

The protective effects of ginseng plant extract (ginsana) and garlic powder against the bad effects of lambda-cyhalothrin insecticide on kidneys of female rats

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ABSTRACT

Ginseng and garlic are well known plants which have anti oxidant effects. The present study investigates the protective effects of ginseng extract and garlic powder on the bad effects of lambda-cyhalothrin insecticides (LTC). Eighty female Wister rats divided into 4 groups as follow; group (1) control group, group (2) injected with(9.3 mg/kg b.wt) LTC alone, group (3) injected with(200 mg/kg b.wt) ginseng extract (G115) followed by (9.3 mg/kg b.wt) LTC, group (4) injected with (100 mg/kg b.wt) garlic extract (GA) followed by (9.3 mg/kg b.wt) LTC All groups were injected intraperitoneally for 15 days (as a short term) and 21 days (as along term).

Histological examination of the kidney sections showed severe congestion, besides cloudy swelling in the proximal and distal convoluted tubules, vacuolar degeneration with necrosis. In addition to edema in the bowman's space, hypercellularity and destruction with renal casts detected in some of renal tubules in animal injected with LTC only for 15 days. While rats injected with LTC for 21 days revealed thickening and edematous in the wall of the blood vessels with proliferation in the endothelial cells, besides thrombosis and hemorrhage. Chronic interstitial nephritis in most rats and perivascularitis were detected. Kidneys of rats pretreated with ginseng followed by LTC for 15 days showed protective effect in the form of disappear of the edema of bowman's space and cloudy swelling except some necrosis and slight congestion. Whereas 21 days showed high protective effects by reducing of cloudy swelling and necrosis. Garlic also diminished the pathological alterations in the kidneys but lower than ginseng.

Histochemical observation revealed, increases in the protein inclusion in rats injected with (9.3 mg/kg b.wt) lambda-cyhalothrin alone in comparison with control, while ginseng and garlic groups almost have normal protein inclusion distributions to some extant.

Key words: Lambda-cyhalothrin (LTC) - Ginseng (G115) - Garlic (GA) - Histology- Histochemistry.

INTRODUCTION

Lambda-cyhalothrin is effective and persistent activity against a large variety of arthropods harmful both to human and animal health, and to vegetal production (who, 1990). LTC is widely used in veterinary products to control lice, flies, and ticks on cattle, sheep, and pigs, as well as used in agricultural formulations to control numerous insect pests on fruits, vegetables, and field crops (leahey, 1985; schenone and rojas, 1992; davies *et al.*, 2000 and kroeger *et al.*, 2003). when given lambda-cyhalothrin to experimental animals at 668 ppm in the drinking water during 21 days showed multiple foci of hemorrhage, tubular dilatation of proximate tubules,

tubular cell desquamation, inflammatory cell infiltration and cloudy swelling of tubules in the kidneys (Fetoui *et al.*, 2009). *Cirrhinus mrigala* fish when exposed to lambda-cyhalothrin at 0.3 ppb and 0.6 ppb LTC exhibited cloudy swelling of epithelial cells of renal tubules, necrosis in tubular epithelium, contraction of the glomerulus's and expansion of space inside the bowman's capsule (Velmurugan *et al.*, 2007). Other researches showed many pathological alteration induced by lambda-cyhalothrin administration (Abdul basir *et al.*, 2011 ;Sakr *et al.*, 2001; Abu EI-Zahab *et al.*, 1993 Abou-Zeid and EI-Balshy, 1995; Abdeen *et al.*, 1994 and Parken *et al.*, 1986).

Panax ginseng (*C.A. Mayer*) which is the protective material in the present work is the most famous plant of all Asian medicinal plants. Panax derives from Greek roots, pan meaning "all" and akos, "cure," referring to the "cure all". Ginseng used in China for over 2,000 years, much of the research has provided a scientific basis for the traditional claims for ginseng use. Ginsengs have some protective effects against toxic substances as reported by (Abdel-Fattah *et al.*, 2010; Fatma, 2002 and 2003).

