

STUDY THE COMPARATIVE EFFECT BETWEEN *CYPERUS ESCULENTUS* SEEDS EXTRACT AND GENTAMICIN ON INDUCED ENDOMETRITIS IN MICE

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ABSTRACT

Objectives of this project were to study the effect of 60% crude alcoholic extract of the seeds of *Cyperus esculentus* induced endometritis in the mice. The plant of *Cyperus esculentus* was extracted by preparing Alcoholic extract 60%. One hundred microliters of saline containing *Escherichia coli* (10^6 cfu) was used to induce endometritis, by a single intracervically injection, and endometritis developed after 2 days from injection. The mice were divided into five groups, The first group were treated with alcoholic extract of *Cyperus esculentus* extract 150mg/kg body weight, the second group was treated with a daily 3mg per kg body weight of gentamicin given intra peritoneal, The third group was treated by 75mg/kg of *Cyperus esculentus* extract and 1.5mg/kg of gentamicin, The fourth group was treated by distilled water given orally by stomach tube, treatment of mice in these groups continued for 14 days while the fifth group was (negative control group). The results of the packed cell volume (P.C.V. %), hemoglobin (Hb)g/dL of mice in alcoholic extract group (T1) after 7 days of treatment showed significant improvement as compared with mice in induction group (T4) and after 14 days of showed no significant increase ($P \leq 0.05$) as compared with mice in control group (T5). While in gentamicin (T2) treated group after 7 and 14 days of treatment showed significant decrease ($P \leq 0.05$) as compared with mice in (T4) and (T5), furthermore the mice in alcoholic extract and gentamicin treated group (T3) after 7 and 14 days showed significant decrease as compared with (T5) in addition showed significant increase ($P \leq 0.05$) as compared (T2). Total white blood cell count and all differential white blood cell of alcoholic extract group (T1) after 7 days of treatment showed significant improvement as compared with (T4) and after 14 days of treatment showed no significant increase ($P \leq 0.05$) as compared with (T5). While the (T2) after 7 and 14 days of treatment showed significant decrease ($P \leq 0.05$) as compared with (T4) and (T5) while the eosinophil percentage showed significant increase ($P \leq 0.05$) as compared with mice in (T5). While (T3) after 7 days showed no significant important as compared with control group, but after 14 days of treatment showed significant decrease as compared with control group, in addition showed significant increase ($P \leq 0.05$) as compared with mice in (T2). the results of fertility index shown in alcoholic extract treated group (T1) and alcoholic extract and gentamicin treated group (T3) was increased as compared gentamicin treated group (T2). The results of body weight and uterine weight showed significant increase ($P \leq 0.05$) in alcoholic extract treated group as compared with control group and mice in gentamicin group. After 7 and 14 days of induction with *E. coli* intrauterine infection revealed presence of inflammatory cells within the endometrial glands and surrounding glands, glandular epithelium is degenerating and the lumen contains cellular debris, while the groups that treated with both extract and gentamicin showed cellular debris within the glandular endometrium. group treated with the extract for 14 days noticed cellular debris within the glandular endometrium and few inflammatory cells around the endometrial glands. The group treated with Gentamicin for 14 days of induction showed also degenerating of glandular epithelium and aggregates of inflammatory cells surrounding degenerating glands and presence of hemosiderin around glands, the control group showed normal uterine tissue.

1. INTRODUCTION

Many disease were treated by administration the herbs and medical plant, *Cyperus esculentus* contained high percentage of alkaloids, saponins and tannins are known to have antimicrobial activity, as well as other physiological activities (1; 2). *Cyperus esculentus* was reported as healthy and helps in preventing heart, thrombosis and activates blood circulation and are responsible for preventing and treating urinary tract infections and other bacterial infections (3). The *Cyperus esculentus* is rich in energy content (starch, fat, sugars and protein), mineral (phosphorus, potassium) and vitamins E and C (4). The researches indicated that the plant is play an important role in enhancement fertility so it might be improve reproductive system maturity (5), in addition the plant use for treating infections such as endometritis caused by bacteria (3). Endometritis was defined as inflammation of the endometrium (uterine lining), the most common symptoms are abdominal distention or swelling, abnormal vaginal discharge, fever, uterine pain (6) to treat endometritis we may use antibiotic e.g. tetracycline or gentamicin and this groups cause side effect like nephrotoxicity, anemia and decrease immunity (7), because of many studies have been found various constituents of *Cyperus esculentus* exhibit a variety of therapeutic effect with little or no

associated toxicity so this herb was considered as potential therapeutic effect to treat endometritis without side effect (8). This study was aimed to treat of induced endometritis in mice by using 60% crude alcoholic extract of *Cyperus esculentus* and to compare between treatment of endometritis of the *Cyperus esculentus* and antibiotic (gentamicin) in addition to reduce the side effect of gentamicin by adding the alcoholic extract.

