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EFFECT OF LIDOCAINE , DICLOFENAC AND THEIR MIXTURE ON SOME BLOOD PARAMETERS IN EXPERIMENTAL MICE

Shereen Jawad Kadhim Al-Ali
Department of Biology, College of Sciences, University of Basrah,Basrah,Iraq
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ABSTRACT

The study included the evaluation of the haematological effects resulting from intramuscular injection of (1 mg/kg) of Lidocaine (group A) , Diclofenac (group B) and Lidocaine – Diclofenac mixture (group C) in experimental mice , as a result there was a significant decrease in haemoglobin concentration and reached it's lowest value in group C (7.04 gm/100ml) . There was also a significant increase in both total WBC_s count and eosinophils number reached it's highest number in group C (9.88 × $10^3/mm^3$, 11.2 %) respectively , while the highest number basophils appeared in group B and reached (8 %) , the same group showed a significant decrease in neutrophils number which reached to (17.6 %) . the a granulocytes (lymphocytes and monocytes) showed no changes in number in all groups .

INTRODUCTION

Lidocaine (lignocaine or xylocaine) is a class IB anesthetic which is particularly useful in treating ventricular arrhythmias [1] and it is used to relive or prevent pain such as minor-burn pain, itching and irritation and also used to anesthetize area before injection [2] As it commonly used to decrease pain on injection with propofol [3] and also we could use nebulized Lidocaine to provide alternatives in patients with severe asthma [4] it works by blocking impulse transmission across the nerve cell membrane [2] the adverse effect of Lidocaine include a little impairment of left ventricular function while the cental nervous system effects include drowsiness slurred speech paresthesia , agitation , confusion and convulsions , cardiac arrhythmias may also occur [1].

Diclofenac (Voltaren) is a drug used for moderate pain and inflammation due to rheumatoid disease , musculoskeletal disorders , renal colic and postoperation pain [5] , it is a cyclooxygenase inhibitor [1] which belong to the non-steroid anti-inflammatory drugs ($NSAID_S$) that often results in relief of pain for significant periods [6] it's mechanism of action is not entirely known , but researchers believe that $NSAID_S$ inhibit prostaglandin synthetase , retard polymorphnuclear leukocyte (PMN) motility and affect the release and activity of lysosomal enzymes [2] it's adverse effect occur in approximately 20% of patients and include gastrointestinal distress , occult gastrointestinal bleeding , and gastric ulceration [6] , also patient on long term treatment with $NSAID_s$ including Diclofenac should have their haemogloin checked if

they exhibit any signs or symptoms of anemia [7] . Other adverse effects include anaemias, thrombocytopenia, neutropenia, eosinophilia, agranulocytosis [8].

The multiuse of diclofenac injection to relief pain and in another hand the multiuse of lidocain to reduce the pain of the needles use to inject diclofenac may results in an unscientific use of Lidocaine by mixing it with diclofenac which react with the local anesthetic, it had been notes that a misty compound formed as a result of mixing Lidocaine with Diclofenac. So the aim of this study is to evaluate and determine the side effects of this resulting compound.

MATERIALS AND METHODS

Animals

The experiment was performed with 20 Balb/c mice their mean weight was about 20 gm, they were fed with the standard fodder.

Dosage

Animals were divided into four groups (five animals for each group), group A had been injected with Lidocaine, group B had been injected with Diclofenac, group C had been injected with Lidocaine – Diclofenac mixture and group D represented as control group. Each group received a dosage of (1 mg/kg) for each of Lidocaine, Diclofenac (UNIQUE PHARMACEUTICAL LABS) and Lidocaine – Diclofenac mixture.

Blood Parameters Analyses

The animals were autopsied after 24 h. from the last injection, they were anesthetized by chloroform , the thoracic cage was opened by surgical scissor and direct aspiration of blood from the heart using 2 ml syringe , blood immediately transferred to an EDTA tube (AFMA-DISPO) and mixed gently , then blood parameters analyses performed according to as following [9] :

1 – haemoglobin concentration

Sahli method was used to measure haemoglobin concentration.

2 – Total white blood cells count

The white blood cells count had been performed by the haemocytometer and the diluent solution (Turk's solution) which prepared from :

3 – Differential Leukocyte count

the Differential Leukocyte count was performed by preparing blood smears , air drying , staining with Wright – Gimsa stain (Gugol Blue) then smears were examined by light microscope under an oil immersion objective (100 ×) [9].

Statical Analysis

The statical analysis performed by Minitab program using ANOVA test under probability ($p< 0.05$) .

RESULTS

1 – Haemoglobin concentration

The results showed a statically significant decreasing in Haemoglobin concentration under probability ($p<0.05$) in the treated groups (A , B and C) and the lowest value was in group C and it reached (7.04 gm / 100ml) compared with the group D (control) which reached (11.72 gm/100ml) , table (1) and figure (2).