Garlic (*Allium Sativum*) which is the second protective material in the present study is a common food spicy used all over the world. Garlic were studied by (Chung, 2006) which described the role of garlic organosulfur compounds in free radical scavenging. Omurtag *et al.*, 2005; Chaverri *et al.*, 2008 and Wongmekiat *et al.*, 2004 noted that garlic and garlic extracts used for millennia in folk medicine and reported to provide protection against free radical damage in the body through their antioxidant activities.

MATERIAL AND METHOD

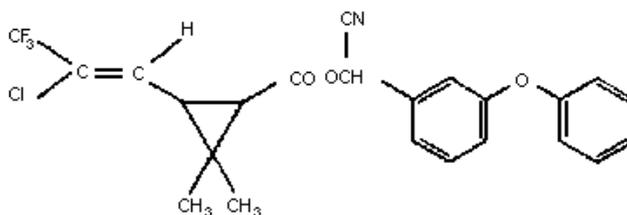
Chemicals:

1-Lambda-cyhalothrin:

Empirical formula: (C₂₃H₁₉ClF₃NO₃). Its commercial name called Lambda super fog and chemical name is [α -cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-methylcyclopropanecarboxylate] (LTC). It is a synthetic pyrethroid, yellow-brown viscous liquid with a mild odor. It obtained from Adwia industries company, Egypt.

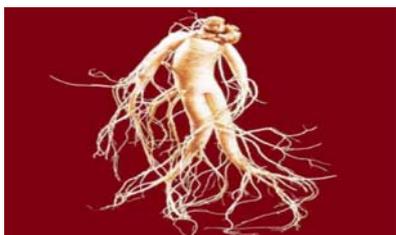
LD50 of LTC = 56 mg/kg for female rat (Extension Toxicology Network)

Structural formula of lambda-cyhalothrin:



2-Panax Ginseng (GINSANA) (G115):

Panax Ginseng (G115) was purchased from EIPICO Pharmaceutical Company, Egypt.



Photograph of Panax Ginseng plant

3-Garlic (*Allium Sativum*) (GA):

The garlic plant (*Allium Sativum*) (GA) was purchased as a powder from National Foods Company, Egypt.



Photograph of garlic cloves

Animals:

Eighty adult female albino rats weighing 200 ± 20 gm at age 4 months. The animals were obtained from Abu Rawash breeding House, Giza, Egypt. Animals divided into 4 groups.

Experiment design

Each group was divided into two subgroups (A & B), 10 rats in each subgroup. Group (1): injected intraperitoneally with normal saline solution and used as a control. Group (2): injected intraperitoneally with lambda-cyhalothrin at 9.34 mg/kg b.wt. Group (3): injected intraperitoneally with panax ginseng at (200 mg/kg) followed by lambda-cyhalothrin at 9.34 mg/kg b.wt. Group (4): The rats injected intraperitoneally with garlic (100 mg/kg), followed by lambda-cyhalothrin at 9.34 mg/kg b.wt. Animals of subgroups (A) were sacrificed after 15 days of treatment and Animals of subgroups (B) were sacrificed after 21 days of treatment. Specimens from the kidneys, from the sacrificed animals were collected, then fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections about 5μ m thickness were prepared and stained with H&E for histopathological examinations according to Drury and Willington, (1980). Specimens from kidneys were fixed in formol alcohol for histochemical examination, dehydrated in ascending concentrations of ethanol, then cleared in methylbenzuate and embedded in paraffin wax. Sections 5μ thick were prepared and stained with bromophenol blue (BP) for protein demonstration according to (Mazia *et al.*, 1953).

RESULTS

I-Histopathological Results

The Insecticide Toxicity:

Kidneys of control rats seen in the normal structure .The cortex consisted of normal renal corpuscles (glomeruli) with adjacent proximal and distal convolution of

nephrons (the epithelial cells in both appeared with normal architecture, besides normal cells displayed in the collecting tubules in the medulla (Fig. 1, A). Injection with (9.3 mg/kg) lambda-cyhalothrin LTC daily for 15 days as a (short term) showed severe congestion in renal and glomerular capillaries, besides cloudy swelling in the proximal and distal convoluted tubules. Vacuolar degeneration with necrosis showed in some renal tubules in the cortex. In addition to, edema in the bowman's space caused pressure atrophy on the glomerular tuft (Fig. 1, B). In other hand, hypercellularity showed in most glomeruli. Destruction with renal casts detected in some of renal tubules, while kidneys of (subgroup B), showed congestion in glomerular and renal blood vessels. Thickening and edematous was detected in the wall of the blood vessels with proliferation in the endothelial cells, manifested with endotheliosis, besides thrombosis and hemorrhage. Edema displayed in the bowman's space resulting in pressure on the glomerular cells. Chronic interstitial nephritis in most rats, manifested with severe aggregation of the inflammatory cells (mainly macrophages cell, lymphocytes and fibroblast) replaced large areas of the degenerated renal tubules (Fig. 2, B). Cloudy swelling showed in all tubular cells, causing atrophy in the glomeruli, besides destruction in some of the tubules. Perivascularitis characterized destruction and edematous in the wall of blood vessels with aggregation of round cells mainly macrophage cells surrounded it.

Ginseng Protective Effect:

Ginseng has protective effect more than garlic. Pretreated with ginseng LTC for 15 days showed reduced in, the histopathological alteration induced by LTC in the form of normal glomeruli and disappearance of edema except moderate necrosis in the other of the glomerular tuft. Mild fibrous tissue proliferation showed among the degenerated renal tubules. Slight Congestion in some of renal blood vessels in cortex and medulla were detected. Focal areas of the necrotic tubules (Fig.1,C)but most tubules displayed mild degeneration, whereas the 21 days treated rats with ginseng before LTC revealed that there is no Chronic interstitial nephritis, Perivascularitis or destruction in the tubules but few rats in this group showed contraction and shrinkage in glomeruli inside the bowman's space. On other hand, Hypercellularity was detected in the glomeruli in other rats (Fig. 2, C). Cloudy swelling was seen in the renal tubules in few rats. Slight Congestion was detected in the renal blood vessels in some rats. Necrosis was noticed in a few renal cells in the collecting tubules.

Garlic Protective Effect:

Garlic have some protective effect observed in diminishing the sever changes in the kidneys tissues caused by (LTC), so that pretreated with garlic before(LTC) for 15 days revealed there is no edema but the cortex has moderate destruction and necrosis in some of renal tubules with renal casts in other (Fig.1,D), besides few fibrous tissues proliferated among it. Thickening in the wall of the congested blood vessels was noticed. Moderate swelling in the epithelial lining showed in most of the renal tubules in the cortex compressed on the glomeruli which appeared congested in the glomerular capillary. Hypercellularity and congestion were seen in the glomeruli. Whenever 21 days treated rats with garlic showed that most of the collecting tubules appeared apparently normal with renal casts in few cells with regeneration the epithelial cells of the tubules but congestion in the glomerular blood vessels was seen in the cortex of some rats. Mild dilation in some of renal tubules (Fig. 2, D) but the remaining tubules appeared normal. Few inflammatory cells scattered among renal tubules.

HISTOCHEMICAL RESULTS

Normal distribution of protein inclusion was observed in the normal kidney section. The glomerular tuft and the basal lamina of the different types of the tubules are denoting their richness in this inclusion that appears as bluish granules with broom phenol blue. While the nuclei of all component cells, and the brush borders of the proximal convoluted tubules appeared moderately stained (Fig. 3, A). 15 Days following the application of repeated (9.3 mg/kg) b. wt of (LTC), an obvious increase sign of protein content mainly in the glomerular tuft, the basal lamina of the different types of the tubules (proximal, distal and collecting tubules) (Fig3, B), while treated with LTC for 21 days showed slight increase in the protein content in the basal lamina of the different types of renal tubules unlike the group treated 15 days (Fig. 4, B).

The kidneys section examined after treatment with ginseng extract (G115) used as protective material followed by(LTC) for 15 days , showed redundant sign of protein level improvement mainly in the glomerular tuft and distal tubules comparing with the treated one and control one (Fig3, C), while ginseng extract (G115) followed by LTC for 21days brought into vision a certain develop the picture of normal distribution of protein materials in the kidneys tissue to some extant comparing with those treated with LTC alone(Fig. 4, C).