2. MATERIAL AND METHODS

Extraction of *Cyperus esculentus* nut:

The nuts were cleaned from soils and dust and dried in shadow in room temperature and then converted to powder by grinder and then kept in plastic sac. hundred grams of the powder was dissolved in 200 ml of 60% methanol (v/v) and put in flask. The flask was placed on hot plate magnetic stirrer. The temperature of extraction was 40-45°C. The solution was left stirring for 72 hours and then sieved by using steriagaze to get rid of coarse particles. The solution then filtered through Whatmann filter. The filtrate was poured in clean and sterile petridishes (12x2 cm) and kept in incubator at temperature of 45°C until dryness (9).

Preparation of stock solution for the extract of the plant and gentamicin :

150mg of the dried extract were dissolved in distilled water until complete the volume to 10 ml to make a concentration of 15mg/ml, 0.1 ml of the stock solution was given to each 10gm mouse (5). Stock solution of 0.3mg/ml of gentamicin was prepared from ampoule of 40mg/ml.

Experimentally Animals: fifty seven albino Swiss mice weighting 30g were obtained from the animal house of Biotechnology central-Al-Nahrain university. Mice were housed plastic cage 30x10x10 cm placing in the room until the beginning of experiments. Standard rodent diet (commercial feed pellets) and Tap ad.lib. Water was freely available.

Induction of estrus cycle: After isolated grouped females for period 4-5 days from males (synchronization), the grouped females are exposed to male; the majority is stimulated into estrus with a high percentage occurring on the third day (10). Vaginal smear was taken to ensure and detect estrus cycle.

Induction of endometritis: Mice were placed in a dorsal supine position and restrained with paper tape, and the perineal area was washed with 70% isopropanol. One hundred microliters of saline containing *Escherichia coli* (10^4 cfu), LPS (5 or 7.5 mg/kg), or sterile saline (vehicle), was injected intracervically by using a needle, attached to the syringe was guided through the vagina and visually advanced 3 mm into the cervix. After two days from injected of bacteria group of mice were killed and endometrial swabs were inoculated onto MacConkeys agar to confirm the presence of an *Escherichia coli* infection (11).

Experimental designs : fifty seven mice were divided into five groups (15 mice in each one), the period of treatment in all groups were (1-7) days and (1-14) days :

- 1- The first group (T1) was treated by daily dose of 150mg/kg body weight of *Cyperus esculentus* extract. given orally by stomach tube
- 2- The second group (T2) was treated with a daily 3mg per kg body weight of gentamicin given intra peritoneal.
- 3- The third group (T3) was treated by 75mg/kg of *Cyperus esculentus* extract and 1.5mg/kg of gentamicin.
- 4- The fourth group (T4) was treated by distilled water given orally by stomach tube.
- 5- The fifth group (T5) was (negative control group).

Parameters used in this experiments:

Blood tests :

- **Haemoglobin test :** the procedure was according to the method mentioned by (12) .
- **Packed Cell Volume (P.C.V.) :** this is according to the method mentioned by (13) .
- **White Blood Cells Counting :** the procedure was according to the method mentioned by colse, (14) .
- **Differential Counting of WBCs :** The method is mentioned by (14) .

Fertility index: parameter was calculated according to (15):

Fertility index = total numbers of females pregnant ÷ total numbers of females mated x 100.

Body weight and uterus weight:

The body weight of females was measured at first day before dosage and after dosage for 14 days by using a balance. In addition the uterus was measured after dosage.

Histological study: all groups were taken the parts from uterus after killed it. These samples were taken for histological study and these were kept in 10% formalin solution until the time of sections (16). The sections were worked in the dental medicine college, university of Baghdad.

