to cadmium treated rats.

Malarvizhi (2017) has also expressed the reduction in body weight on cadmium chloride treatment and alleviation of this change to a certain extent on supplementation treatment with conjugated silver and gold nano particles. A general decrease in body weight of male rats treated by cypermethrin has been reported by El-Sheshtawy *et al.*, (2016).

Different pyrethroids have been reported to cause body weight change following their exposure to rats (Delport *et al.*, 2007). Lowering of food consumption may be due to toxic effect causing decreased intake and absorption of nutrients by gastrointestinal tract and thus altered efficiency of food conversion as observed Ball and Chhabra (1981). A varied response

of male and female rats to aqueous Annona muricata leaf extract has been observed by Arthur (2011).

In the present study, on comparison with control group, there was no significant increase in bodyweight of any of the treatment groups, except for a significant decrease in MTX alone given group. When compared to CYP treatment, no significant decrease in body weight can be seen in both the AMLE as well as MTX supplemented groups.

EFFECT ON TESTIS WEIGHT (TABLE 2)

Organ weight is a basic parameter for toxicological studies (Takahosbi and Bishi, 2001). Hypertrophy or reduction in weight of organs is the first-hand indications of toxicity of any chemical or biological substance. The decrease in organ indices like testiculo-somatic index, prostatic index and epididymal index on cypermethrin induction and their elevation on treatment with zinc has been reported by Das *et al*., (2016).

Fang *et al*.,(2013) have reported about the absence of significant difference in testicular and accessory organ weights in any of the treatment groups on comparison with control on cypermethrin administration. But El-Sheshtawy *et al*., (2016) have observed a significant dose dependent increase in testes and epididymis weight in male rats on oral administration of two doses of cypermethrin. As testicular and epididymal weights are a valuable index of reproductive health, accumulation of cypermethrin in reproductive organs and resultant decrease in organ weight on xenobiotic exposure may be due to reduced tubule size and decrease in number of germ cells (Choudhory *et al*., 2008).

As the testicular weight is generally dependent on the mass of differentiated spermatogenic cells, decrease in germ cell number and inhibition of subsequent process may be cause of testis weight reduction.

In the present study, when compared to control, a significant decrease in weight of testis can be observed in all the treatment groups with the decrease being less in the cypermethrin treated methotrexate supplemented group, On comparison with cypermethrin treatment no significant change in the weight of testis is observed on the treatment with either AMLE of MTX alone or

in AMLE supplemented group, but methotrexate supplementation seems to be effective in increasing the testis weight to a certain extent.

TABLE 1: EFFECT OF *ANNONA MURICATA AQUEOUS* LEAF EXTRACT ON BODY WEIGHTOFCYPERMETHRIN INDUCED ALBINO RATS

BODY WEIGHT	CONTROL	CYPERMETHRIN	AMLE	METHOTREXATE	CYP+AMLE	CYP+MTX
INITIAL	248±4.636	141.2±1.772	95.4±1.720	113±2.549	105.4±1.720	103±2.097
FINAL	321±3.316	188.2±0.860	138.4±1.630	104.4±2.315	148±2.549	136±1.370
% CHANGE	29.43	33.05	45.07	-7.61	40.4	32

Group I- Normal Control, Group II –Cypermethrin, Group III – Leaf Extract of *Annona muricata*, Group IV – Methotrexate, Group V – Cypermethrin+ Leaf Extract of *Annona muricata*, Group VI Cypermethrin+Methotrexate.

TABLE 2: EFFECT OF AQUEOUS *ANNONA MURICATA* LEAF EXTRACT ON TESTIS WEIGHT OF CYPERMETHRIN INDUCED ALBINO RATS

GROUPS	TESTIS WEIGHT (Grams)	ORGAN INDEX(Grams)
CONTROL	3.368±0.063	1.053±0.019
CYP	1.3±0.0170*	0.692±0.010*
AMLE	1.258±0.063*	0.963± 0.050
MTX	1.302±0.061*	1.252±0.060*
CYP + AMLE	1.226±0.055*	0.827±0.033*a
CYP + MTX	2.03±0.068*	0.961±0.007*bdes

Values are expressed as mean ± S.E.M of five rats.

Group I- Normal Control, Group II –Cypermethrin, Group III – Leaf Extract of *Annona muricata*, Group IV – Methotrexate, Group V – Cypermethrin+ Leaf Extract of *Annona muricata*, Group VI Cypermethrin+Methotrexate.

*Significance at 5% level of Group I Vs All groups, [a]Significance at 5% level of Group II Vs Group III, [b]Significance at 5% level of Group II Vs Group VI, [c]Significance 5% level of Group III Vs Group V, [d]Significance 5% level of Group IV Vs Group VI, [e]Significance 5% level of Group V Vs Group VI.

