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IMMUNOPATHOLOGICAL STUDIES ON IMMUNOSTIMULANT PLANT [Astragalusmembranaceus]

Thabet A. Mohamed Nagah , Mohamed F. salem zlitni, Naima Mohamed Omer Elkakalli.Ahmed M. Elmesalati .

Higher Institute for Technical Medical Science, Tripoli-Libya
Higher Institute for Technical Medical Science, Tripoli-Libya
Tripoli Diabetic Centre
College of dentistry/ Tripoli university
Attahadi University.

دراسات مناعية مرضية عن النبات المحفز للمناعة في الفئران البيضاء

ثابت الهاني محمد نجاح — المعهد العالي للعلوم والتقنيات الطبية /طرابلس
أحمد ايهاب محمد المسلاتي — جامعة التحدي الطبية الاهلية
محمد فرج الزليطني — المركز الوطني لأمراض السكر والغدد الصماء
نعيمة محمد الككلي — جامعة التحدي الطبية الاهلية

Abstract

The present work was performed to study the effect of Astragalusmembranaceus on immunity of healthy albino rats.. The comprised 36 rats subdivided into four groups. Each group was consisted of nine rats. The first group received daily oral administration with 0.25g/kg/day of Astragalusmembranaceus for two and four weeks, the 2nd group received daily oral administration of 0.5g/kg/day of Astragalusmembranaceus for two and four weeks, while the 3rd group received daily oral administration with 1.0g/kg/day of Astragalusmembranaceus for two and four weeks, the 4th group kept as control.

Regarding the cellular immunity results, astragalusmembranaceus was found to have stimulatory effect on lymphocytic transformation rate at the end of 2nd and 4th weeks in groups A2 and A3. Significant increase in phagocytosis in group A3 (2nd week) and groups A2 and A3 (4th weeks).

Bacterial killing showed a significant increase in groups A1, A2 and A3 at the end of 4th weeks and in groups A2 and A3 at the end of 2nd and 6th weeks. Chemotaxis showed a significant increase in groups A2 and A3 at the end of 2nd and 4th weeks and in group A3 at the end of 6th week.

Results of humoral immune response, Astragalusmembranaceus significant increased of both IgG and IgM at all concentration at the end of 2nd and 4th weeks except group A1 did not show significant increase at the end of 2nd week in IgG.

منذ القدم استخدم الانسان الأعشاب والنباتات المحلية لتخفيف العديد من أمراض واضطرابات الجسم. والتداوي بالأعشاب عريق في الصين منذ قديم الزمان ، ومعرفة هذا النوع من التداوي قد تدرجت من سلالة الى سلالة حتى كونت عندهم ما يسمى بالطب الشعبي في هذه المنطقة من العالم ، وتعزى قيمة النباتات كعقاقير الى وجود مواد فعالة في هذه النباتات تحدث آثارا عضوية فى جسم الانسان ، ذلك ما شجعنا على دراسة بعضا من تلك النباتات الطبية أو ما يسمى بالعقاقير الشعبية المستخدمة على نطاق واسع.

أجريت هذه الدراسة على نوع من النباتات الطبية المعروفة فى الصين والمستعمل كمحفز للجهاز المناعي وهو**(الاستراجلوسممبراناسيوس)**

والذي يستعمل كمضاد حيوي " طبيعي " فهو يعمل كالبنسلين فى الجسم بدون آثار جانبية ، ويعمل كمخفف للحمّى ، والاصابات ، والتنفس السيّيء ، وتعزيز الاغشية المخاطية ، ويعتبر من أفضل النباتات التى تقوم بتطهير وتنظيف الدم والغدد الليمفاوية.
تم إجراء هذا البحث في تجربة على ستة وثلاثين فأرا ابيضا ، وتحتوى على مجموعة الاستراجلوس. قسمتا لمجموعة الى اربع مجموعات (1) ، (2) ، (3) ، (4) يتكون كل منها من تسعة فئران وذلك على النحو التالي:
التجربة :-
قسمت المجموعة الرئيسية الى المجموعات الفرعية التالية:
مجموعة(1)- تم تجريع هذه المجموعة يوميا ب(0.25 جرام كيلوجرام/ يوم) من الاستراجلوس . وذلك على فترات مختلفة 14 و 28 يوم على التوالي.
مجموعة(2)- تم تجريع هذه المجموعة يوميا ب(0.5 جرام / كيلوجرام / يوم) من الاستراجلوس . وذلك على فترات مختلفة 14 و 28 يوم على التوالي.
مجموعة(3)- تم تجريع هذه المجموعة ب(1.0 جرام / كيلوجرام / يوم) من الاستراجلوس . وذلك لفترات 14 و 28 يوم على التوالي.
مجموعة(4)- أعتبرت هذه المجموعة كمجموعة ضابطة.
تم أخذ عينات دم عند نهاية كل فترة من التجريع (الاسبوع الثانى والرابع) وأخذت عينات دم عند نهاية الاسبوع السادس وذلك لاجراء الاختبارات المختلفة على الدم والمصل .
النتائـــج:
لم يلاحظ وجودا أ ى من ا لأعراض الاكلينيكية أو وفيات تستحق أن تسجل.
وبالفحص الخلوي للدم وجد أن تجريع الفئران بنبات الاستراجلوس لم يحدث اى زيادة معنوية فى العدد الكلى ولا بالفحص النوعي لكرات الدم البيضاء.
وبالنسبة للمناعة الخلوية وجد زيادة معنوية فى معدل تحول الخلايا الليمفاوية في المجموعتين الثانية والثالثة عند نهاية فترة الاسبوعين والأربعة اسابيع كما وجد زيادة معنوية في نسبة الخلايا البلعمية فالمجموعة الثالثة (الاسبوع الثاني) والمجموعتين الثانية والثالثة (الاسبوع الرابع). بالإضافة الى وجود زيادة معنوية في المجموعتين الثانية والثالثة (الاسبوع الثاني والسادس) والمجموعات الثلاث (الاسبوع الرابع) بالإضافة الى زيادة معنوية فى نسبة الانجذاب الكيميائي فى المجموعتين الثانية والثالثة (الاسبوع الثاني- الاسبوع الرابع) وزيادة فى المجموعة الثالثة (الاسبوع السادس- بعد توقف التجريع بأسبوعين).
كما لوحظت زيادة معنوية ملحوظة فى معدل امينوجلوبيولينGفى المجموعتين الثانية والثالثة (الاسبوع الثانى) و فالمجاميع الثلاث (الاسبوع الرابع)، وزيادة معنوية ملحوظة فى معدل الامينوجلوبيولينMفى المجاميع الثلاث وذلك فى نهاية (الاسبوع الثانى– الرابع).
الامينوجلوبيولينM فى المجاميع الثلاث وذلك فى نهاية (الاسبوع الثانى– الرابع).
نستخلص من هذا البحث : 1:- أن إعطاء العشبين الاستراجلوس عن طريق الفم بالتركيزات 25. ، 5. و 1.0جم / كيلوجرام/يوم لمدة 14، 28 يوم يحفز الجهاز المناعى متمثلا فى المناعة الخلوية والجاماجلوبيولين دون آثار جانبية على الكبد والكلية.
2:- أفضل الجرعات لزيادة المناعة 5، ، 1.0 جرام/كيلوجرام يوميا من كلا العشب.

Introduction

Astragalusmembranaceus is most commonly used as a general tonic for those practicing Chinese herbal medicine, and specifically for immune enhancement **(Yoshida et al 1997)**. It is a staple of Traditional Chinese Medicine (TCM), where it is also known as Huang Qi. It is considered a sweet, warming herb with particular effects on the lung, spleen and heart meridians **(Jin et al 1994)**. Astragalusmembranaceus is a component of numerous TCM tonics and is often combined with ginseng, angelica and other herbs. Traditionally it is used in the treatment of fatigue, decreased appetite, general debility, susceptibility to viral infections, non- healing wounds, fever, sweating , uterine prolapses, uterine bleeding, edema

(nephritis), numbness, muscle pain, diabetes mellitus, and uterine, ovarian or colon cancer **(Hou et al1981)** . Western herbalists began using the roots of A. membranaceus in The main aim of the present work is to study and to throw more light on the immunological stimulant effect of Astragalusmembranaceus on humoral and cellular immune response and to choose the best immunostimulant dose.

Review of literature

1-ASTRAGALUS MEMBRANECEUS [Huang Qi] :

Astragalusmembranaceous (A.M.) is the botanical name for the Chinese herb, huang means--- yellow, which refers to the yellow interior of the root. Qi - means {Leader} which signifies. ***(Li et al 1998)*.**

COMMON NAME

Goat's horn, green dragon, gum dragon, gummi tragacanthae , hog gum , huang Qi , membranous milk vetch , Syrian tragacanth . It uses as superior tonics.***(Ehling , Dagmar 1996).***

MODE OF ACTION

Hikino . et al .(1976)mentioned that Astragalusmembranaceus stimulates virtually every phase of immune system activity. It increases the number of stem cell in the marrow and lymph tissue, and it stimulates their development into active immune cells that are released into the body. It could promote or tigger immune cells from the resting state into high tend activity. Astragalus has also been found to stimulate the production of interferon and increase its affects in fighting disease.

Material and Methods

I- Material:

1-Experimental animals:

A total of Thirty sex two of apparently clinically healthy male albino rats (200 gm in weight) was used in our experiment .

2- Crudeherbs :

Astragali root (AstragalusMembranaceus Root Powder, 4 oz. Bulk Herb, 100% organic- code 5530b-Noupc) . Then 50 g of crude herb was boiled with 1000 ml of distilled water until the volume was reduced to 500 ml. The supernatant fluid was filtered and lyophilized. The concentration of the herbal extract was given orally to rat

After adjusted the concentration of the extract to (0.25g, 0.5g, 1.0g, per day per Kg per day as extract) . The control group was given water. The herbal medicines were administered consecutively for two and four weeks.

Experimental design:

Seventy two of male albino rats was divided into 36 rats . and the rats was subdivided into four subgroups (A1,A2,A3,and A4) each subgroup was consisted of nine rats, assigned for daily oral administration with (0.25 ,0.5, and 1.0 g) of AstragalusMembranaceus administered by a stomach tube for two and four weeks respectively.

The rats in subgroup A4 were kept as a control groups and were orally received water for two, four and six weeks. The administration of herb will stopped after four weeks and the samples will collected from remaining rats after six weeks.

METHODS

Experimental design:

Seventy two rats were divided one group (each of 36) rats, and again divided into four subgroups each of nine rats.

1-The first subgroup (A1) from each main group received 0.25 g of herb for two and four weeks.
2-The second subgroup (A1) from each main group received 0.5 g of herb for two and four weeks.
3-The third subgroup (A1) from each main group received 1.0 g of herb for two and four weeks.
4-The fourth subgroup (A4) ***(Healthy control subgroup)*** received no treatment and left as control.

Blood sampling:

The blood samples collected for studding cellular as well as the humoral immune response.

Blood samples:

1-Sample number 1:

Three ml of blood were collected from each rat in sterile plastic centrifuge tubes containing heparin (50i.µ/ml) to be used for separation of mononuclear leukocytes.

Haemagglutination test (HA test): The slow haemagglutination test was carried out according to **Anon(1970)** for detection of haemagglutinins in the allantoamniotic fluids using 1% suspension of washed rats erythrocytes.