The kidneys section of the experimented animals that received the other protective material (garlic) followed by LTC for 15 days were found to have acquired a few of the protein figure of the normal one. Normal distribution of protein was observed only in the glomerular tuft and the basal lamina of the proximal convoluted tubules that appeared moderately stained(Fig3, D), while rats treated with garlic followed by LTC for 21 days showed no variation in the protein content was observed comparing between those treated with LTC or control group(Fig. 4, D).

DISCUSSION

Pathologically, in the present study the kidneys in group treated with (LTC) alone for 15days showed severe congestion in renal and glomerular capillaries, besides cloudy swelling in the proximal and distal convoluted tubules. Vacuolar degeneration with necrosis showed in some renal tubules in cortex. Edema in the bowman's space causing pressure atrophy on the glomerular tuft. In other hand, hypercellularity showed in most golmeruli. Destruction with renal casts detected in some of renal tubules. While, in 21 days showed mild congestion in glomerular and renal blood vessels. Focal areas of macrophage cells replaced the necrotic renal cells with destruction in the renal blood vessels resulting in hemorrhage. Thickening and edematous in the wall of the blood vessels with proliferation in the endothelial cells, manifested with endotheliosis, besides thrombosis. Edema displayed in the bowman's space resulting in pressure on the glomerular cells. Interstitial nephritis in most rats manifested with severe aggregation with inflammatory cells among the degenerated renal tubules. Cloudy swelling showed in all tubular cells causing atrophy in the glomeruli, besides destruction in some tubules. Perivascularitis characterized with aggregation of mononuclear mainly macrophage cells surrounded the blood vessels. Vacuolation in the glomerular cells and dilation in the blood vessels. These results were emphasizes by Sakr and Hanafy, (2002) which recorded that the Kidneys of Toads (*Bufo egularis*) intoxicated with a pyrethroid insecticide "fenvalerate" dissolved in tap water at a dose level of 0.5 mg/ kg b. wt (1/10LD₅₀/4 days) once per day for 3 weeks showed congestion and dilatation of the renal vessels at the first week. But at the second week of the treatment, the intertubular spaces were infiltrated

by leucocytes, besides cloudy swelling of the renal tubules and shrinkage of the glomeruli was observed. Necrosis of the renal cells and cytoplasmic vacuolations with atrophy of the glomeruli was detected at the third week of the treatment.

The present finding in the kidneys agreed with other authors in different animals and this results agreed with (Fetoui. *et al.*, (2009) which reported that rats given lambda-cyhalothrin (668ppm) through drinking water during 21 days showed that LTC induced multiple foci of hemorrhage, tubular dilatation of proximate tubules, tubular cell desquamation, inflammatory cell infiltration and cloudy swelling of tubules in the kidney. Also among of these authors Velmurugan *et al.*, (2007) in tissues of kidneys of *Cirrhinus mrigala* fishes that exposure to 0.3 ppb and 0.6 ppb lambda-cyhalothrin. Abdul basir *et al.*, (2011) found that lambda-cyhalothrin induced congestion and hemorrhage of blood vessels were seen only on serosal surface in groups B, C and D, while, renal parenchyma and stroma were free of any pathology. Abu EI-Zahab *et al.*, (1993) found that kidneys of rats inhaled pyrethroids induced congestion of blood vessels, hemorrhage, necrosis and inflammatory leucocytes. Abdeen *et al.*, (1994) reported that treating mice with fenvalerate induced renal damage of the epithelial lining of the renal tubule, ruptured of the distal tubules and enlargement of the glomeruli with hydropic degeneration. Abou-Zeid and EI-Balshy (1995) recorded that inhalation of Eazalo (a synthetic pyrethroid) caused acute tubular necrosis and glomerulonephritis in kidneys of new born mice. Parken *et al.*, (1986) observed that sub chronic feeding of decaboxy fenvalerate induce glomerulonephrosis in kidney of rats. Sakr *et al.*, (2001) found that inhalation of tetramethrin showed many histopathological changes in the kidney of rats. Tamang *et al.* ,(1991) reported that acute cypermethrin toxicity in Black Bengal goats by drenching cypermethrin at 600 mg/kg b. w kidneys showed necrosis of lining epithelial cells of proximal convoluted tubules.