2 – Total white blood cells count

From the results it appeared that there was a statically significant increasing in total WBC_s count under probability ($p<0.05$) in the treated groups (A , B and C) and the highest number of WBC_s was (9.88×10^3 /mm^3) in group C compared with the group D (control) which reached (2.26×10^3 /mm^3) , table (1) and figure (3).

3 – Differential leukocyte count

1. **Granulocyte**

The results illustrated that there was a statically significant increasing in number of eosinophils and basophils under probability ($p<0.05$) in the treated groups (A , B and C) , the highest number of eosinophils appeared in group C and it reached (11.2 %) while the highest number of basophils appeared in group B and it reached (8 %) compared with their numbers in group D which was (2.6 % and 1.4 % , respectively) , there was a decrease in neutrophils number but not statically significant in both group A and C while there was statically significant decreasing in neutrophils number in group B and it reached (17.6 %) compared with group D which reached (35.2 %) , table (1) and figure (4).

2. A granulocytes

There was no statically significant changes in number of monocytes and lymphocytes in all groups of the experiment ,table (1) .

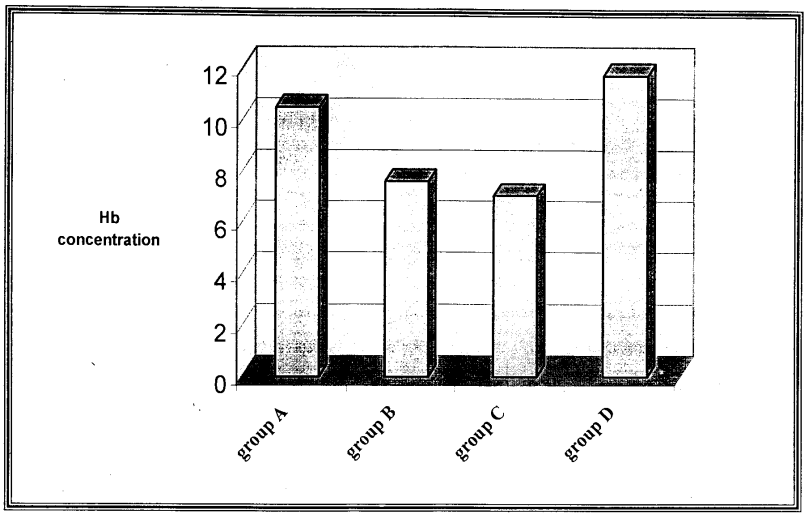

Figure (1) : Haemoglobin concentration in the four groups of the experiment