Determination of immunoglobulins titre:

The developed sandwich ELISA was carried out according to **Erhard et al.(1992)**as follows:

Cellular immune response:

1- **Lymphocyte transformation**

***A- Separation of lymphocytes:* (Boyum, 1968 and Burrels and Wells, 1977).**

***B-Total lymphocyte count* : (Hudson and Hay, 1980).**

C-Standardization oftymphocyte concentration for blastogenesis:

D-Preparation of mitogens (non-specific mitogen):

E- Standardization of NDV antigen concentration (specific mitogen):

F- Standardization of Brucella abortus antigen concentration (specific mitogen):

Setting up of lymphocyte culture:

Lymphocytic transformation assay using MTT staining

Chemotaxis under agarose:

b) Preparation of agarose plates:

***c) Leucocyte chemotaxis assay* (Nelson et al., 1975):**

Phagocytosis and Killing assay (Woldehiwet and Rowan, 1990);

***Staphylococcus aureus:* Assay of phagocytosis:**

Assay for bacterial killing:

Results

Clinical Results

Rats treated with 0.25g , 0.5g and 1.0 g of AstragalusMembranaceus for Two and Four weeks.

a-Clinical signs and mortality rate:

Our results revealed that the rats appeared healthy during the

Whole experimental time. No mortalities were recorded with this group.

b- Cellular immunity results:

The effect of three dosages of AstragalusMembranaceus Herb on lymphocyte

transformation rate, Phagocytosis, bacterial killing activities and

chemotaxis activity respectively. The results showed significantly

increases ($p > 0.05$) in lymphocyte transformation rate in both subgroups

A2 and A3 at the end of 2^{nd} and 4^{th} weeks. Moreover the levels of Phagocytosis activity in mice given 1.0 g of AstragalusMembranaceus
Subgroup A3 was significant increased at the end of 2^{nd} week, while Significantly increases were observed in subgroups A2 and A3 at the end
of 4^{th} weeks by ($p > 0.05$). A significant increase ($P > 0.05$) in bacterial
Killing percent was recorded in both subgroups A2 and A3 at the end of
2^{nd} week. And at the end of 4^{th} week the significantly increases in bacterial
killing activity were observed in all subgroups A1, A2 and A3 . While the
effect of AstragalusMembranaceus Herb were statistically significant increases in chemotaxis percent in subgroups A2 and A3 by the end of 2^{nd}
and 4^{th} week.

e- Humoral immunity results:

The proteinogram results showed a significantly increases in total gamma globulin in subgroups A2 and A3 compared with the control group at the end of 4th week. There were non-significant alteration were observed in total protein, albumin ,total alpha globulin and total beta globulin during the whole period of the experiment. Significant increases of both IgG and IgM antibodies was observed . Where IgG were significantly increases by ($P > 0.05$) in Subgroups A2 and A3 at the end of 2nd week and in all subgroups A1, A2 And A3 at the end of 4th week. While IgM antibody was higher than those in the controls ($P > 0.05$) in all subgroups A1, A2 and A3 at the end of 2nd and 4th week of the experiment.

Discussion

A traditional medicine used to treat viral infections, the root of ***Astragalusmembranaceus***has been shown in clinical studies to reduce the incidence and shorten the course of the common cold. In scientific studies, extracts of Astragalus have been shown to increase macrophage, T cell and natural killer cell activity as well as to enhance the antibody response. It was also found to stimulate the production of Interferon (a type of cytokine with antiviral properties), helping to protect the body against viruses.

The root of Astragalusmembranaceus is a crude drug in traditional medicine. It has been reported that it has an effect on circulatory, immune systems and enhanced the cell metabolism in vitro **Song et al(2000).**Astragalusmembranaceus showed an effect on the various immune responses such as T-Lymphocytes blastogenesis, cytokine production of lymphocytes and macrophages and cytotoxicity of natural killing cells (NK) or lymphokine activated killer cells from the depression induced by carcinogen or anticancer agent **(Jin et al 1994b; Jin and Kurashige 1996 and Kurashige et al 1999;;).** It has

also been reported that astragalusmembranaceus can enhance macrophage activation in the immune system **Lau et al (1989).**

Our results of daily oral administration of astragalusmembranaceus to rats on lymphocyte transformation rate showed significant increase at concentration of 0.5g and 1.0g at the end of 2nd and 4th week. On the same ground , our results were parallel to the result obtained by **Yoshida et al(1997)** who recorded that astragalusmembranaceus seem to have immunostimulating activity and enhance lymphocyte transformation rate not only in vitro but also in vivo.

On contrary, our results is disagreed with **Liang H et al (1994)** who reported that the administration of astragalusmembranaceus to burned mice has significant suppressive activity of lymphocyte transformation rate.

Our results revealed significant increase in phagocytosis activity at the dose of 1.0 g at the end of 2nd week, and in both doses of 0.5 and 1.0g at the end of 4th weeks. This finding agreed with that results obtained by **Fany et al (1985)and Xie et al (1989)** who reported that polysaccharide fraction of Astragalusmembranaceus enhanced the phagocytic activity of macrophages and inhibited the growth of transplanted tumors in rats. Moreover our results are agreed with those mentioned by **Wang et al (1992)** .

Bacterial killing showed a significant increase in all groups at the end of 4th week and in groups A2 and A3 at the end of 2nd and 6th weeks.

Some investigators showed that some kind of crude drugs and traditional Chinese medicine activated NK cells **Zee et al (1992);Yamaoka et al (1996) , and Biron (1997).** On the same ground, our results were paralled to the finding of **Hong (1992),** who reported that NK activity of spleen cells in orally astragalusmembranaceus administered mice, was significantly higher than in control mice.

These results were confirmed in another study where Astragalusmembranaceus was shown to potentiate the lymphokine-activated killer cell **Wang et al (1992)**.

Results of daily oral administration of 0.25g, 0.5g and 1.0g of Astragalusmembranaceus for two and four weeks on IgG and IgM were in accordance with the results reported by **He et al (1992)** who reported an elevation in serum IgG level in mice fed by 9g / kg, 20g / kg of Astragalusmembranaceus, Fructusligustrilucidi and Eclipta prostrate for seven days. The same results were observed by **Yoshida et al (1997)** who recorded the infusion of Astragalusmembranaceus stimulated polyclonal Ig production in male and female mice 6-8 weeks of age treated with extracts of both herbs Astragalusmembranaceus and Oldenlandandiadiffusa, also our results were in agreement with those of **Huang et al (1995).**

Similar observation was seen by **(Duane and Wang 2003)** who observed increased of IgG and IgM in tumor patients given Astragalusmembranaceus intravenously.

Moreover our results are in agreement with **Lin et al (2003)** who reported that injection of Astragalusmembranaceus to patient with congestive heart failure (CHF) was elevated the activity of humoral immunity (IgG, IgA and IgM).

Our results agreed with that obtained by **Gue et al (2004)** who concluded that supplementation with mushroom and herb extracts resulted in enhancement of both IgG and IgM. These increases may be attributed to the immuno-stimulatory effect of Astragalusmembranaceus.

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The effect of artificial Corridors on inter-patch movement of The silver-studded blue (*Plebejus argus*) in fragmented habitat

Othman a dakhli. ***Asmhan belgasem***

The higher and intermediate institute of agricultural technology, AL Ghyran – Tripoli- Libya

تأثير الممرات الاصطناعية على تبادل حركة الطيران لفراشة (*Plebejus argus*) جوالس الزرقاء الفضية بين الموائل المشتتة

عثمان سالم الدخلى اسمهان بلقاسم عطية

المعهد العالي والمتوسط للتقنيات الزراعية بالغيران – طرابلس – ليبيا

Abstract

in this study , We examined artificial corridors effects on inter-patch movement of the butterfly (Plebejus argus) in two habitat types by tested the hypothesis that the extent to which corridors increase inter-patch movement of The silver-studded blue butterfly .the results of mark-recapture study showed that , The longest dispersal distance recorded was 359m among the butterflies' movement. 64.35% of all movements were found between 0-50m, 12.8% were between 50-100m, and 2.97% were greater than 250m. the result was showed that there was a significant difference in mean movement between butterflies,. 11 individual butterflies were detected and moved between patches by used corridors. Of these, 64.4% were males and 36.6 % were females. 54.5% were initially moved from the GO2 patch where they were marked to patch GO1 and 45.5% were initially moved from patch GO1 to GO2. , this study found the corridor effect was not significant for this species,by increase emigration from a its fragmented patches,Our results suggest that increased density of its host and nectar resourcethrough corridors will have positive impacts on butterfly populations and community interactions in fragmented landscapes.

الملخص

هدفت الدراسة لمعرفة تأثيرات الممرات الاصطناعية على معدل وتبادل الطيران لفراشة بين أثنين من الموائل باختبار الفرضية الى أى مدى يمكن للممرات أن تزيد من معدل تبادل النوع بين البيئات. وقد بينت النتائج المتحصلة من الاختبار (MRR) بأن أطول معدل طيران لفراشة تم تسجيله كان (359 متر) بين مجموع الكلى للفراشات . حيث سجلت حوالى (64.35%) من أجمالى معدل طيران الفراشات كان ما بين (0-50 متر) وكانت نسبة (12.8%) من أجمالي معدل الطيران كانت مابين (50-100 متر) وتعتبر (2.97%) من أجمالى الفراشات التطارت أكثر (250 متر) بين نوعين من الموائل . كما بينت النتائج بأنه توجد فروقات معنوية بين متوسط معدل طيران الفراشات فى كلا من البيئات المذكورة . حيث وجد فقط حوالى (11) فردا من مجموع الفراشات تم تسجيل حركتها بين الموائل باستخدام الممر المعد فى هذى الدراسة . حيث كانت نسبة الذكور حوالى (64.4%) ونسبة الاناث (36.6%) .حيث وجد (54.5%) فراشات زارت الموقع (GO2) حيث سجلت ورقمت الى موقع (GO1)بينما (45.5%) من (GO1) الى الموقع (GO2). ، وقد بينت الدراسة بأن الممرات ليس لها تأثير على هذا النوع من الفراشات من خلال زيادة معدل الهجرة

والطيران بين بيئاتها المشتتة .وتوصى هذى الدراسة الى زيادة النباتات المفضلة لهذا نوع من الفراشات من ضمن الممرات والتيسوف تكون لها تأثير أيجابى على حياتية مجتمعات الفراشات فى البيئات المشتتة.

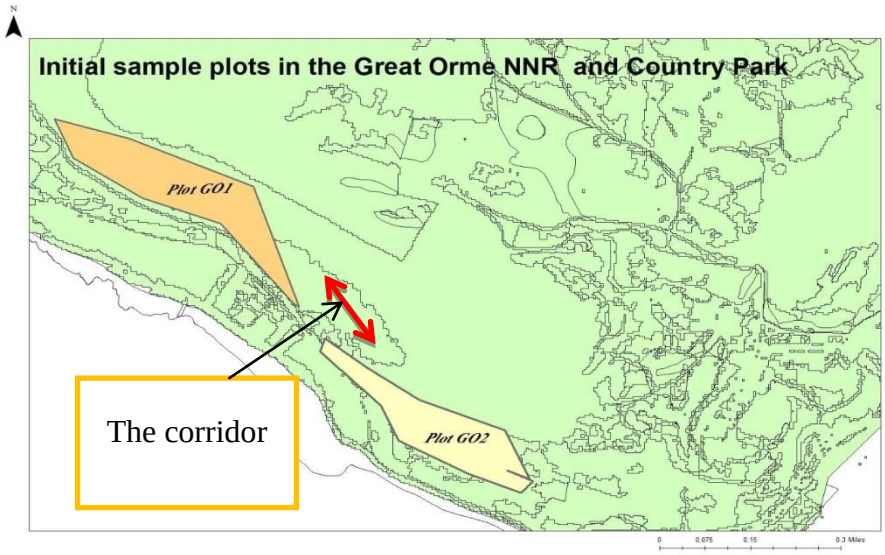
Introduction

Human activities and anthropogenic pressures have become one of the major causes of the loss of biodiversity and natural habitats around the world, including grasslands, forests, woodlands and wetlands (Bergman and Landin, 2002). In addition, agricultural practice and the other forms of land use are the greatest cause of habitat loss and fragmentation (Andren, 1994). Furthermore, habitat fragmentation and isolation result from these activities, which has led to the separation of a huge population into several small populations (Hanski and Giplin, 1997). Habitat fragmentation can lead to the extinction of many species, because the population cannot survive in an isolated area due to the increase of inbreeding and the reduction of gene flow (Schmitt et al., 2006). Another problem is that the barriers are a result of the effects of habitat fragmentation and isolation which may occur in natural habitats such as forests, seas and rivers or by created man-made structures such as roads and dams within the habitats network. These barriers play a significant role in the extinction of many species such as butterflies by isolating the appropriate habitats and also reducing the dispersal ability of these species (Conradt et al., 2000; Davies et al., 2001).