In present study Group 3; pretreated with ginseng extract improve toxic effects of LTC in the kidneys due to their anti oxidant effects .This results revealed by (Chang *et al.*, 1999) which stated that ginseng can induce the antioxidant enzymes which are important for maintaining cell viability by lowering the level of oxygen radical generated from intercellular metabolism.

Also, Group 4; pretreated with garlic have some protective effects against LTC toxicity through their antioxidant effects previously noted by (Chung, 2006) which described that the role of garlic organosulfur compounds in free radical scavenging. Allicin is a major component of garlic organosulfurs and its antioxidant properties has already been confirmed. In addition to allicin, other garlic organosulfurs, such as alliin, allyl cysteine, allyl disulfide and diallyl disulfide have antioxidant properties and can neutralize several types of reactive oxygen species.

Omurtag *et al.*, (2005) noted that garlic and garlic extracts used for millennia in folk medicine and reported to provide protection against free radical damage in the body through their antioxidant activities.

Histochemically, Kidneys of treated rats for 15 days with LTC showed an obvious increase sign of protein content mainly in the glomerular tuft, the basal lamina of the different types of the tubules (proximal, distal and collecting tubules), the nuclei of all component cells, and the brush borders of the proximal convoluted tubules, whereas animals treated for 21 days showed slight increase in the protein content of the basal lamina of the different types of renal tubules unlike the group treated 15 days.

(Group 3); showed that animals treated with ginseng prior to LTC for 15 days revealed redundant sign of protein level improvement mainly in the glomerular tuft

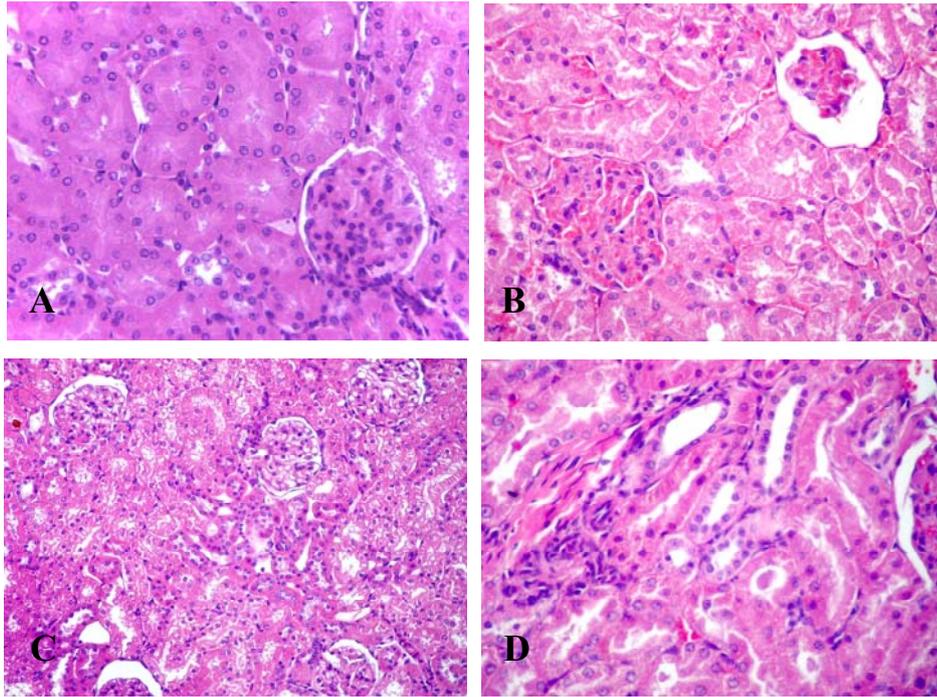
and distal tubules comparing with the normal group, while the application of LTC plus G115 daily for 21 days has brought into vision a certain develop the picture of normal distribution of protein materials in the kidneys tissue to some extent comparing with those treated with LTC alone.