Statistical analysis: the ready program SAS (17) was used in statistical analysis for study the effect of different treated in adjective studies and the significant between medium was compared with less significant LSD.

3. RESULTS**Hematological parameters:**

The hematological parameters are shown in table (1), The results of the packed cell volume (P.C.V.)%, hemoglobin (Hb)/dL of mice in alcoholic extract group(T1)after 7 days of treatment showed significant improvement as compared with mice in induction group (T4) and after 14 days of showed no significant increase ($P \leq 0.05$) as compared with mice in control group (T5). While in gentamicin treated group (T2) treated group after 7 and 14 days of treatment showed significant decrease ($P \leq 0.05$) as compared with mice in induction group (T4) and control (T5), furthermore the mice in alcoholic extract and gentamicin treated group(T3) after 7 and 14 days showed significant decrease as compared with control (T5) in addition showed significant increase ($P \leq 0.05$) as compared gentamicin treated group (T2).

Total white blood cell count and all differential white blood cell of alcoholic extract group(T1)after 7 days of treatment showed significant improvement as compared with induction (T4) and after 14 days of treatment showed no significant increase ($P \leq 0.05$) as compared with control (T5). While the gentamicin treated group (T2)after 7 and 14 days of treatment showed significant decrease ($P \leq 0.05$) as compared with induction group (T4) and control (T5) while the eosinophil percentage showed significant increase ($P \leq 0.05$) as compared with mice in control group (T5). While extract and gentamicin treated group (T3)after 7 days showed no significant important as compared with control group, but after 14 days of treatment showed significant decrease as compared with control group, in addition showed significant increase ($P \leq 0.05$) as compared with mice in gentamicin treated (T2).

Table(1): Effect of alcoholic extract of *Cyperus esculentus*, gentamicin, *Cyperus esculentus* extract with gentamycin and distilled water on P.C.V., Hb and total WBCs count :

(A) : after 7 days of the treatment.

groups	P.C.V.	Hb	WBCs	N%	L%	M%	E%	B%
T1	31.4 ± 0.34 c	11.0± 0.20 c	7011 ± 7.66 c	59.7 ± 0.59 c	39.1 ± 1.40 c	5.4 ± 0.51 c	3.2 ± 0.20 ab	0.8 ± 0.27 c
T2	26.5 ± 2.73 d	8.6± 0.47 d	5311 ± 8.42 d	53.2 ± 0.50 d	34.3 ± 1.90 a	4.0 ± 0.56 a	3.4 ± 0.31 cb	0.5 ± 0.23 d
T3	28.0 ± 2.10 b	10.0 ± 0.36 e	6311 ± 9.03 a	54.0 ± 2.21 d	36.6 ± 1.81 a	5.1 ± 0.70 a	3.2 ± 0.40 ab	0.7 ± 0.28 a
T4	28.8 ± 0.96 b	9.5 ± 0.39 b	8301 ± 8.91 b	62.5 ± 1.50 b	42.8 ± 0.32 b	6.4 ± 0.82 b	3.3 ± 0.28 b	1 ± 0.29 b
T5	34.2± 1.40 a	12.0± 0.31 a	6210 ± 12.58 a	56.7 ± 0.67 a	35.2 ± 0.51 a	4.7 ± 0.31 a	3.1 ± 0.26 a	0.6 ± 0.26 a

(B): After 14 days of the treatment.

Groups	P.C.V.	Hb	WBCs	N%	L%	M%	E%	B%
T1	35.0 ± 0.52 a	12.3 ± 0.38 a	6241 ± 8.92 a	56.9 ± 0.58 a	35.0 ± 1.04 a	4.1 ± 0.55 a	3.0 ± 0.21 a	0.6 ± 0.25 a
T2	25.0 ± 2.59 c	7.8 ± 2.73 c	4820 ± 8.10 c	51.9 ± 0.52 c	31.1 ± 1.80 c	3.4 ± 0.52 c	3.8 ± 0.38 c	0.4 ± 0.22 c
T3	32.9 ± 2.10 d	11.5 ± 2.91 d	5924 ± 9.29 d	54.8 ± 1.90 d	33.0 ± 1.73 d	3.9 ± 0.77 d	3.6 ± 0.40 d	0.5 ± 0.23 c
T4	28.1 ± 0.90 b	9.3 ± 0.46 b	8704 ± 7.96 b	62.1 ± 1.01 b	41.9 ± 0.26 b	6.3 ± 0.71 b	3.3 ± 0.27 b	1 ± 0.27 b
T5	35.4 ± 1.63 a	12.7 ± 0.42 a	6121 ± 12.49 a	56.3 ± 0.57 a	35.9 ± 0.52 a	4.5 ± 0.30 a	3.1 ± 0.24 a	0.6 ± 0.21 a