Nature corridors are used to overcome difficulties experienced by species as result of habitat fragmentation (Diamond, 1975). However ,Ecological corridors increase connections between isolated patches, promoting dispersal between patches and gene flow (Simberloff et al., 1992). The results of mark-release-recapture studies demonstrated that corridors are contributing to increased inter-patch movement rates (Haddad, 1999; Haddad and Baum, 1999; Gilbert et al., 1998). Sufficient dispersal or migration between habitat patches is a necessary condition for persistence (Hanski et al., 1995). The main objective of the study was to investigate the dispersal distances of butterflies by using Corridors to contact between two patches.

Methodology

The experiment, conducted at the The Great Orme is located at the north-western tip of the Creuddyn Peninsula near Llandudno, which is in Conwy County (North Wales , UK), Study plots were shown in (Fig. 1). The sample plots which include the FfynnonGogarth and Pen y Ffriedd (GO1) and the CreigiauCochion (GO2). The distance between these plots were about 210m apart from each other which was used as Corridor in this study , It is known that patches are considered separate if the distance between the patches is more than 100 m **(***Gustafson*, et al., 1992**).** The distances between patches, are separated by sets of tall standing trees and some private land, which may acts as an additional barrier to dispersal. In this study We were created corridors by harvesting pine forest between plots as shown in (Fig. 1).



(Fig. 1) shown the study plots andThe corridorat The Great Orme country park.

Mark- release- recapture: We used The MRR techniqueto test dispersal distances between two habitat patches (G01 and GO2). Mark-release-recapture was conducted during the summer of June 2016. In that period, these plots were visited daily which depended on suitable weather, and walked by random at each

plot for captured butterflies. In this experiment, butterflies were caught (by using a Watkins and Doncaster kite net) and marked individually by special code (1-2-4-7), which involved a marking system of a set of dots on the butterfly's underwing using a different colour for each location, with a permanent marker pen (StaedtlerLumocolour@) following Brussard's (1971) method (Fig.2). For each captured butterfly, the location of its capture was recorded using a handheld Garmin GPS unit with other information (time, day, and sex). The marked and recapture sampling were divided into three events as showed in (Table 1),. There were one or two days as interval days between occasions to allow butterflies to mingle and move between plots and the longevity of this butterfly to be between 3 to 4 days (Lewis et al., 1997). Each marking occasion was started in different plots in the sequence, in order to avoid bias due to time and day.

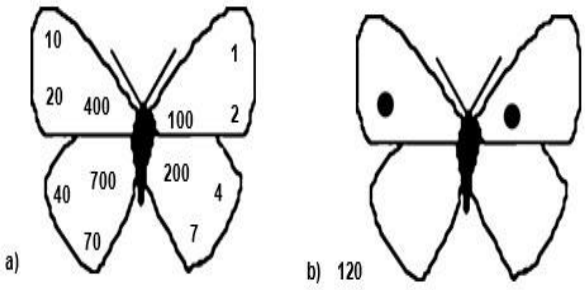


Fig.2.Brussard's (1971). (A) *The 1-2-4-7 marking system is a mark with single dots on the left side wing and on the right side wing Butterfly (B) showed the example the number ("120") would be marked as 20 on the left side wing and100 on the right side wing.*

Table 1. Days devoted to marking and capturing occasions.

Occasions	Day	Dates
Event 1	Marking day	03/06/2016
	Marking day	04/06/2016
	Marking day	05/06/2016
	Recapture day	06/06/2017
	Recapture day	07/06/2016 **

Event 2	Marking day	09/06/2016
	Marking day	10/06/2016
	Marking day	11/06/2016
	Recapture day	12/06/2016
	Recapture day	13/06/2016
Event 3	Marking day	17/06/2016
	Marking day	18/06/2016
	Marking day	19/06/2016
	Recapture day	20/06/2016
	Recapture day	21/06/2016
event 4	Marking day	23/06/2016
	Marking day	24/06/2016
	Marking day	25/06/2016
	Recapture day	26/06/2016
	Recapture day	27/06/2016

The 07/06/2016** no butterflies were recaptured

Data analysis

Locations:Mann-Whitney U test in the SPSS statistical package version 22 (2016) was used to compare between plots of the site on the numbers of butterflies. They were used to determine which plot differed significantly from the other plot in terms of number of butterflies.

Dispersal distances:

The MRR data was imported into the ArcGIS Desktop 10.3 software. All data from the MRR were converted into an Excel spreadsheet for every GIS point of capture with easting and northing as the X and Y field in the ArcGIS file. All GIS points were plotted on different maps for different occasions of marked and recaptured butterflies (Howson, 2010). The distances between capture and recapture butterflies were estimated by using line measurement in ArcGIS Desktop 9.3 for every individual butterfly that was captured more than once, which was labelled with the letter suffix "A" and the second capture was labeled with "B". Frequencies of dispersal distances were analyzed in SPSS 22.0 (statistical package version).

Inter -patch movement:Dispersal trajectories were examined in the ArcGIS Desktop 10.3 software. By determination if there is any inter-patch movement between plots.

Results :

There was no statistically significant difference between plots GO1 and GO2 in butterfly number (table 2) , p-value=. 130 is greater than .005. The frequency of the number of butterflies on both plots is given in Fig. 3.

Table 2. Mann-Whitney U test results for comparison butterfly number between plots.

SITE	N	Mean	Std. Deviation	Mean Rank	Mann-Whitney U	P-Value
1	21	6.71	5.021	17.83	143.500	0.130
2	19	8.68	3.742	23.45		
* Significant at the 0.05 level						

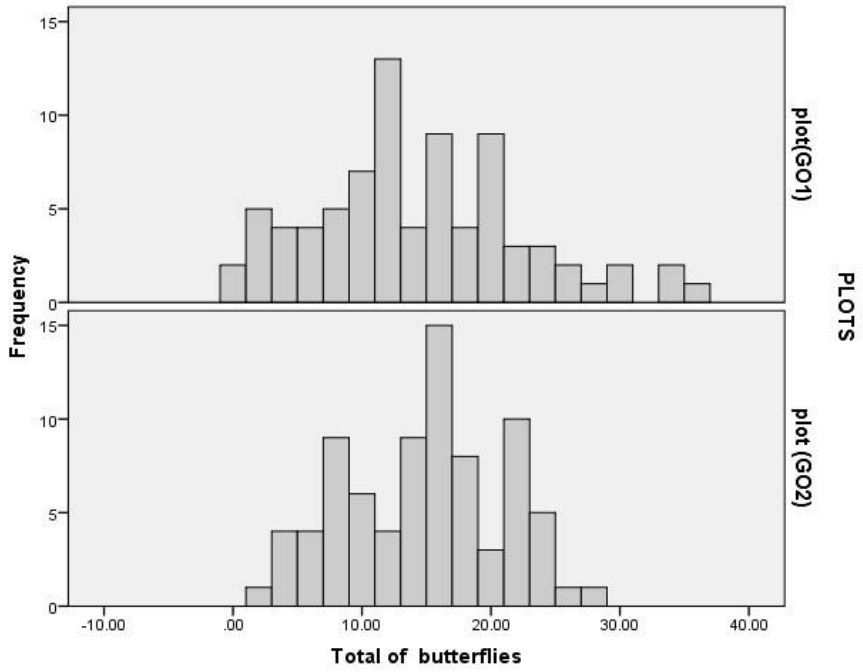


Fig. 3. The frequency of the number of butterflies on both plots.

Dispersal distances :

The movement of each individual *Plebejus argus*captured and recaptured during the mark-recapture study was plotted on the map and their movement was calculated as straight lines between capture points (Fig. 4) . The longest dispersal distance recorded was 359m among the butterflies' movement. 64.35% of all movements were found between 0-50m, 12.8% were between 50-100m, and 2.97% were greater than 250m. Table (4) showed that there was a significant difference in mean movement between butterflies, t (97) = -7.891, p = <0.001.. The frequency of dispersal distances is given in Fig 5.

Table 6. The means differences in distances between butterflies.