(Group 4); treated with garlic followed by LTC for 15 days were found to have acquire a few of the protein figure of the normal one. Normal distribution of protein was observed only in the glomerular tuft and the basal lamina of the proximal convoluted tubules that appeared moderately stained compared with treated one and control one, while 21 days following the utilization of LTC plus garlic, no variation in the protein content was observed comparing between those treated with LTC or control group.

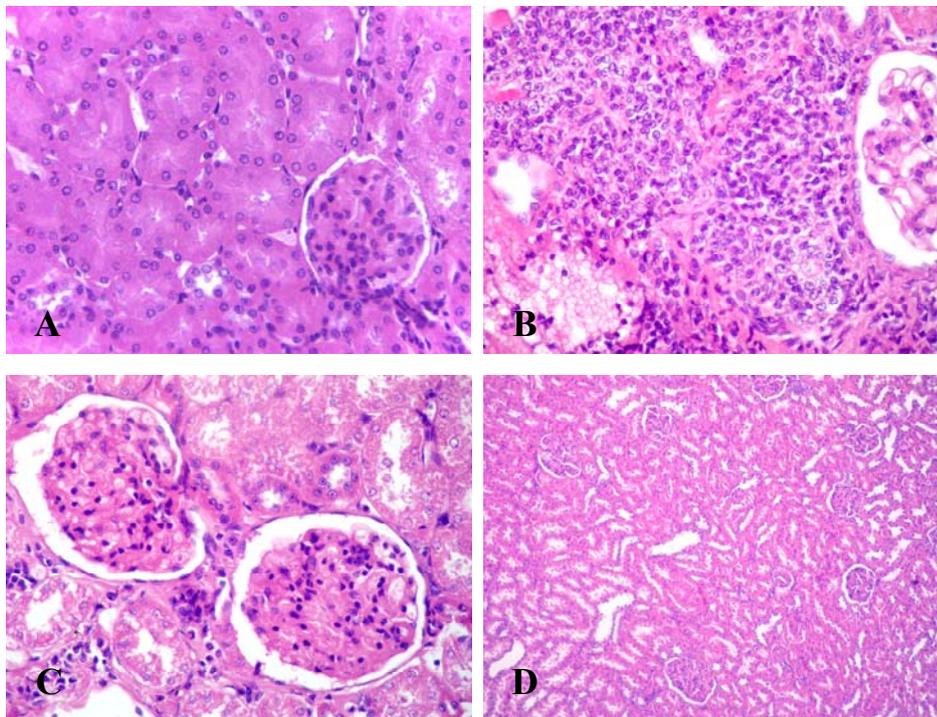
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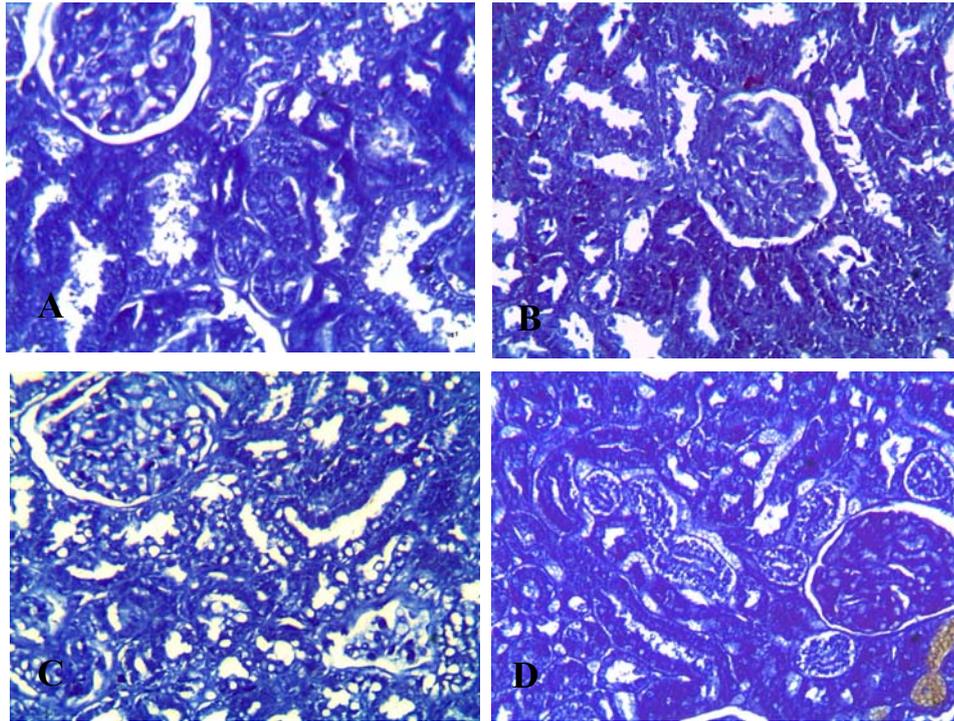