*T1= treated with *cyperus esculentus* extract

*T2=treated with gentamicin

*T3=treated with *cyperus esculentus* extract and gentamicin

*T4=treated with distilled water

*T5=control group (negative)

Fertility index

The result shown in table (2), Fertility index the result shown in table (2), the fertility index of mice in alcoholic extract treated group (T1) and mice in alcoholic extract and gentamicin treated group (T3) was increased as compared mice in gentamicin treated group (T2).

Table (2): Effect of alcoholic extract of *cyperus esculentus*, gentamicin, *Cyperus esculentus* extract with gentamicin and distilled water on fertility index (%) after 14 days of treatment:

Group	T1	T2	T3	T4	T5
Fertility index	95%	30%	70%	0%	100%

Body weight and uterine weight

The result in table (3) showed significant increase ($P \leq 0.05$) in body weight and uterine weight of mice in alcoholic extract treated group as compared with mice in control group and mice in gentamicin group.

Table (3): Effect of alcoholic extract of *cyperus esculentus*, gentamicin, *Cyperus esculentus* extract with gentamicin and distilled water on body weight and uterus weight after 14 days of treatment:

Group	T1	T2	T3	T4	T5
Body weight	33.308 ± 0.70 c	27.533 ± 0.73 d	29.051 ± 0.74 a	25.752 ± 0.75 b	30.102 ± 0.70 a
Uterus weight	0.141 ± 0.0022 c	0.060 ± 0.0012 d	0.080 ± 0.0011 a	0.057 ± 0.0017 b	0.082 ± 0.0024 a

*T1= treated with *cyperus esculentus* extract

*T2=treated with gentamicin

*T3=treated with *cyperus esculentus* extract and gentamicin

*T4=treated with distilled water

*T5=control group (negative)

Histopathological changes:

After 7 and 14 days of induction with E.coli intrauterine infection revealed presence of inflammatory cells within the endometrial glands and surrounding glands, glandular epithelium is degenerating and the lumen contained cellular debris (Fig.1,2), in (Fig.3) group treated with the extract for 14 days noticed cellular debris within the glandular endometrium and few inflammatory cells around the endometrial glands. The group treated with gentamicin for 14 days of induction showed degenerating of glandular epithelium and aggregates of inflammatory cells surrounding degenerating glands and presence of hemosiderin around glands in (Fig.4). while the groups that

treated with both extract and gentamicin showed cellular debris within the glandular endometrium (Fig.5). the control group showed normal uterine tissue in (Fig.6).

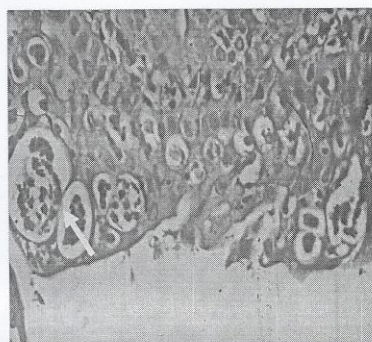


Fig 1 : Histopathological section in uterine of mouse infected with *E.coli* for one week showed distinguished by variable numbers of inflammatory cells within the endometrial glands. Glandular epithelium is degenerating and the lumen contains cellular debris (→). (H&EX400).

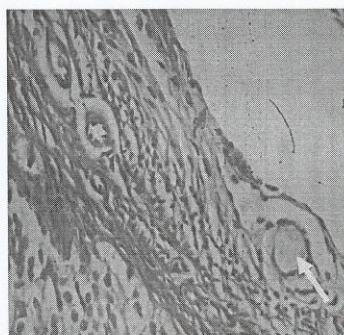


Fig 2 : Histopathological section in uterine of mouse infected with *E.coli* for 2 weeks showed dense aggregates of inflammatory cells surrounding glands (→), glandular epithelium is degenerating and the lumen contains cellular debris (→). (H&EX400).