	MEAN	STD. D.	T	DF	SIG. (2-TAILED)
DISTANCES	64.27	80.624	7.891	97	<0.001

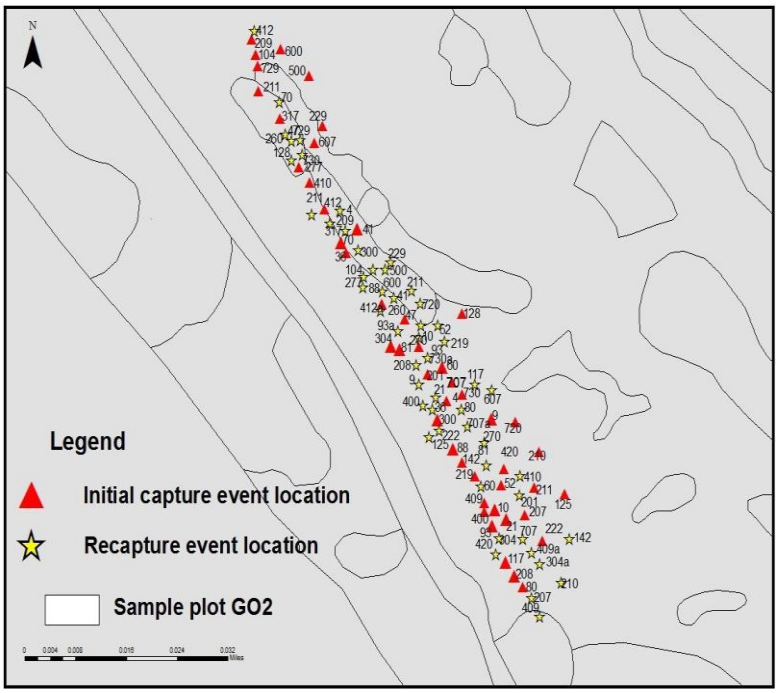


Fig. 4. Distribution of marked and

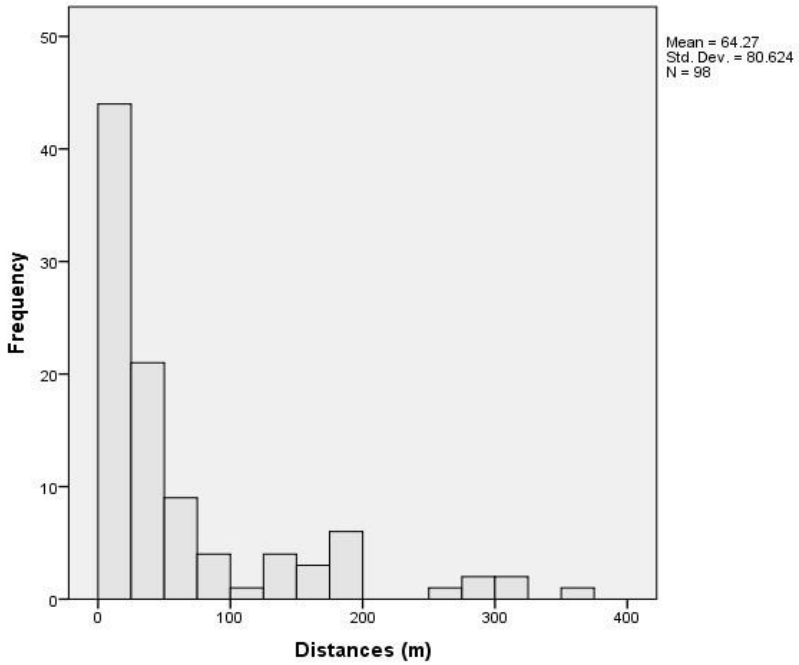


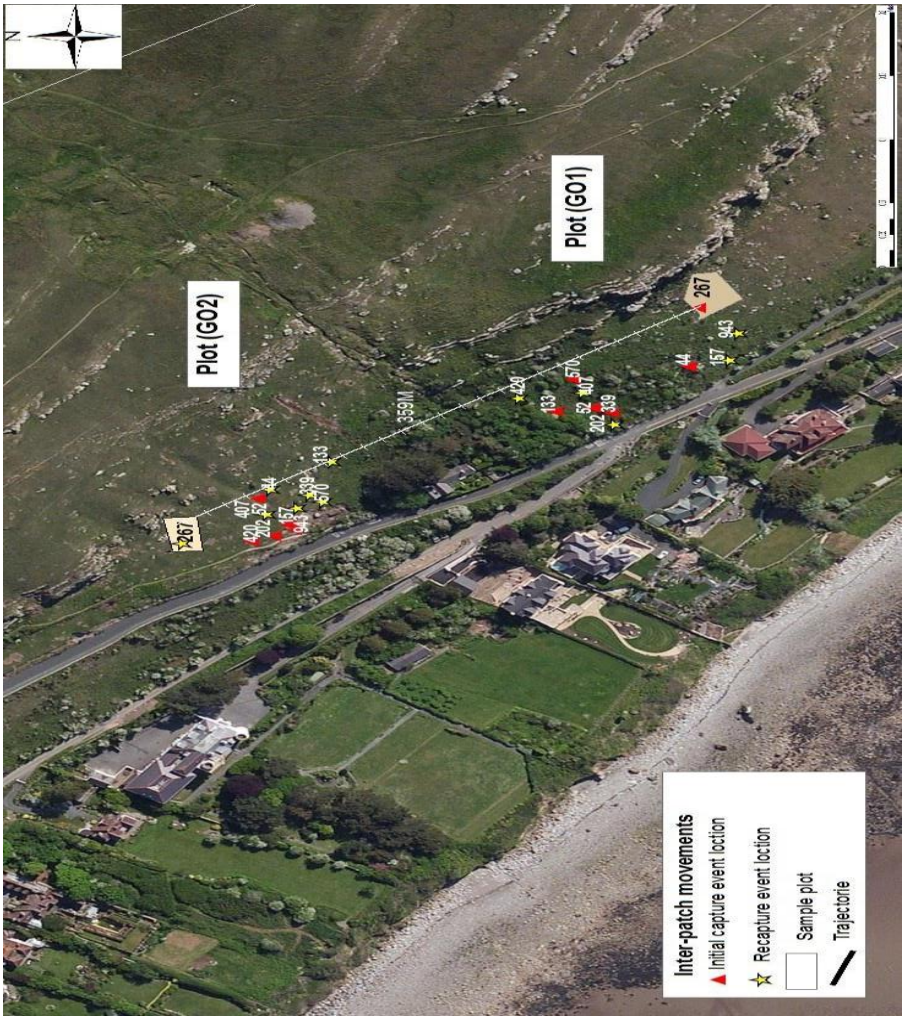
Fig. 5. The frequency of dispersal distances between butterflies.

Inter-patch movements:

The movement between patches was carried out by plotting the flight butterflies in ArcGIS Desktop 10.3 (Fig. 6). 11 individual butterflies were detected and moved between patches. Of these, 64.4% were males and 36.6 % were females. 54.5% were initially moved from the GO2 patch where they were marked to patch GO1 and 45.5% were initially moved from patch GO1 to GO2 (see Table 8).

Table 8. Number of males and females, which were interchanged between the two plots.

PLOTS	MALES	FEMALES	TOTAL
PLOT GO1 TO GO2	4	1	5
PLOT GO2 TO GO1	3	3	6
TOTAL	7	4	11



(Fig. 6).showed Inter-patch movements of *Plebejus argus* between study plots

Discussion

Based on these results, Four hundred and one individual butterflies were captured and marked over the 4-week capture period. , there is no difference in the number of butterflies in the two main patches and from that there are two colonies which are balanced in butterfly number and are located close to each other. However, in the past, the study by Thomas (1985b) in the Great Orme site for the Status and

Conservation of *Plebejus argus,* found ten colonies on limestone habitat, six of them consisted of between 40-4,000 individuals and the four colonies are more than 20,000 individuals at the peak of the flight period, in addition to the Sandlings of East Anglia, England. The colony consisted of more than 5,000 *Plebejus argus* on heathland habitat within 0.07 ha (Ravenscroft, 1990).

The results of this study show that, The population size of *Plebejus argus* is considered to be quite small compared to many others studies. However, the patch size is more likely to be reflected in the smaller population in both two patches, which are no more than 1km in size. In the Dulas Valley in North Wales, the population of *Plebejus argus* size was estimated to be 144 individuals in 0.22ha of habitat patch and 1975 individuals in a 3 ha patch, whilst another study found that the population of this species reached 20,000 adults in 8 hectares in North Wales (Thomas, 1985a; Lewis *et al*., 1997).

The longest dispersal distance recoded at the Great Orme was 359m among all the butterflies' movement. 64.35% of all movements were found between 0-50m, 12.8% were between 50-100m, and 2.97% were greater than 250m. In a similar finding from other studies done on the *Plebejus argus* in the Dulas Valley in North Wales by Lewis *et al*. (1997), the maximum distances were 395m, 2% of individual butterflies moved more than 100m, were only 1.4% were moved between patches, which separated (13-200m) . Moreover, a total of 663 individuals of the Woodland Brown butterfly were marked in 1989 in Sweden, only 11% of them were moved between their habitats (Bergman and Landin, 2002).

Inter-patch movements were conducted between the two main patches for *Plebejus argus.* They were found to have generally low mobility and the majority of their movement occurred within their habitats, a few butterflies were found to move for longer distances and interchanged between patches G1 and G2. The 11 individual butterflies were detected by movement between patches. Of these, It seems likely that the low movement between habitats can be behind several reasons that were stated to contribute to a lower movement rate. Although the population size of *Plebejus argus*is found to be very low when compared to other studies, this would not have affected a lot of movement between patches. For example, the study by Lewis *et al*. (1997) that found that from a large population of *Plebejus*

argus(which was nearly 10,000 individual butterflies) only 1.4% were detected by movement between patches. In another example of that in a dispersal study for two species of diurnal burnet moth, 8·5% were recorded that had moved between habitat patches for both species (Franzén and Nilsson, 2007).

The interchange between patches was conducted between the two nearest colonies (GO1-GO2) which are separated by 200m between each other within the actual range of *P.argus*. Navigation in the area surrounding the two study plots are so difficult to reach for butterflies that fly there and it can lead to a reduction in the number of butterflies captured. This could be the main factor that affects the low interchange between patches within this site as it consists of private house which act as barriers to dispersal. The fact that the butterfly has to fly so close to the ground and is unlikely to fly over, suggests that no butterflies were observed or recaptured over that area.

Conclusions and recommendation the corridors have been found to be a perfectly beneficial way to increase connectivity between isolated habitats, which can aid species' survival by exchange between local populations (Haddad, 1999; Cross, 2004). In future management of the site, the corridors are increasingly necessary to aid these colonies' survival and the aim to increase their number as a result shows that the area between patch 1 and patch 2 is more likely to act as a barrier to this species.

As result of the present study, few *Plebejus argus* were found to have moved between their colony. The current situation of colonies of *Plebejus argus* were not located close enough due to this butterfly not being expected to fly more than 50-100m in most of their movement, and a migration rate of more than 1km is thought to be very rare. Where these colonies are separated by 200 m, in a conservationist view, the area needs to shrink to fit closely to allow these populations to exchange or expand the colony to the surrounding area, however the area between these colonies is outside the Great Orme site. This may involve the purchasing of more land as part of the site. In future research for dispersal in this area should be included as part of the study by getting permission from the land owner which should lead to getting the full picture of dispersal and metapopulation structure. As the main patches are located closely to marine drive, which is used as a road for cars and the effect of the road is that it acts as a barrier to *Plebejus argus*was not as part of this study, however the findings of

this study will strongly recommend that the effect of roads should be taken into account in future research.

An alternative perspective on the impact of shrub and tree cover can be seen in research carried out by Dennis & Sparks (2006) at Great Orme's Head in North Wales. The study found that tall, vegetation structures, surrounding or close to host plants, were essential requirements for *P. argus*caernensis facilitating activities such as roosting, mating, courtship and shelter. An additional study by Dennis (2003) found that in coastal locations, *P. a.caernensis* was more likely to be abundant on taller shrubs than on host plants unless the percentage of sunshine was consistently high and wind speed low. He pointed out that as *P. a.caernensis* use different vegetation for different purposes throughout the day, it is difficult to establish the habitat preferences of the butterfly with any accuracy as it will depend on the time of day and weather conditions the survey is undertaken in.

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BIOCHEMICAL STUDIES ON MALATHION [ORGANOPHOSPHOROUS COMPOUND] IN ALBINO RATS

Thabet A. Mohamed Nagah , Mohamed F. salem zlitni, Naima Mohamed Omer Elkakalli
.Ahmed M. Elmesalati .
Higher Institute for Technical Medical Science, Tripoli-Libya
Higher Institute for Technical Medical Science, Tripoli-Libya
Tripoli Diabetic Centre
College of dentistry/ Tripoli university
Attahadi University.

دراسات على كيمياء الدم في الفئران البيضاء المجّرعة بمبيد الملاثيون

ثابت الهاني محمد نجاح — المعهد العالي للعلوم والتقنيات الطبية /طرابلس
أحمد ايهاب محمد المسلاتي — جامعة التحدي الطبية الاهلية
محمد فرج الزليطني — المركز الوطني لأمراض السكر والغدد الصماء
نعيمة محمد الككلي — جامعة التحدي الطبية الاهلية

Abstract

Insecticides are widely used in agricultural fields to achieve greater production, protection and conservation of food products. This results in many different human and animal infections. These infections are usually caused by mouth, skin, or inhalation.
Malathion is one of those pesticides that are used extensively in agricultural fields for the protection of agricultural crops such as vegetables or fruit from agricultural pests that may be caused by mosquitoes, manure, or some other insects.
This study aimed to shed more light on some of the expected side effects due to the excessive use of this pesticide.
The study was conducted on one hundred and forty-four white mice divided into major groups (A, B, C, D) each consisting of 36 mice. The main groups are divided into three small groups (1), (2), (3) and each consists of twelve groups as follows:
The first group: its smallest daily dose (1/10) of the half-lethal dose of melatonin dissolved in oil was reduced to three different periods of 7 days, 21 days, 90 days respectively.
The second group: The smallest group (1/50) of the half-lethal dose of melatonin dissolved in oil was mined on three different periods of 7 days, 21 days, and 90 days respectively.
Group 3: The smallest group (1/100) of the half-lethal dose of melatonin dissolved in oil was mined at three different intervals of 7 days, 21 days, and 90 days respectively.
Group 4: This group was considered as an control group, and its smaller group was restricted to oil only for the same periods.
Samples were taken at the end of each period of the dosage alone to conduct various tests on blood, serum and liver, kidney, heart, brain, intestines, and spleen samples for histopathological tests:.
The results were as follows:
Clinical symptoms:
Increased salivation, tears, and nose were observed with lack of appetite for food and decreased weight with bays, imbalance and swaying. These symptoms were severe in group

(A) while the symptoms were similar in group (B) but with minimal reduction. While these symptoms are absent in group (c)..

Effect on liver function:

Serum analysis showed a significant increase in liver enzyme, total bilirubin, direct and indirect, and total cholesterol. There was also a significant reduction in total protein, albumin and globulin in the large dose group, while no change was observed in the two lowest concentration groups.

Effect on kidney function:

There was an increase in urea, creatinine, and calcium, with a decrease in phosphorus in the major dose group. No change was observed in the two least concentrated groups except for the increase in calcium and the decrease in the phosphorus rate.

الملخص

تستخدم المبيدات الحشرية بكثرة في المجالات الزراعية وذلك لتحقيق مزيدا من الانتاج والحماية والحفظ للمنتجات الغذائية ، وينتج عن ذلك الكثير من الاصابات المختلفة للإنسان والحيوان على حد السواء ، وتلك الاصابات تحدث عادة عن طريق الفم ، الجلد، أو عن طريق الاستنشاق.