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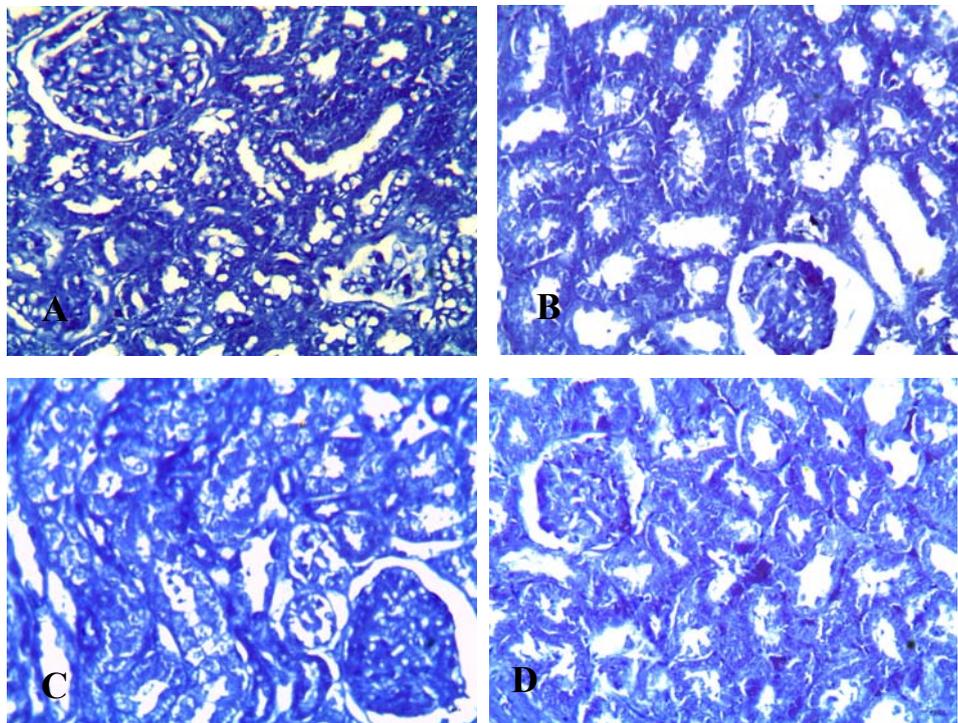
(Fig. 1): Photographs of kidneys of rats treated with LTC for 15 days, A control group, B LTC alone showing severe congestion in renal and glomerular capillaries besides cloudy swelling in the proximal and distal convoluted tubules (H&E., x 300), C Ginseng +LTC showing focal areas of the necrotic tubules but most tubules displayed mild degeneration (H&E., x 300), D Garlic + LTC showing moderate destruction and necrosis in some of renal tubules with renal casts, besides few fibrous tissues proliferated among it (H&E., x 300).