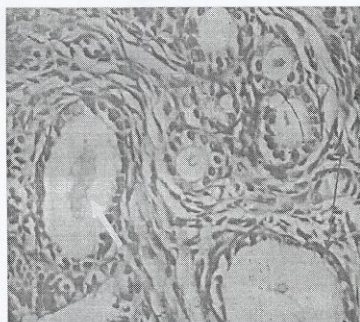


Fig 3: Histopathological section in uterine of mouse treated with the extract for 14 days showed cellular debris within the glandular endometrium (→) and few inflammatory cells around the endometrial glands (↔). (H&EX400)

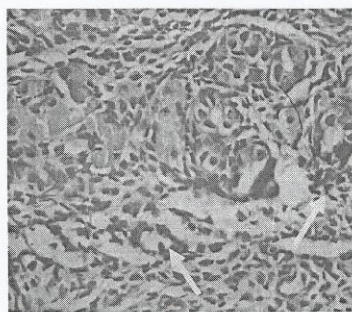


Fig 4: Histopathological section in uterine of mouse treated with gentamicin after one week of induction showed degenerating of glandular epithelium and aggregates of inflammatory cells surrounding degenerating glands (→) and presence of hemosiderin around glands (↔). (H&EX400).

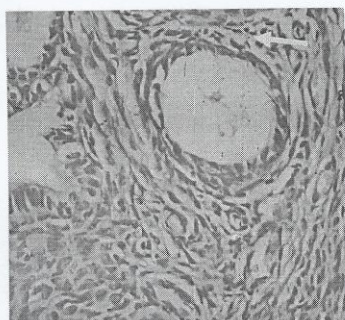


Fig 5: Histopathological section in uterine of mouse treated with the extract and gentamicin showed few inflammatory cells around the endometrial glands (→) (H&EX400).

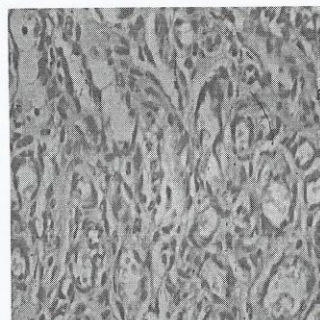


Fig 6: Histopathological section in uterine of control mouse showed normal uterine tissue. (H&EX400).

4. DISCUSSION

The extract of *Cyperus esculentus* may have active constituents to inhibit inflammatory process with low toxicity as compared with antibiotics (35), in addition the extract does not need sophisticated apparatus and complicated procedure, for the above mentioned reasons *Cyperus esculentus* was chosen to treat endometritis. The alcoholic extract of seeds of plant was dried and 12% of extract was used, as dark brown this result agreed with (9).

In this study, injection *Escherichia coli* (10^6 cfu) intracervically to induce endometritis cause infection of uterus appeared when inoculated endometrial swabs onto MacConkeys agar as compared with control group, this results agreed with results reported by (11).

After 7 and 14 days of endometritis induction the blood picture showed decrease in Hb and P.C.V. with increase in total white blood cells count and differential white blood cell might indicate that erythrocytes are being affected or destroyed with the infection to an important cell involved in the immune response lead to bacterial toxins circulating in blood results in some deformities in the shape of red cells so that it is entrapped in the spleen network and the animals go in regenerative anemia, the infection produces more white blood cells and differential cell as immune competent cells due to invade the bacteria the body and is stimulated the bone marrow to produce large number of white blood cell and the blood rise dramatically (18). Also the presence of bacteria stimulate the immune system to produce more phagocytic cells such as neutrophils and monocytes (19).

The hematological parameters, total and differential white blood cells, hemoglobin and packed cell volume rate of mice in alcoholic extract group showed no significant difference as compared with control group this might be attributed to nutritional adequacy of the tuber extract and they did not indicate malabsorption or under nutrition (20). Other research had earlier recorded a strong influence of food components on hematological traits packed cell volume and hemoglobin concentration being very strong indicators of nutritional status of animals (21). It is well known that various antinutritional substances and xenobiotic could cause hemolysis, nutrients malabsorption and abnormal haemopoiesis which could arise from liver damage (22). The result of the total and differential white blood cell count indicate that the animals were healthy because decrease in number of white blood cells is an indication of allergic conditions, anaphylactic shock and certain parasitism while elevated value indicate to the existence of a recent infection, usually with bacteria (23).