والملاثيون هو أحد تلك المبيدات التي تستخدم بوفرة في المجالات الزراعية وذلك من أجل الحماية اللازمة للمحاصيل الزراعية سوآءا كانت خضار أو فاكهة من الآفات الزراعية والتي قد يسببها البعوض ، المن، أو بعض الحشرات المختلفة.

واستهدفت هذه الدراسة إلقاء مزيدا من الضوء على بعض الآثار الجانبية المتوقعة نتيجة الاستعمال المفرط لهذا المبيد.

أجري هذا البحث على مائة واربعة واربعين فأرا ابيض قسمت الى مجموعات رئيسية (أ) ،(ب)،(ج) ،(د) كل منها يتكون من ستة وثلاثين فأر. قسمت المجموعات الرئيسية الى ثلاث مجموعات صغرى (1)،(2)،(3) ويتكون كل منها من اثني عشر فأرعلى النحو التالي:

المجموعة الاولى: وقد تم تجريع مجموعتها الصغرى يوميا (10/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.

المجموعة الثانية: وقد تم تجريع مجموعتها الصغرى يوميا (50/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.

المجموعة الثالثة: وقد تم تجريع مجموعتها الصغرى يوميا (100/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.

المجموعة الرابعة: وقد اعتبرت هذه المجموعة كمجموعة ضابطة، وقد تم تجريع مجموعتها الصغرى بالزيت فقط، وذلك لنفس الفترات السابقة.

تم أخذ العينات عند نهاية كل فترة من التجريع على حده ، وذلك لإجراء الاختبارات المختلفة على الدم ، المصل، وكذلك أخذت عينات من الكبد، الكلية، القلب، الدماغ ، الامعاء ، الطحال لإجراء الفحوصات الهستوباثولوجية:

وقد كانت النتائج على النحو التالي:

أولا: الأعراض الاكلينيكية:- لوحظ زيادة في افراز اللعاب ،الدموع، والأنف مع نقص في الشهية للطعام وانخفاض في الوزن مع خلجان، عدم الاتزان والتمايل . وكانت هذه الاعراض شديدة في المجموعة (أ) بينما تشابهت الاعراض في المجموعة (ب) ولكن بأقل حدية. في الوقت الذي إنعدمت فيه هذه الاعراض في المجموعة (ج).

التأثير على وظائف الكبد:- بأخذ تحاليل المصل وجدت زيادة ملحوظة في انزيم الكبد ، والبيليروبين الكلي ، المباشر والغير مباشر ، والكوليسترول الكلي ، كما سجل انخفاض ملحوظ في معدل البروتين الكلي، والالبيومين والجلوبيولين وذلك في مجموعة الجرعة الكبرى، بينما لم يلاحظ أي تغيير في الجرعتين الأقل تركيز.

التأثير على وظائف الكلية:- لوحظ زيادة في نسبة اليوريا ، الكرياتينين ، والكالسيوم ، صاحب ذلك نقص في الفوسفور وذلك في مجموعة الجرعة الكبرى ، بينما لم يلاحظ وجود أي تغيير في المجموعتين الأقل تركيز بإستثناء الزيادة في نسبة الكالسيوم ونقص في معدل الفوسفور.

Introduction

Organ phosphorus pesticides are a group of compounds with various toxicities to different forms of life. The wide use of these compounds are mostly in the agricultural affairs of the most developing countries which have entailed the extensive use of pesticides for better production , protection and preservation of food grains .

Malathion is one of the most commonly used organophosphorus pesticides in agriculture and is known to be least toxic to mammals as compared to insects (Debruin, 1970). It is used to a few plant – crops and widely used to control plant pests, flies and parasites of livestock

Exposure to these toxic chemicals occurs via oral, dermal and inhalation.

In rats, acute Malathion toxicity severely impairs the digestive and absorptive function of intestine (Chakravarly and Ghosh 1980).

Malathion also causes decrease in the hepatic AST and ALT activities (Bulusu and Chakravarty 1984). And causing severe leukocytosis (Gupta and Paul 1972).

In cockerels malathion led to increase in the serum AST and cholesterol as well as hydropic degeneration and hepatocellular necrosis of the liver lymphoid follicles of spleen necrosis and hyperplastic changes , Degenerated changes of adrenal cortex *(Varshneya , Bahga and Sharma*

Exposure of chicks to malathion significantly decrease erythrocytic count and haemoglobin level while in the later stage, these parameters had returned to normal levels *(Srivastava , Saxena and Sharma 1960*)

MATERIAL AND METHODS

Insecticide

The organophosphorus compound Malathion diethyl (dimethixyphosphinothioy/thio succinate) was purchased from kataw Gonker Bombay Pesticides Company "India The insecticides was dissolved in corn oil, and different dilution (1/10 , 1/50 and 1/100 of acute oral LD50) were prepared . Acute oral LD50 was calculated according to Boyd and Tanikella (1969) (LD50 = 2800mg/kg.b.wt.

The Animals

One hundred and forty four of apparently clinically healthy female albino rats (120 to 150 mg in weight) used in our experiment. The animals housed under suitable lighting, temperature and proper hygienic condition. Food and drinking water available ad libitum.

Diagnostics Kits

Commercial diagnostic kit produced by Sclavo Diagnostic Laboratories Company (Laboratory Reagent and Chemical Products) "Italy" , were used for determination of inorganic phosphorus,bilirubin(total and direct), alanine aminotransferase , aspartate amino transferase , total protein , albumin and globulin blood urea , nitrogen , creatinine and cholesterol .

Experimental Design

One hundred and forty four of female albino rats were divided into four groups (A, B, C and E), each of 36 rats (Table1)

Rats in group "A" were subdivided into three subgroups (A1, A2 and A3) , each subgroup consisted of twelve rats , assigned for daily oral treatment with 1/10 of LD50 of malathion , dissolved in mineral oil (corn oil) and administered by a stomach tube for 7 , 21 and 90 days respectively .

The rats in group "B" were subdivided into three subgroups (B1, B2, and B3) , each subgroup contained twelve rats and assigned for daily oral treatment with 1/50 of LD50 of malathion dissolved in (corn oil) by a stomach tube for 7,21 and 90 days .

The rats in group "C" were subdivided into three equal subgroups (C1, C2, and C3), each of twelve rats and daily orally treated with 1/100 of LD50 of malathion dissolved in mineral oil (corn oil) by a stomach tube for the previous period .

The rats in-group "E" divided into three equal subgroups (E1, E2 and E3), kept as a control and orally received oil alone for 7, 21, and 90 days. Twelve rats from each subgroup sacrificed at the end of their treatment.

groups	Subgroup	No. of animals	Treatment	Days of treatment
A	A1	12	Daily oral treatment	7
	A2	12	with 1/10 LD50	21
	A3	12		90
B	B1	12	Daily oral treatment	7
	B2	12	with 1/50 LD50	21
	B3	12		90
C	C1	12	Daily oral treatment	7
	C2	12	with 1/100 LD50	21
	C3	12		90
E	E1	12	Control untreated	7
	E2	12		21
	E3	12		90

1/10 LD50 = 43.5 mg/Rate
1/50 LD50 = 8.7 mg/Rate
1/100 LD50 = 4.35 mg/Rate

Sampling

Blood samples collected from each rat in a clean tube containing "EDTA" anticoagulant for estimation of Serum for determination of serum biochemical parameters.

Methods of Examination:

Serum Biochemical Parameters:

1. **AST*and ALT****
   Activities of aspartate and alanine aminotranaferase (AST and ALT) were determined calorimetrically according to the method of Reitmans and Frankel (1957)
2. **Blood Urea Nitrogen:**
   Colorimetric determination of urea was done after Fawcett and Scott (1960).
3. **Creatinine**
   This test was determined according to Henery (1974)
4. **Total protein:**
   Colorimetric determination of total protein performed based on the Biuret reaction (Henery 1968).
5. **Total cholesterol:**
   Colorimetric determination of total cholesterol done according to Henery(1974)
6. **Albumin:**
   Colorimetric determinationof albumin done according to Rodkey (1965)
7. **Total Bilirubin, Direct and indirect:**
   Colorimetric determination of total,direct and indirect were described by Winsten-Chehelyk (1969).
8. **Phosphorus:**
   Colorimetric determination of phosphorous done according to Daly and Ertingshausen (1972).
9. **Calcium:**
   Colorimetric determination of calcium done according to Davidsohn and Henery (1974).

RESULTS & DISCUSSION:

Mortalities:

Seventeen rats died from group 'A' during the periods of the experiment, four rats from subgroup 'A1' six rats died from subgroup 'A2' and seven rats died from subgroup 'A3'

. *Biochemical Parameters*

The effect of daily oral administration of 1/10 LD_{50} of malathion on different serum parameters was illustrated in Table (3) and figs .(13-25). It showed significant increase in the level of transaminases , total , direct and indirect bilirubin in both subgroup A_1 and A_2 by +23.11% , +25.53% , 39.58% , +19.05%,+61.54% , +53.06%, +48.68% , +58.42%,+43.33% and +78.26% respectively , without significant changes in subgroup A_3 .

The data showed hypoproteinemia , hypoalbuminemia and hyperglobulinemia in subgroup $A_1$and A_2 by -37.74% , -35.50% , -40.04%, -26.13% , -37.93% and -56.31% respectively without significant changes in subgroup A_3 Total cholesterol , blood urea nitrogen , creatinine and calcium showed significant increase in both subgroups A_1 and A_2 by -31.03% , +6.34% , 44.27%, +30.25% , -52.51% , +36.44% , +117.22% and +188.85% respectively , In subgroup A_3 there was significant increase in calcium levels by 137.85% , without significant changes in T . Cholesterol, BUN and creatinine, while the phosphorus level was significantly decreased in all subgroups A_1 , A_2 and A_3 by -51.73% , -69.93 and -62.12% respectively

The serum biochemical constituents of rats orally treated with 1/10 LD50 of Malathion were greatly influenced. The elevation of serum ALT and AST activities in rats treated for (7^{th} and 21^{st} days) observed. This confirms the idea that the insecticide has a revere damaging effect on body tissues. The present results could e supported by the histopathological observation of lesions in the liver could be supported by the histopathological observation of lesions in liver, kidney, intestine and heart muscles. Our findings agree with Gupta et al. (1981) . The elevation of aminotransferase in serum may be sue to : (i) tissue damage particularly in liver , kidney and heart (Rouiller , 1964) , and (ii) increased permeability of cell membrane , or increased synthesis or decreased catabolism of aminotransferase (Dinman et al . 1963) . Recently, Malik et al. (1979) demonstrated that elevation of

these enzymes in plasma of rats treated with 274 mg/kg of an organophosphorus compound (fenitrothion) orally is due to increased synthesis of those enzymes in brain and liver. Cockerels receiving malathion showed significant increase in ALT and AST. This increase was indicative of damage to internal organs like, liver, myocardium and muscles , as reported by Cornelius (1970) . Similar findings were recorded in buffalo calves following malathion toxicity as state by Uppal and Ahmed (1977) . The same results were observed by Wright (1966) , Luckens& Phelps (1969) , Younger & Wright (1971) , Malik et al . (1980) , Westlake et al . (1981) , Gupta (1984) and AbdalJabbar et al . (1990) . Meanwhile, rats treated for 90 days an the rats treated with 1/50 and 1/100 LD50 during (7,21 and 90 days) showed no changes in serum transaminases These results are in agreement with Roe (1969) .

Significant alteration in the total bilirubin level and both conjugated and unconjugated bilirubin were observed in rats received 1/10 LD_{50} of malathion for 7 and 21 days. Meanwhile, after 90 days and in the doses 1/50 and 1/100 LD_{50} doesn't make any changes in the total direct and indirect bilirubin along the period of experiment (7,21 and 90 days) . The same results agreed with the finding of Varshneya et al .(1988) who stated that the dietary intake of malathion up to 1,600 ppm did not influence several parameters such as total protein , total biliubin and protein bound iodine in serum .