(Fig. 2): Photographs of rats treated for 21 days with LTC, A control group, B LTC alone showing chronic interstitial nephritis in most rats. (H&E., x 300), C Ginseng + LTC the kidneys, showing hypercellularity in the glomeruli (H&E., x 300), D Garlic + LTC showing mild dilation in some of renal tubules (H&E., x 150).



(Fig. 3): Photographs of kidneys of rats treated with LTC for 15 days, A control group showing normal protein inclusion, B showing LTC alone showing increase in protein inclusion, C ginseng + LTC showing decrease in protein inclusion compared with LTC alone, D garlic +LTC showing normal distribution of protein only in the glomerular tuft. The basal lamina of the proximal convoluted tubules that appeared moderately stained. (Bromo phenol blue X400)



(Fig4): Photographs of kidneys of rats treated with LTC for 21 days, A control group showing normal protein inclusion, B LTC alone showing slight increase in the protein content, C ginseng +LTC almost normal distribution of protein content, D garlic +LTC no variation in the protein content comparing between those treated with LTC. (Bromo phenol blue X 400)

ARABIC SUMMARY

"التأثير الوقائي لمستخلص نبات الجنسنج (جنسانا) وبودرة الثوم ضد التأثيرات الضارة لمبيد (لمبادا-سيهالوثرين 5%) على كلى إناث الحرزان البيضاء"

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2 - قسم علم الحيوان، كلية العلوم، جامعة جنوب الوادي، قنا.

من المعروف جيدا أن الجنسنج والثوم نباتات التي لها تأثيرات مضادة للتأكسد. هذه الدراسة تبحث في التأثيرات الواقية لمستخلص نبات الجنسنج، ومسحوق الثوم على الآثار السيئة للمبيد الحشري. للامبادا-سيهالوثرين. ثمانون من إناث الفئران ويستر تنقسم إلى 4 مجموعات على النحو التالي مجموعة (1) مجموعة ضابطة، مجموعة (2) حقنت (9.3 ملغم / كغم من وزن الجسم) بالامبادا-سيهالوثرين وحده، مجموعة (3) حقنت (200 ملغم / كلف ب. وزن) من مستخلص الجنسنج تليها 9.3 ملغم / كلف. من وزن الجسم) لامبادا-سيهالوثرين، مجموعة (4) حقن مستخلص الثوم (100 ملغم / كغم من وزن الجسم t)، يليه بنسبة 9.3 ملغم / كلف من وزن الجسم بالامبادا-سيهالوثرين تم حقن الحيوانات في التجويف البطني 15 يوما (كما هو الحال على المدى القصير) و 21 يوما (كما هو الحال على طول المدى).

وأظهر الفحص الهستوباثولوجية للكلية الاحتقان الشديد، بالإضافة إلى تورم غائم في الأنابيب المتلوية القريبة والبعيدة، تنكس فجوي مع نخر. بالإضافة إلى، وذمة في فرط الخلوية في يوميا، وتكسر مع شوائب كلوية في بعض من الأنابيب الكلوية في الحيوانات التي حقنت بالمبيد فقط لمدة 15 يوما. في حين كشفت الفئران التي حقنت مع المبيد لمدة 21 يوما زيادة سمك واستسقاء في جدار الشعيرات الدموية مع الانتشار في الخلايا البطانية، بالإضافة إلى الجلطة ونزيف. كما تم الكشف عن التهاب الكلية أخلالي المزمن في معظم الفئران والتهاب حوائط الوعاء. وأظهرت الكلية في الفئران التي تعاملت بالجنسنج يليها المبيد لمدة 15 يوما تأثير وقائي في شكل عدم وجود استسقاء في محفظة بومان التورم الغائم باستثناء بعض نخر والاحتقان الطفيف في حين أن 21 يوما أظهرت بعض التأثيرات الواقية من خلال تقليل التورم غائم والنخر ولكن هناك فرط الخلوية. كلما تضاءلت التغييرات المرضية في الثوم.

الفحص الهستوكيميائي لأنسجة الكلية أظهر زيادة في محتوى البروتين في الفئران حقنت مع (9.3 ملغم / كغم من وزن الجسم) بالامبادا-سيهالوثرين وحده بالمقارنة مع المجموعة الضابطة، في حين مجموعات الجنسنج والثوم محتوى البروتين شبه طبيعي.

تم ذبح الحيوانات من المجموعات الفرعية (أ) بعد 15 يوما من العلاج والحيوانات من المجموعات الفرعية (ب) بعد 21 يوما من العلاج. وتعرض عينات أنسجة الكلية للفحوص الهستوباثولوجية والهستوكيميائية