The decrease in Hb, P.C.V., total WBCs and some differential white blood cell as compared with control group might be attributed to that gentamicin caused leukopenia, anemia, and decreased reticulocyte counts, thrombocytopenia, immunologic thrombocytopenia, and suppress or inhibit suppressor immunocytes by the inhibition of phospholipases activities accumulates phospholipids and formation of lysosomal myeloid bodies (24). Gentamicin inhibits mitochondrial respiration and Ca^{2+} transport or lipid peroxidation that causes irreversible cell damage. Gentamicin was shown to chelate mitochondrial iron to catalyze the formation of free oxygen radicals, forming a very oxidant Fe (II)-gentamicin complex capable of causing cell death (25), while the blood picture of mice in gentamicin and alcoholic extract showed improvement as compared with gentamicin add alone this might be indicated to nutritional benefits of tiger nut on the immune cells in health and diseases by exhibits selective effects

on T lymphocytes, that was it inhibits the proliferation of the inflammatory lymphocytes, but increases the proliferation of naive lymphocytes (26).

Fertility index: the increase fertility of mice in alcoholic extract group and in alcoholic extract with gentamicin group as compared with mice treated with gentamicin alone this might attributed to the seeds of *Cyperus esculentus* established as a very nutritious (27), rich mineral content especially vitamin E, phosphorus and potassium, oil resistance to peroxidation and fatty acid (palmitic acid, stearic acid, oleic acid and linoleic acid), alkaloids that prolonging the action of cAMP, they also affect glucagons and thyroid stimulating hormones, saponins and tannins are known to have antimicrobial activity, as well as other physiological activities (1; 2). The extract play an important role in enhancement fertility so it may be improve reproductive system maturity (5), while the gentamicin lead to inhibits spontaneous as well as oxytocin and PGF₂-induced contractions of myometrium, this may be of importance considering the potentially negative effect of gentamicin sulfate on uterine with puerperal endometritis, resulting in impairment of fertility performance (28). While the fertility index of induction group might attributed to the bacterial products had adverse effect on the reproductive function via suppressed pituitary LH secretion and perturb postpartum ovarian follicular growth, function and ovulation (29). The endometritis delays uterine involution and perturbs fertility (30). Thus, uterine disease is associated with lower conception rate, induce failure to conceive (31).

Body weight and uterine weight: the increase in body weight and uterine weight might attributed to the nutrient rich extracts of the tigernut tuber extract might have allowed proper absorption of the nutrients which have allowed proper utilization of the nutrients. Low level of active/toxic principles may have stimulated appetite and increased feed utilization resulting in increased weight gain. (32).

While the gentamicin suggested cause decrease in final weight gain percent, food intake, food efficiency ratio (FER) and protein efficiency ratio (PER). It has been demonstrated that gentamicin reduced cellular protein synthesis and total cellular proteins synthesis was inhibited to the same extent as brush border membrane protein synthesis in addition the inhibition of phospholipid degradation was quantitatively the major contributor to the effects of gentamicin on phospholipid metabolism (24).

Histopathological change: After one and two weeks of induction with *E. coli* intrauterine infection revealed presence of inflammatory cells within the endometrial glands and surrounding glands may result from the bacteria stimulated the immune system to produce more phagocytic cells such as neutrophils and monocytes (19). In addition that inflammatory diseases are associated with enhanced oxidative reactions and reduced antioxidant defense capabilities lead to initiate tissue damage, these results are in agreement with (33).

Whereas after studying histopathological change of uterine mice in the extract group for 14 days noticed cellular debris within the glandular endometrium and few inflammatory cells around the endometrial glands this may regarded to that extract contained alkaloids, saponins and tannins are known to have antimicrobial activity, as well as other physiological activities (1; 2). Saponins have been reported to be useful in reducing inflammation; tannins have astringent properties that hasten the healing of wounds and prevention of decay. Tannin compounds have antimicrobial activities and are responsible for preventing and treating urinary tract infections and other bacterial infections (34).