In the present experiment, hypoproteinemia, hypoalbuminemia and hpoglobulinemia were observed in rats treated with 1/10 LD_{50} of Malathion for 7 and 21 days if compared with control group. This decrease can be attributed to either in appetence and poor absorption of dietary constituents from intestinal tract as reported by Coles (1980) or to the effect on the liver as said before. Similar findings were obtained by Bulusu et all. (1984), Amer and Abdul El – all (1987) and Abdul Jabbar et al . (1990) . Earlier reports have also shown the reduction in protein concentration in liver of rats and fish post the administration of malathion and other organphosphates (Varshenyer and Sharma 1986; and Akhtar 1985) .

In the present study, no changes were observed in the proteinogram after either 90 days or post 1/50 and 1 /100 LD_{50} in the different periods. The obtained results agreed with those previously recorded by Varshney et al . (1988) , and by Thatoo and Prasad (1989) .

Rats given 1/10 LD_{50} of malathion revealed significant increase in the level of total cholesterol after 7 and 21 days of treatment . Hyperchlestrolemia is expected to occur in some diseases of the liver , nephritic syndrome , diabetes mellitus and acute pancreatitis . It is suggested that impairment of liver , kidneys and pancreas by the insecticide had resulted in the observed changes of serum cholesterol as reported by Nariman (1994) . The hypercholesterolemia was consistently present with hyperlipodaemia , this could occur due to inhibition of 7 – hydroxylation of cholesterol of liver microsomes , as stated by Shefer et al . (1968) . The increase in cholesterol may also be sue to the constriction of bile – suct because of the accumulation of acetylcholine under the influence of anticholinesterase action of malathion as reported by Hothi and Kwatra (1972) . The same results were reported by Varshneya et al . (1988) and Thatoo and Prasad (1989) .

Measurement of blood urea nitrogen and creatinie is helpful to detect efficiency of glomerular filtation rate and renal blood flow as reported by Medway et al .(1969) . In the present study , BUN and creatinine concentration were increased in rats treated with 1/10 LD_{50} for 7 and 21 days . The obtained results together with the observation of kidney lesion by histopathologic mean would indicated involvement of the kidney in the excretion of the insecticide . It is suggested that uremia was due to increased catabolism of body proteins , decreased renal blood flow as a result of the general circulatory distress , or renal damage from the insecticide . The present finding agree with those of Naherman et all . (1974) who recorded elevation of serum urea nitrogen in case of acute toxicity of rats with the organphosphorus compound dichlorocos . Ali (1983) also recorded an increase in serum urea nitrogen in goats received cyolane orally for 32 weeks. These results were also reported by Abdel Salam et al . (1982) and Saad (1992) . These results agreed with the findings of Thatoo and Prasad (1989) who fed lambs with malathion for 2,4 and 6 months . They found that the level of total serum protein and BUN did not show much variation and experimental groups. Our results also agree with those of Dheranetra et all. (1988) who stated that given of Malathion orally to rat caused reduction of BUN-creatinine ratios.

Measurement of calcium level in rats treated with all different concentrations of malathion 1/10 , 1/50 and 1/100 LD_{50} showed significant increase during all the period of the experiment (7,21 and 90 days) . The same results agreed with the finding of Chanadra et al et all . (1981) who recorded an increase in serum calcium after acute Malathion toxicity in poultry. Similar results were shown by Amer and Abdel – All (1987).

Phosphorus level revealed significant decrease due to 1/10, 1/50 and 1/100 LD_{50} of Malathion at 7, 21 and 90 days after treatment. Our results disagreed with Amer and Abdel – all (1986) who recorded non-significant variation between treated and intoxicated animals the level of inorganic phosphorus.

CONCLUSION

Experiment 1 (Group A)

Activities of serum enzymes, total, direct indirect bilirubin, total cholesterol, urea, creatinine and calcium were markedly elevated. While hypoproteinemia, hypoalbuminemia and hyperglobulinemia were recorded after 7 and 21 days. Moreover, phosphorus level decreased.

Experiment 2 (Group B)

Biochemical parameters showed an increase in the level of calcium and decreased level of phosphorus.

Experiment 3 (Group C)

Results of rats received 1/100 LD_{50} of Malathion showed no changes in the serum biochemical parameters except that the values of calcium were markedly elevated and the level of phosphorus decreased.

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HITOPATHOLOGICAL STUDIES ON MALATHION [ORGANOPHOSPHOROUS COMPOUND] IN ALBINO RATS

Thabet A. Mohamed Nagah , Mohamed F. salem zlitni, Naima Mohamed Omer Elkakalli.Ahmed M. Elmesalati .

Higher Institute for Technical Medical Science, Tripoli-Libya
Higher Institute for Technical Medical Science, Tripoli-Libya
Tripoli Diabetic Centre
College of dentistry/ Tripoli university
Attahadi University.

دراسات الأمراض النسيجية الناتجة عن مبيد الملاثيون في الفئران البيضاء

ثابت الهاني محمد نجاح — المعهد العالي للعلوم والتقنيات الطبية /طرابلس
أحمد ايهاب محمد المسلاتي — جامعة التحدي الطبية الاهلية
محمد فرج الزليطني — المركز الوطني لأمراض السكر والغدد الصماء
نعيمة محمد الككلي — جامعة التحدي الطبية الاهلية

Abstract

The present work was carried out to study the effect of short term administration of the insecticides "Malathion" on histopathological structure of the liver, kidney, intestine, spleen, heart and brain of albino rats.

Thirty six albino rats of about 120-150 gm average weight were used in this work, and were divided into three subgroup each of 12 rats assigned for daily oral treatment with C1/10, LD50 for 7 days. Rats in group E represents the normal control group and received corn oil only. Tissue samples were collected for histopathological studies.

Microscopical examination of liver showed congestion of central, portal veins and blood sinusoids, small focal area of lymphocytic aggregation, mononuclear cellular aggregation in the portal area with vacuolation and hydropic degeneration of the hepatocytes were noticed. Section of kidneys revealed dilation of blood vessels of medulla and cortex, periglomerular lymphocytic aggregation, focal area of mononuclear cellular aggregation.

الملخص

تستخدم المبيدات الحشرية بكثرة في المجالات الزراعية وذلك لتحقيق مزيدا من الانتاج والحماية والحفظ للمنتجات الغذائية ، وينتج عن ذلك الكثير من الاصابات المختلفة للإنسان والحيوان على حد السواء ، وتلك الاصابات تحدث عادة عن طريق الفم ، الجلد، أو عن طريق الاستنشاق. والملاثيون هو أحد تلك المبيدات التي تستخدم بوفرة في المجالات الزراعية وذلك من أجل الحماية اللازمة للمحاصيل الزراعية سوآءا كانت خضار أو فاكهة من الآفات الزراعية والتي قد يسببها البعوض ، المن، أو بعض الحشرات المختلفة. واستهدفت هذه الدراسة إلقاء مزيدا من الضوء على بعض الآثار الجانبية المتوقعة نتيجة الاستعمال المفرط لهذا المبيد. أجري هذا البحث على مائة واربعة واربعين فأرا ابيض قسمت الى مجموعات رئيسية (أ) ،(ب)،(ج) ،(د) كل منها يتكون من ستة وثلاثين فأر. قسمت المجموعات الرئيسية الى ثلاث مجموعات صغرى (1)،(2)،(3) ويتكون كل منها من اثني عشر فأر على النحو التالي:

المجموعة الاولى: وقد تم تجريع مجموعتها الصغرى يوميا (10/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.

المجموعة الثانية: وقد تم تجريع مجموعتها الصغرى يوميا (50/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.

المجموعة الثالثة: وقد تم تجريع مجموعتها الصغرى يوميا (100/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.

المجموعة الرابعة: وقد اعتبرت هذه المجموعة كمجموعة ضابطة، وقد تم تجريع مجموعتها الصغرى بالزيت فقط، وذلك لنفس الفترات السابقة.

تم أخذ العينات عند نهاية كل فترة من التجريع على حده ، وذلك لإجراء الاختبارات المختلفة على الدم ، المصل، وكذلك أخذت عينات من الكبد، الكلية، القلب، الدماغ ، الامعاء ، الطحال لإجراء الفحوصات الهستوباثولوجية:

وقد كانت النتائج على النحو التالي:

أولا: الأعراض الاكلينيكية:-

لوحظ زيادة في افراز اللعاب ،الدموع، والأنف مع نقص في الشهية للطعام وانخفاض في الوزن مع خلجان، عدم الاتزان والتمايل . وكانت هذه الاعراض شديدة في المجموعة (أ) بينما تشابهت الاعراض في المجموعة (ب) ولكن بأقل حدية. في الوقت الذي إنعدمت فيه هذه الاعراض في المجموعة (ج).

التغيرات الباثولوجية:-

أولا: الكبد:- كانت التغيرات الباثولوجية أكثر حدية في المجموعة ذات الجرعة الكبرى ، حيث تمثلت في وجود إحتقان في الاوردة الرئيسية والجيبانيات الدموية ، مع وجود مساحة بؤرية صغيرة من تجمعات للخلايا الليمفاوية مع تجمعات خلوية أحادية الخلايا في المساحات البابية. مع وجود تجوف وفساد استسقائي في خلايا الكبد.

ثانيا : الكلية:- شوهد إمتلاء عروق النخاع والقشرة بالدم، مع وجود تجمعات للخلايا الليمفاوية تحيط بالكبيبات الكلوية، اضافة الى تجمعات أحادية الخلايا في المساحات البابية.

ثالثا: القلب:- لوحظ إحتقان في لعروق الدموية لعضلة القلب ، مع وجود نزيف دموي بين العضلات.

رابعا: في الامعاء:- لوحظ وجود إتشاحات خلوية ليمفاوية مع وجود تنكس مخاطي في النسيج الظهاري.

خامسا: في الدماغ:- لوحظ وجود تنكس عصبي مع وجود تجمعات لخلايا ليمفاوية حول وعائية

سادسا: في الطحال:- لوحظ وجود تكثر نسيجي في اللب الابيض.

أما في المجموعتين الاخريين فقد وجد تشابه في التغيرات الباثولوجية في بعض الاعضاء وإن كانت أقل حدة، واختلفت في البعض الآخر من الأعضاء.

OBJECTIVE. The aim of the present study is to investigate the effect of short – and long – term administration of Malathion to investigate the histopathological alteration in the liver, kidney, intestine, spleen, heart and brain of albino rats, we hope that this work would throw a more light on the toxic effect of this pesticide.

INTRODUCTION: Organophosphorus pesticides are a group of compounds with various toxicities to different forms of life. The wide use of these compounds are mostly in the agricultural affairs of the most developing countries which have entailed the extensive use of pesticides for better production , protection and preservation of food grains .

Malathion is one of the most commonly used organophosphorus pesticides in agriculture and is known to be least toxic to mammals as compared to insects (Debruin, 1970). It is used to a few plant – crops and widely used to control plant pests, flies and parasites of livestock Exposure to these toxic chemicals occurs via oral, dermal and inhalation.

Malathion toxicity in human being revealed miosis, excessive secretion of the salivary, sweat, bronchial and lacrimal glands, Bronchoconstriction, pulmonary edema, cyanosis, hypermotility of the digestive and urinary tracts resulting in vomiting and involuntary defecation and urination. On the other hand, Malathion through its effect on central nervous system and myoneural junction may result in

headache, restlessness, anxiety, tremors, convulsions, muscular twitches, drowsiness and coma (Goldman; Teitel; and Manhasset, 1958).

The clinical signs reflect the great disturbances in the functional status of different organs as brain, heart, liver, pancreas, kidney, intestine and other organs.

In dog, Malathion causes gastritis, enteritis, colitis, diffuse lung congestion, convulsion, vomiting and malaise {McCurnin and D.V.M. 1969}.

Hypersecretion of adrenaline, which lead to hyperglycemia, reported in lambs subjected to Malathion [Thatoo and Parasad 1989].

In rats, acute Malathion toxicity severely impairs the digestive and absorptive function of intestine (Chakravarly and Ghosh 1980).