EFFECT ON TESTICULAR HISTOLOGY

Al-Shaikh (2013) has reported about the adverse effect of cypermethrin on testicular tissue and alleviation of this toxicity on vitamin E supplementation. Fang *et al.*, (2013) have reported about the distortion of seminiferous tubules, atrophy and deformed disordered arrangement of germ cells as well as impairment of Sertoli and Leydig cells on cypermethrin treatment in a dose dependent manner. A significant damage of testicular tissue on weekly treatment of 20mg/kg MTX when compared to control and post treatment with carvacrol was observed to reduce testicular damage (Daggulli *et al*., 2014).

Intraperitoneal injection of methotrexate caused a decrease of seminiferous tubular diameter, increased interstitial space as well as distorted the morphology of Leydig cells (Shreshthaet *et al*., 2007). Battan *et al., (*2015) have also observed the marked histopathological alterations in seminiferous tubules and Leydig cells on MTX administration.

Administration of curcumin protected testis of mice exposed to cadmium as evidenced by appearance of about 75% normal structures of seminiferous tubule of testis showing the ongoing process of spermatogenesis as evidenced by the presence of spermatids and spermatozoon, and lack of exfoliated cells in the luminal space (Singh *et al*., 2012).

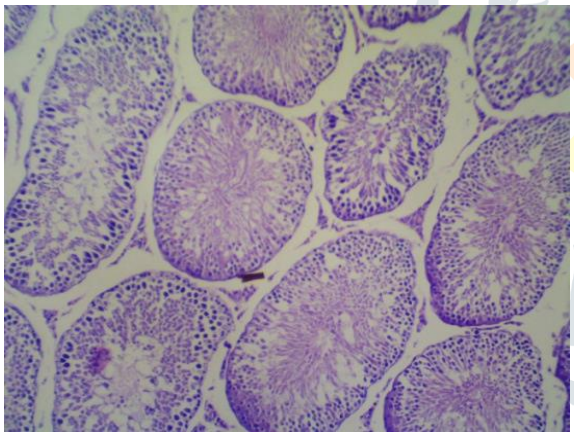
Testicular tissue of animals treated with Azathioprine revealed moderate degenerative changes in most seminiferous tubules. Vitamin C provided significant protection of testicular tissue and spermatogenesis when administered along with Azathioprine (Karawya and El-Nahas, 2006).This is in agreement with the finding of Das *et al*., (2002) who proved the testicular protection against cyclophosomide toxicity by vitamin C administration.

Aqueous Annona muricata leaf extract and cypermethrin treated testis showed testicular damage seen to be rectified by the leaf extract by appearance of seminiferous tubules enclosing most of the stages of germ cells, and spermatids in the lumen, while slight vacuolations are still evident. Cypermethrin treated methotrexate supplemented testis showeddamage to seminiferous tubules being rectified to a certain extent as testicular histoarchitecture is observed to have part of the germinal stages, while basement membrane is seen to be disrupted with lymphocyte infiltration in interstitial spaces.

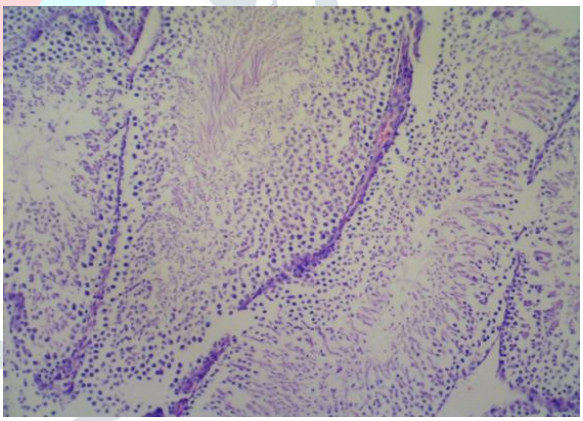
Thus, supplementation with methotrexate provided a near normal reversal from the cypermethrin induced damage even though vacuolations in the spermatogenic layers as well as slightly disorganized tubular epithelium, increase in interstitial space with appearance of edema as well as reduction in the number of spermatozoa. When compared to methotrexate extract supplementation, AMLE shows a positively better protecting ability, as partial revival of spermatogenesis is evident, thus proving its anticancer activity against cypermethrin.

The results of the present study, reveal that cypermethrin induced severe alterations in histopathological profile of testis as manifested by disarrangement of morphology in of Leydig cells and 100% seminiferous tubular damage within which spermatogonia, spermatocytes and differentiating spermatids were severely affected and were lost in the luminal space of the tubules culminating in total suppression of spermatogenesis, thus inducing azoospermia.