The tissue section of uterine mice in the gentamicin group showed degenerating of glandular epithelium and aggregates of inflammatory cells surrounding degenerating glands might attributed to gentamicin is bactericidal and acts by inhibiting protein synthesis in susceptible bacteria. Cell death results, furthermore it cause inhibition of phospholipase activities accumulates phospholipids and formation of lysosomal myeloid bodies (24). Furthermore it inhibits mitochondrial respiration and Ca²⁺ transport or lipid peroxidation that causes irreversible cell damage and chelate mitochondrial iron to catalyze the formation of free oxygen radicals, forming a very oxidant Fe (II)-gentamicin complex capable of causing cell death (25).

The histopathological change of groups that treated with both extract and gentamicin might be indicated to effect of extract in reduce side effect of gentamicin by it possess some biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine. These phytochemicals exhibit diverse pharmacological and biochemical actions when taken by animals (35).

5. REFERENCES

- [1]. Sofowora, E.A. (1993). Medicinal plants and traditional medicine in Africa. Ibadan-Owerri-Kaduna-Lagos Spectrum Books Limited, pp: 159-238.
- [2]. Evans, N.S. (2005). Trease and Evans. Pharmacognosy, 15th Ed., Elsevier, India, pp: 1-24.

- [3]. Adejuyitan, J.A.; Otunola E.T.; Akande, E.A.; Bolarinwa, I.F. and Oladokun, F.M. (2009). Some physicochemical properties of flour obtained from fermentation of tigernut (*Cyperus esculentus*) sourced from a market in Ogbomoso, Nigeria. *Afr. J. Food Sci.*, 3: 51-55.
- [4]. Belewu, M.A. and Belewu, K.Y. (2007). Comparative physicochemical evaluation of tigernut, soybean and coconut milk sources. *Int. J. Agric. Biol.*, 5: 785-787.
- [5]. Almashhadani, A. A.M. and Al Essawe, M. A. (2010). The Effect of *Cyperus esculentus* on sperm function parameters in prepubertal mice as a model for Human. *Journal of Baghdad for Science* 7: 389-393
- [6]. Hubert, G.; Baggish, M. S.; Valle, R. H. (2007). Hysteroscopy: visual perspectives of uterine anatomy, physiology, and pathology. Hagerstown, MD: Lippincott Williams & Wilkins. p. 488.
- [7]. Sundin, D.P.; Sandoval, R. and Molitoris, B.A. (2001). Gentamicin inhibits renal protein and phospholipid metabolism in rats: Implications involving intracellular trafficking. *J. Am. Soc. Nephrol.* 12:114-123.
- [8]. Shilenko, M.; Kalacheva, G.; Lisovskii, G. and Trubachev, I. (1979). Chufa (*Cyperus esculentus*) as a source of vegetable fat in seal life-support system. *Kosmobiol. Aviakosm. Med.* 13:70-74.
- [9]. Monago, C.C. and Uwakwe, A.A. (2009). Proximate composition and *in-vitro* anti sickling property of Nigerian *Cyperus esculentus* (tiger nut sedge) Trees for Life Journal a forum p.55.
- [10]. Whitten, W.K. (1956). Modification of the oestrous cycle of the mouse by external stimuli associated with male. *J. Endocrinol.* 13:399-404.
- [11]. Leonid, L.; Reznikov, G.; Fantuzzi, L.; Craig, H.; Selzman, K.; Brian, D.; Shames, Hazel A.; Barton, L.; Hobart Bell, James, A.; McGregor, J. and Charles A. D. (1999). Utilization of endoscopic inoculation in a mouse model of intrauterine infection-induced preterm birth: Role of Interleukin 1 β . *Biology of reproduction* 60, 1231-1238.
- [12]. Varley, H.; Gonlock, A. and Bell, M. (1980). *Practical clinical biochemistry* 5th ed. Williu Heinemann. Medical books. Ltd. London.
- [13]. Archer, R.K. (1985). *Hematological techniques for use on animals*. Oxford; Blackwell, Scientific publication.