REVIEW OF LITERATURE:

TOXICITY OF ORGANOPHOSPHOROUS COMPOUNDS:

Goldman Teitel and Manhasset (1958) reported the early symptoms of the accidental Malathion poisoning after the ingestion of about 8cc of 50% Malathion by thirty-four months old child weighing twenty-one kg. They found that the symptoms began fifteen minutes after ingestion and consisted of coma, pulmonary edema, miosis, hypersalivation, muscle flaccidity, involuntary defecation and urination.

Bourke, Broderick, Hackler and Lippold (1968) stated that rat eliminated the bulk of ingested radioactive Malathion in the urine within twenty-four hours.

Mccurnin (1969) observed the clinical signs of Malathion intoxication in twenty-four inadvertently military scout dogs, which were exposed, to one or more inadequately diluted malathion-dip solution. The signs were of extreme weakness, excessive salivation, anorexia, muscle tremors, convulsion, malaise, arched back, elevated body temperature and some vomiting.

Gupta and paul (1979) studied the bioavailability and metabolism of [P32] malathion after a single oral dose of 262.4 mg/kg in adult hens (1.2 to 1.8 kg in wight). They suggested that thecompound was absorbed rapidly from gastrointestinal tract and serious signs observed.

Amer and Abd El-all (1987) observed that the accidental Malathion toxicity signs in ewes included profuse salivation, diarrhea, ataxia and tremors of the skeletal musles.

Thatoo and Prasad (1988) observed the influence of daily oral administration of (20 mg/kg B.W.) of Malathion in twelve months pregnant sheep (1.5 years old). They described the syndromes, which were ataxia, hind leg weakness and depression; abotyion, birth, dystocia, and retention of placenta and all lambs born were below normal weight.

Muan and Nafstad (1989) studied the distribution pattern of Malathion after intravenous administration of [C14] Malathion to rats.Highest levels of radioactivity detected in the liver and the kidneys.

Abo-Zeid, El-Barouty, Abdel-Reheim, Blancato. Dary, El-Sebae and Saleh (1993) recorded that more than 90% of C14-methoxy-malathion released with urine after 24 hours from administration to male albino rats by oral or dermal route. The rest of C14 detected in the feces, blood, intestine, liver and kidney in descending order. Moreover, no significant C14 detected in other organs.

MATERIAL AND METHODE:

MATERIAL: ***Insecticide*** The organophosphorus compound Malathion (diethyl (dimethixyphosphinothioy/thio) succinate) was purchased from katawGonker Bombay Pesticides Company "India The insecticides was dissolved in corn oil, and different dilution (1/10 , 1/50 and 1/100 of acute oral LD50) were prepared . Acute oral LD50 was calculated according to Boyd and Tanikella (1969) (LD50 = 2800mg/kg.b.wt.

The Animals: One hundred and forty four of apparently clinically healthy female albino rats (120 to 150 mg in weight) used in our experiment. The animals housed under suitable lighting, temperature and proper hygienic condition. Food and drinking water available ad libitum.

Experimental Design: Thirty six of female albino rats were divided into four groups (A, and E), each of 36 rats (Table1) Rats in group "A" were subdivided into three subgroups (A1, A2 and A3) , each subgroup consisted of twelve rats , assigned for daily oral treatment with 1/10 of LD50 of malathion , dissolved in mineral oil (corn oil)

and administered by a stomach tube for 7 , 21 and 90 days respectively .

The rats in-group "E" divided into three equal subgroups (E1, E2 and E3), kept as a control and orally received oil alone for 7, 21, and 90 days. Twelve rats from each subgroup sacrificed at the end of their treatment.

Groups	Subgroup	No. of animals	Treatment	Days of treatment
A	A1 A2 A3	12 12 12	Daily oral treatment with 1/10 LD50	7 21 90
E	E1 E2 E3	12 12 12	Control untreated	7 21 90

1/10 LD50 = 43.5 mg/Rate
1/50 LD50 = 8.7 mg/Rate
1/100 LD50 = 4.35 mg/Rate

Sampling: Specimens from liver, kidneys, brain, intestine, heart and spleen obtained in 10% formalin for histopathological examination after carful gross pathological examination.

METHOD: Specimens from liver, kidneys, brain, intestine, heart and spleen from scarified rats fixed in 10% formalin, embedded in paraffin, sectioned at 5 microns thickness and stained with hematoxylin and Eosin according to Thomas and Richter (1984).

Results: Pathological Finding: Macroscopical Examination: The gross changes observed in Group A (received 1/10 LD50 Malathion). The examined livers slightly enlarged in size and pale in colour in many cases. In some cases, the liver and kidneys were congested. The coronary vessels of the heart showed congested. Moreover, excessive amount of mucous seen on the intestinal mucosa.

Microscopical Picture:

Subgroup A1 (received 1/10 LD50 Malathion for 7 days).

Liver: The examined livers showed congestion of central veins, portal vein and blood sinusoids. Small focal areas of lymphocytic aggregation commonly observed in between the hepatic parenchyma. Mononuclear cellular aggregation also

detected in the portal area (fig). Moreover, vaculation and hydropic degeneration of the hepatocytes noticed in many cases.

Kidneys: The blood vessels of the cortex and medulla dilated and engorged with blood. Slight periglomerular lymphocytic aggregation was prominent in few cases (Fig). Focal areas of small mononuclear cellular aggregation detected in between the renal tubules in some cases. The epithelium of the proximal and distal convoluted tubules revealed degenerative changes in the form of cloudy swelling and vacuolar degeneration. Moreover, eosinophilic cast also observed in the lumena of some renal tubules.

Heart: The microscopical examination of the heart revealed congestion of myocardial blood vessels> Intermuscular haemorrhage were also found in some cases.

Intestine: Lymphocytic cellular infiltration of the lamina propria and mucous degeneration of the lining epithelium were detected in the intestine of most of examined cases

Brain and spleen: No microscopical changes were seen in examined cases.

Subgroup A_2 (administered 1/10LD_{50}malathion for 21days): The histopathological changes detected in the animals were nearly similar to those seen in the subgroup A_1.

Liver: Its revealed congestion blood vessels and sinusoid. Hydropic degeneration of the hepatocytes were detected in many cases. The portal area revealed congested blood vessels with mononuclear cellular infiltration.

Kidneys: Histopathological examination of the kidneys revealed congestion blood vessels and intertubular haemorrhages. Cloudy swelling of the proximal and distal convoluted tubules was noticed in most of the examined cases. Eosinophilic casts were detected in the lumen of many tubules. Dilation of some renal tubules was observed.

Heart: Congested of myocardial blood vessels and intermuscular haemorrhages were prevalent in many cases. Myomalacia of some muscle fibers with leucocytic cellular infiltration were also observed in some cases.

Intestine: Mucous degeneration of the lining epithelium with lymphocytic infiltration of the lamina propria were observed.

Spleen: No histopathological changes were observed in the spleen.

Brain: Degenerated neurons and perivascular lymphocytic aggregation were detected in the brain in few cases.

Subgroup A3 (administered 1/10 Ld50 malathion for 90 days):

Similar to those in subgroup A1 and A2.

Discussion: With an increasing world population , use of insecticides is unavoidable. However, indiscriminate use of these chemical agents has resulted in several incidence of human intoxication,Bulusu and charavarty (1984). Toxic effect of human exposure may be influenced by the nutritional status. Liver, kidney are the major organs of detoxification and excretion of these toxins. Damage to such tissues would likely cause changes in blood parameters.

Our pathological results revealed moderate microscopical changes in the treated rats particularly those administered 1/10 LD50 of malathion. The liver and kidney were grossly enlarged in size and congested, while the liver were also pale in color in many cases. Congestion of coronary blood vessels and presence of catarrhal exudate on the intestinal mucosa were also observed in this work. These results were in partial agreement with those of Abol-ghar et al. (1968), Talose et al.(1968), Antsiferov et al. (1972), Clark and Clarke (1975) and Mohamed et al. (1989), where no pulmonary lesions were seen in our study, while our results were not in agreement with the results of Amer and Abdel-All (1987) who recorded severe gastroenteritis with

ecchymotic and petechial hemorrhage on the serous membrane of ewes subjected to malathion toxicity.

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HEMATOLOGICAL STUDIES ON MALATHION [ORGANOPHOSPHOROUS COMPOUND] IN ALBINO RATS

Thabet A. Mohamed Nagah , Mohamed F. salem zlitni, Naima Mohamed Omer Elkakalli.Ahmed M. Elmesalati .

Higher Institute for Technical Medical Science, Tripoli-Libya
Higher Institute for Technical Medical Science, Tripoli-Libya
Tripoli Diabetic Centre
College of dentistry/ Tripoli university
Attahadi University.

دراسة تأثير مبيد الملاثيون على كريات الدم الحمراء وخلايا الدم البيضاء في الفئران البيضاء

ثابت الهاني محمد نجاح — المعهد العالي للعلوم والتقنيات الطبية /طرابلس
أحمد ايهاب محمد المسلاتي — جامعة التحدي الطبية الاهلية
محمد فرج الزليطني — المركز الوطني لأمراض السكر والغدد الصماء
نعيمة محمد الككلي — جامعة التحدي الطبية الاهلية

Abstract

Insecticides are widely used in agricultural fields to achieve greater production, protection and conservation of food products. This results in many different human and animal infections. These infections are usually caused by mouth, skin, or inhalation.

Malathion is one of those pesticides that are used extensively in agricultural fields for the protection of agricultural crops such as vegetables or fruit from agricultural pests that may be caused by mosquitoes, manure, or some other insects.

This study aimed to shed more light on some of the expected side effects due to the excessive use of this pesticide.

The study was conducted on one hundred and forty-four white mice divided into major groups (A, B, C, D) each consisting of 36 mice. The main groups are divided into three small groups (1), (2), (3) and each consists of twelve mice as follows:

The first group: its smallest daily dose (1/10) of the half-lethal dose of melatonin dissolved in oil was reduced to three different periods of 7 days, 21 days, 90 days respectively.

The second group: The smallest group (1/50) of the half-lethal dose of melatonin dissolved in oil was mined on three different periods of 7 days, 21 days, and 90 days respectively.

Group 3: The smallest group (1/100) of the half-lethal dose of melatonin dissolved in oil was mined at three different intervals of 7 days, 21 days, and 90 days respectively.

Group 4: This group was considered as an control group, and its smaller group was restricted to oil only for the same periods.

Samples were taken at the end of each period of the drug alone to conduct various tests on blood, serum, and liver, kidney, heart, brain, intestines, and spleen samples were taken for histopathological tests.

The results were as follows:

Clinical symptoms:Increased salivation, tears, and nose were observed with lack of appetite

for food and decreased weight with bays, imbalance and swaying. These symptoms were severe in group (A) while the symptoms were similar in group (B) but with minimal reduction. While these symptoms are absent in group (c).
Effects on red blood cells:It was observed that malathion showed the highest reduction in the number of erythrocytes and the proportion of jaundice. These results were evident in the major therapeutic dose group, while no significant change was observed in the lower concentration groups.
Third: Effect on white blood cells: -It has been found that the dosage of mice with the pesticide large dose has caused a clear increase in white blood cells. While no change was recorded in the other two groups.