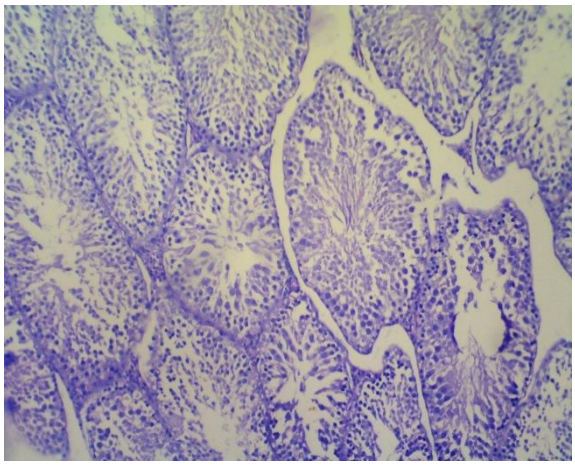
FIG 1: EFFECT OF *ANNONA MURICATA AQUEOUS* LEAF EXTRACT ON TESTIS HISTOLOGY CYPERMETHRIN INDUCED ALBINO RATS (FIG A, B, C, D, E, F)



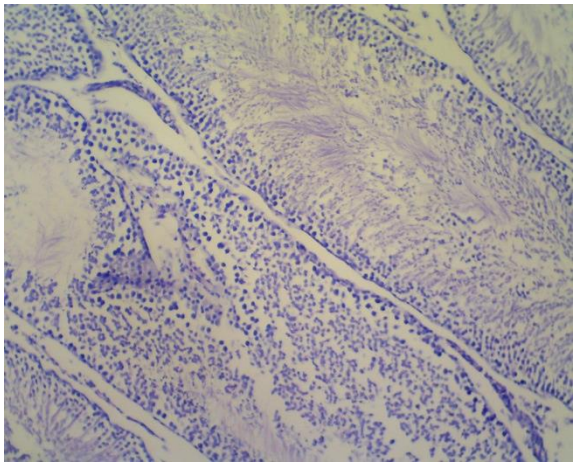
A. CONTROL TESTIS



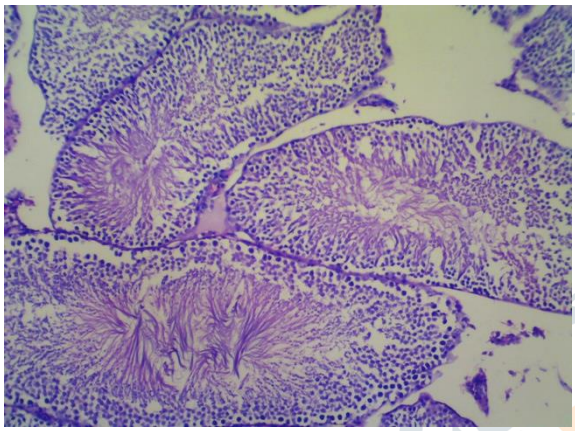
B. CYPERMTHRIN TREATED TESTIS



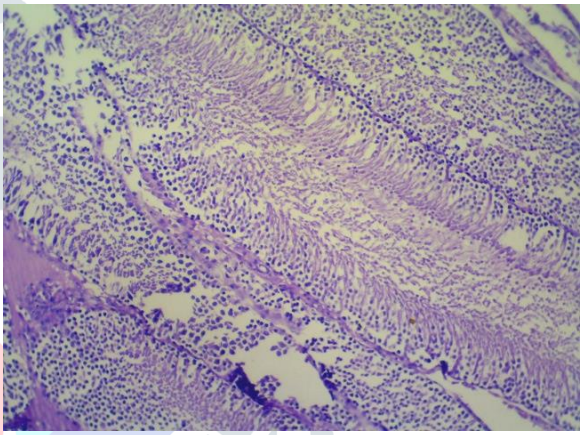
C. AMLE TREATED TESTIS



D. MTX TREATED TESTIS



E. CYP + AMLE TREATED TESTIS



F. CYP + MTX TREATED TESTIS

The results of present experiment also correlate well with other reports where cadmium has been shown to induce testicular damage in rat and mice, (Mathur *et al*., 2010). Our observations are also similar to the observation of Adamkovicova *et al*., (2010) where, similar to our results, cadmium chloride has been shown to cause rapid testicular edema, hemorrhage, necrosis, and degeneration of testicular membrane tissue.

V. CONCLUSION

The present study was undertaken to establish the testicular toxicity of the type - II pyrethroid cypermethrin and the possible alleviation of its toxicity by AMLE, as it is said to have anti tumor effects both *in vivo* and *in vitro* and its capacity in modulating the innate immune system. Cypermethrin was observed to bring about a reduction in body and organ weight as well as damaged the histoarchitecture of the testis. Supplementation with both AMLE and MTX seems to be beneficial, but AMLE seems to be the better alleviator of cypermethrin

toxicity than MTX. Perhaps, an increase in duration and should do the magic in proper reversal of induced toxicity.

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