- [14]. Coles, E.H. (1974). *Veterinary clinical histology*. 2nd ed. W.B. Saunders company.
- [15]. Reshu, M. and Patwant, K. (2007). Antifertility effect of melia azadirachlin (dharek) seed extract in female albino rat. *Indian Journal of experimental biology*. 45: 853-860.
- [16]. Luna, L.G. (1968). *Manual of histologic staining methods of the armed .Institute of pathology*. 3rd., McGraw-Hill Book company, N.Y., Toronto, London, Sydney; 12-31
- [17]. SAS institute. SAS/ATAT, (2001) Guide for personal computers version, 9th Ed. SAS instyiteinc; Cary NC, USA.
- [18]. Dacie, J.V. and Lewis, S.M. (1994). *Practical Haematology* 8th ed. Churchill Livingstone Edinburgh.
- [19]. Jain, N.C. (2000). *Schalm's Veterinary Hematology*. 5th Edn. Lee and Febiger, Philadelphia, USA.
- [20]. Church, J.P.; Judd, J.T.; Yomg, C.W.; Kebay, T.L. and Kim, W.W. (1984). Relationship among dietary constituents and specific serum clinical components of subjects eating self-selecting diets. *Am. J. Clin. Nutr.*, 40: 1338-1344.
- [21]. Hackbath, H.; Buron, K. and Schimansley, G. (1983). Strain differences in inbred rats: influence of strain selection and diet on haematological traits. *Laboratory Anim.*, 17: 7-12.
- [22]. Chubb, L.G. (1982). Recent advances in animal nutrition. W. Harvesign Butterworths, London, pp: 21-37.
- [23]. Ahamefule, F.O.; Obua, B.E.; Ukwani, I.A.; Oguike, M.A. and Amaka, R.A. (2008). Haematological and biochemical profile of weaner rabbits fed raw and processed pigeon pea seed meal based diets. *Afr. J. Agric. Res.*, 3: 315-319.
- [24]. Kaloyanides, G. (2000). Aminoglycoside induced functional and biochemical defects in the renal cortex. *Fundam. Appl. Toxicol.*, 4: 930-943.
- [25]. Dominguez, J.H.; Hale C.C. and Qulali, M. (1996). Studies of renal injury, gentamicin toxicity and expression of basolateral transporters. *A.J.P.-Renal Physiol* 2: 245-253.
- [26]. Mokady, S.H.; Dolev, A. (1970). Nutritional evaluation of tubers of *Cyperus esculentus* L. *J. Sci. Fd. Agric.* 21:211-14.
- [27]. Paigen, B.; Morrow, A.; Holmes, P.A.; Mitchell, D. and Williams, R.A. (1987). Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis*. 68(3):231-40.
- [28]. Halis, O.; Murat, Y. and Ahmet, A. (2004). Effects of gentamicin sulfate on the contractility of myometrium isolated from non-pregnant cows. *Animal Reproduction Science* 84: 269-277.
- [29]. Moghaddam, A.A.I. and Mamoei, M. (2004). A survey on some of the reproductive and productive traits of the buffalo in Iran, 23rd World Buiatrics Cong. Qu. and Eacute. be, pp: 1910
- [30]. Foldi, J.; Kulcsar, M.; Pecsai, A.; Huyghe, B. De Sa, C.; Lohuis, J.A.; Cox, P. and Huszenicza, G.Y. (2006). Bacterial complications of postpartum uterine involution in cattle. *Animal Reproduction Science*, 96: 265-281.
- [31]. Sheldon, I.M. and Dobson, H. (2004). Postpartum uterine health in cattle. *Animal Reproduction Science*, 82: 295-306.
- [32]. Belewu, M.A. and Abiodun, A.O. (2006). Preparation of kuunu from unexploited rich food source, Tigernut (*Cyperus esculentus*). *Pak. J. Nutr.* 7: 109-111.
- [33]. Behiman, H.I.; Kodman, P.H.; Preston, S.L. and Gao, S. (2001). Oxidative stress and the ovary. *J. Soc. Gynecol. Investigations*, 8: 540-542.
- [34]. Frantisek, S.S. (1991). *The natural guide to medicinal herbs and plants*. Tiger Barks Cast, Twinkemhan, United kingdom, pp: 1-5.
- [35]. Amadi, B.A.; Ibegbulem, C.O. and Egbebu, A.C. (2006). Assessment of the effect of aqueous extract of (*Asimnatriloba*) root on organ weights and liver function of albino rats. *Int. J. Nat. Appl. Sci.*, 2: 79-81.