الملخص

تستخدم المبيدات الحشرية بكثرة في المجالات الزراعية وذلك لتحقيق مزيدا من الانتاج والحماية والحفظ للمنتجات الغذائية ، وينتج عن ذلك الكثير من الاصابات المختلفة للإنسان والحيوان على حد السواء ، وتلك الاصابات تحدث عادة عن طريق الفم ، الجلد، أو عن طريق الاستنشاق.
والملاثيون هو أحد تلك المبيدات التي تستخدم بوفرة في المجالات الزراعية وذلك من أجل الحماية اللازمة للمحاصيل الزراعية سوآءا كانت خضار أو فاكهة من الآفات الزراعية والتي قد يسببها البعوض ، المن، أو بعض الحشرات المختلفة.واستهدفت هذه الدراسة إلقاء مزيدا من الضوء على بعض الآثار الجانبية المتوقعة نتيجة الاستعمال المفرط لهذا المبيد.أجري هذا البحث على مائة واربعة واربعين فأرا ابيض قسمت الى مجموعات رئيسية (أ) ،(ب)،(ج) ،(د) كل منها يتكون من ستة وثلاثين فأر. قسمت المجموعات الرئيسية الى ثلاث مجموعات صغرى (1)،(2)،(3) ويتكون كل منها من اثني عشر فأر على النحو التالي:
المجموعة الاولى: وقد تم تجريع مجموعتها الصغرى يوميا (10/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.
المجموعة الثانية: وقد تم تجريع مجموعتها الصغرى يوميا (50/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.
المجموعة الثالثة: وقد تم تجريع مجموعتها الصغرى يوميا (100/1) من الجرعة نصف القاتلة من الملاثيون المذاب في الزيت ، وذلك على ثلاث فترات مختلفة من 7 أيام، 21 يوما ، 90 يوما على التوالي.
المجموعة الرابعة: وقد اعتبرت هذه المجموعة كمجموعة ضابطة، وقد تم تجريع مجموعتها الصغرى بالزيت فقط، وذلك لنفس الفترات السابقة.
تم أخذ العينات عند نهاية كل فترة من التجريع على حده ، وذلك لإجراء الاختبارات المختلفة على الدم ، المصل، وكذلك أخذت عينات من الكبد، الكلية، القلب، الدماغ ، الامعاء ، الطحال لإجراء الفحوصات الهستوباثولوجية.
وقد كانت النتائج على النحو التالي:
أولا: الأعراض الاكلينيكية:-
لوحظ زيادة في افراز اللعاب ،الدموع، والأنف مع نقص في الشهية للطعام وانخفاض في الوزن مع خلجان، عدم الاتزان والتمايل . وكانت هذه الاعراض شديدة في المجموعة (أ) بينما تشابهت الاعراض في المجموعة (ب) ولكن بأقل حدية. في الوقت الذي إنعدمت فيه هذه الاعراض في المجموعة (ج).
ثانيا: التأثيرات على كريات الدم الحمراء:-
لوحظ أن الملاثيون أحدث انخفاضا في عدد الكريات الحمراء ونسبة اليحمور وهذه النتائج كانت واضحة في مجموعة الجرعة العلاجية الكبرى بينما لم يلاحظ أي تغيير معنوي في المجموعتين الأقل تركيز.
ثالثا: التأثير على خلايا الدم البيضاء:- لقد وجد أن تجريع الفئران بالمبيد بالجرعة الكبرى قد أحدث زيادة واضحة في خلايا الدم البيضاء. بينما لم يسجل أي تغيير في المجموعتين الاخريين.

OBJECTIVE. The aim of the present study is to investigate the effect of short – and long – term administration of Malathion on hemogramof albino rats,we hope that this work would throw a more light on the toxic effect of this pesticide.

INTRODUCTION:

Organ phosphorus pesticides are a group of compounds with various toxicities to different forms of life. The wide use of these compounds are mostly in the agricultural affairs of the most developing countries which have entailed the extensive use of pesticides for better production , protection and preservation of food grains .

Malathion is one of the most commonly used organophosphorus pesticides in agriculture and is known to be least toxic to mammals as compared to insects (Debruin, 1970). It is used to a few plant – crops and widely used to control plant pests, flies and parasites of livestock Exposure to these toxic chemicals occurs via oral, dermal and inhalation.

In rats, acute Malathion toxicity severely impairs the digestive and absorptive function of intestine (Chakravarly and Ghosh 1980).

Exposure of chicks to malathion significantly decrease erythrocytic count and haemoglobin level while in the later stage, these parameters had returned to normal levels *(Srivastava , Saxena and Sharma 1960*).

MATERIAL AND METHODS ***Insecticide*** The organophosphorus compound Malathion (diethyl (dimethixyphosphinothioy/thio) succinate) was purchased from katawGonker Bombay Pesticides Company "India The insecticides was dissolved in corn oil, and different dilution (1/10 , 1/50 and 1/100 of acute oral LD50) were prepared . Acute oral LD50 was calculated according to Boyd and Tanikella (1969) (LD50 = 2800mg/kg.b.wt.

The Animals One hundred and forty four of apparently clinically healthy female albino rats (120 to 150 mg in weight) were used in our experiment. The animals were housed under suitable lighting, temperature and proper hygienic condition. Food and drinking water available ad libitum.

Diagnostics Kits Commercial diagnostic kit produced by Sclavo Diagnostic Laboratories Company (Laboratory Reagent and Chemical Products) "Italy" , were used for determination of inorganic phosphorus,bilirubin(total and direct), alanine aminotransferase , aspartate amino transferase , total protein , albumin and globulin blood urea , nitrogen , creatinine and cholesterol .

Experimental Design One hundred and forty four of female albino rats were divided into four groups (A, B, C and E), each of 36 rats (Table1)

Rats in group "A" were subdivided into three subgroups (A1, A2 and A3) , each subgroup consisted of twelve rats , assigned for daily oral treatment with 1/10 of LD50 of malathion , dissolved in mineral oil (corn oil) and administered by a stomach tube for 7 , 21 and 90 days respectively .

The rats in group "B" were subdivided into three subgroups (B1,B2, and B3) , each subgroup contained twelve rats and assigned for daily oral treatment with 1/50 of LD50 of malathion dissolved in (corn oil) by a stomach tube for 7,21 and 90 days .

The rats in group "C" were subdivided into three equal subgroups (C1 , C2, and C3) , each of twelve rats and daily orally treated with 1/100 of LD50 of malathion dissolved in mineral oil (corn oil) by a stomach tube for the previous period .

The rats in-group "E" were divided into three equal subgroups (E1, E2 and E3), kept as a control and orally received oil alone for 7, 21, and 90 days. Twelve rats from each subgroup were sacrificed at the end of their treatment.

groups	Subgroup	No. of animals	Treatment	Days of treatment
A	A1	12	Daily oral treatment with	7
	A2	12	1/10 LD50	21
	A3	12		90
B	B1	12	Daily oral treatment with	7
	B2	12	1/50 LD50	21
	B3	12		90
C	C1	12	Daily oral treatment	7
	C2	12	with 1/100 LD50	21
	C3	12		90
E	E1	12	Control untreated	7
	E2	12		21
	E3	12		90

1/10 LD50 = 43.5 mg/Rate
1/50 LD50 = 8.7 mg/Rate
1/100 LD50 = 4.35 mg/Rate

Sampling

Blood samples collected from each rat in a clean tube containing "EDTA" anticoagulant for estimation of erythrocytecount,

haemoglobin concentration, packed cell volume, total leucocytes count and differential leucocytescount.

Methods of Examination:

1. Hemogram: Erythrocytic Leucocytic counts, Haemoglobin concentration, packed cell volume, and differential Leucocytic count were determined according to the standard technique described in Davidsohn and Henry(1974)

RESULTS & DISCUSSION: Mortalities:

Seventeen rats died from group 'A' during the periods of the experiment, four rats from subgroup 'A1' six rats died from subgroup 'A2' and seven rats died from subgroup 'A3'

Hemogram:

1-The Eryhocytic Parameters: The effect of daily oral administration of 1/10 LD_{50} of Malathion on erythrocytic parameters were recorded. The results showed microcytic hypochromic anemia , in which RBCs counts , Hb concentration , PCV,MCV,MCH and MCHC were significantly decreased in subgroup A_1 by – 12.53% - 16.53% , - 29.45% , - 8.53%, -11.55% ,-15.96% , and in subgroup A_2 by – 6.65% , -12.51% , -17.30% , -8.00% , -15.80% and -19.25% respectively . Normocytic normochromic anemia observed in subgroup A_3, in which RBCs 9.13 and -6.82% significantly decreased counts and Hb concentration, while the PCV, MCV, MCH and MCHC were without significant changes.

2-***Total and Differential Leucocytic Count:***

The effect of daily oral administration of 1/10 LD_{50} of malathion on the total and differential Leucocytic count was illustrated in Table (2) and Figs . (7-12) .

Total Leucocytic count showed significant increase in subgroup A_1 and A_2 by + 48.000% and + 20.41 % , while no significant changes were recorded in subgroup A_3 . Mature neutrophils showed significant increase in subgroup A_2 by + 20.41% without significant changes in both group A_1 and A_3. Meanwhile stab neutrophils revealed significant increase in subgroup A_1 and A_2 by + 84.82% and +20.45% without any significant changes in subgroup A_3. Eosinopenia and lymphopenia noticed by significant decrease in subgroup A_1 and A_2 by -65.75%,-9.24%, +46.35% and -22.03% respectively without significant changes in subgroup A_3. Monocytosis observed in

subgroup A_1 and A_2 by significant increase by +96.08% and 134.23% and no significant changes observed in subgroup A_3.

The Erythrogram of treated rats with 1/10 LD_{50} of Malathion showed microcytic hypochromic anemia. It was clearer after 7 and 21 days.

Our results of that hemogram agreed with the finding of srivastava et all. (1960) who observed significant lower haemoglobin and erythrocyte in Malathion administration to chick. Similar results were observed by Rajini et all. (1987) who stated that pirimphos methyl administration to rats results in decreased number of red cells, haemoglobin and packed cell volume.

Shill (1987) studied the toxicity of primiphos methyl in laying hens and found reduction in haemoglobin content and erythrocytic count. Meanwhile, the same changes observed by most of the investigators such as Hothi (1970),Verkhovskii et al. (1981), and Masoud et al. (1985), while rats treated with Malathion for 90 days showed significant decrease in erythrocytic count and haemoglobin concentration without changes in MCV, MCH and MCHC.

The leukogram of rats orally administered with 1/10 LD_{50} of malathion for 7 and 21 days showed significant leucytosis with stress manifestations illustrated by increase in the total Leucocytic count , stab neutrophils and monocytes and lymphocytes . Gupta and Paul (1972) reported no change in hematological parameters except that a slight increase in total Leucocytic countnoticed. They attributed that to some internal manifestation produced by Malathion that is not sufficient to produce any apparent symptoms in the birds. Similar increase in Leucocytic count has been reported in buffalo calves after Malathion treatment by Hothi (1970). The occurrence of leukocytosis in a variety of toxic conditions has also been reported by Wintrobe (1961). Florey (1962) mentioned that the exact mechanism for the increase in W.B.C. because of insecticidal toxicity is not understood.

Lymphopenia and Eosinopenia occurred in rats treated with the insecticide after 7 and 21 days without any changes after 90 days. Lymphopenia could have been occurred due to effect of the insecticide on circulating lymphocytes of lymphoid tissue, sequestration of lymphocytes in different body tissues, or a response to stress.

Neutrophil cells were elevated after 7 and 21 days after treatment with insecticide. Neutrophilia could be a compensatory to decrease for lymphocytes, a response to chemical mediators from damaged tissues,

or a response to stress as reported by Emtenan (1992) . Similar finding to the present results, were observed by Rajini et al. (1987) who administered pirimiphos methyl to rats.

- Monocytosis observed after 7 and 21 days. The obtained results agreed with those of Vartic et al. (1972) after administered the organophosphorus compound (Bublin) to pigs and observed a steady rise of monocytes, basophils and lymphocytes. In the present study, the erthrogrm and leucogram showed no changes in the other doses (1/50 and 1/100 LD_{50}) during the different periods of experiment (7^{th}, 21^{st}, and 90^{th}days). These results agreed with the findings of Lai and Virdis (1969) after administration of parathion to lactating sheep. Similar results shown by Rehfeld (1969) who did not find any change in blood pictures post administration of Malathion to chicks at the level of 1000 ppm

CONCLUSION

Experiment 1 (Group A)

Blood picture revealed the development of microcytic hypochromic anemia in rats treated for 7 and 21 days. Leukocytosis,Monocytosis and neutrophilia were noticed after 7 and 21 days. While Eosinopenia and lymphopenia were also observed after 7 and 21 days.

Experiment 2 (Group B) Erythrogram and leucogram revealed no significant changes in rats received the different doses of Malathion throughout the experiment.

Experiment 3 (Group C) Results of rats received 1/100 LD_{50} of Malathion showed no changes in the hemogram.

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