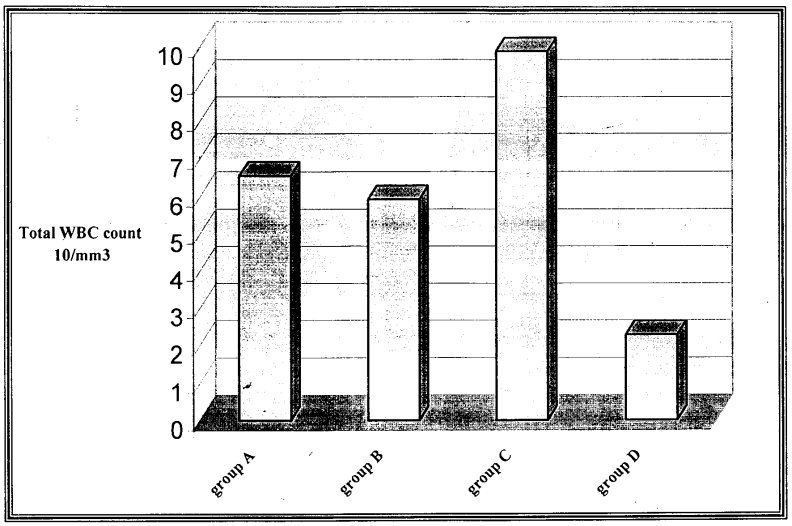

Figure (2) : Total white blood cells count in the four groups of the experiment

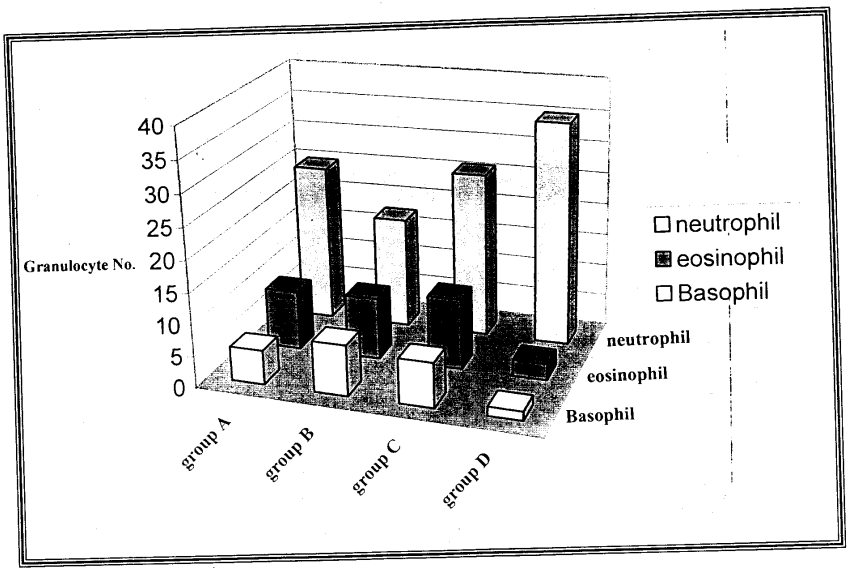

Figure (3) : Differential Granulocytes count in the four groups of the experiment

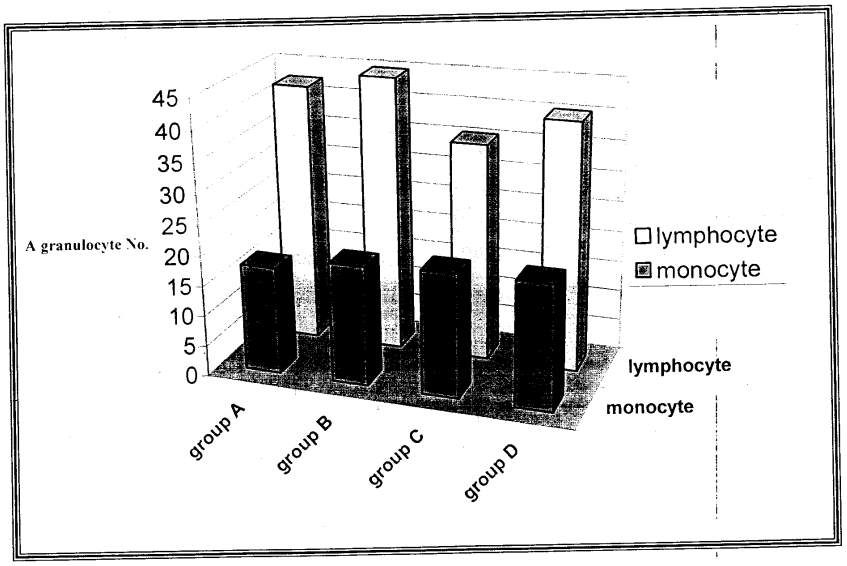

Figure (45) : Differential A granulocytes count in the four groups of the experiment

Table (1) : values of blood parameters of the four groups of the experiment

Differential leukocytes count (%)					WBC $10^3/mm^3$	Hb gm/100ml	Mice
Lympho.	Mono.	Baso.	Eosino.	Neutro.			
٤٢,٦ ± ٥,١٢٠	١٧,٦ ± ٤,٨٧٨	٥,٤ ± ٢,٣٠٢	٩,٤ ± ٣,٥٠٧	٢٥ ± ١١,٨٩	٦,٥٤ ± ٢,٩٩٣	١٠,٤٨ ± ١,٤١٨	Group A N = 5
٤٥ ± ١١,٨١١	١٩,٦ ± ٥,٨٥٦	٨ ± ١,٢٢٤	٩,٨ ± ٥,٣٥٧	١٧,٦ ± ٦,٦٥٥	٥,٩١ ± ٢,٦٧٤	٧,٦٦ ± ١,٧٦٨	Group B N = 5
٣٥,٦ ± ١٥,٤٣٧	٢٠,٢ ± ٧,٠٤٩	٧ ± ١,٤١	١١,٢ ± ٥,٦٣٠	٢٦ ± ١٦,٨٢٢	٩,٨٨ ± ٣,٨٢٥	٧,٠٤ ± ١,١٨٦	Group C N = 5
٤٠,٤ ± ٣,٥٧٧	٢٠,٤ ± ٥,١٢٨	١,٤ ± ٠,٥٤٧	٢,٦ ± ٢,٣٠٢	٣٥,٢ ± ٥,٤٠٣	٢,٢٦ ± ٠,٢١٩	١١,٧٢ ± ٠,٣٠٣	Group D N = 5

DISCUSSION :

The present study shown that the haemoglobin concentration declined in the treated groups (group A , B and C) the decline in group A may be result from the use of Lidocaine which is one of many drugs that cause methemglobinemia as an idiosyncratic reaction , it do so by oxidize the iron of haemoglobin from ferrous form (Fe^{+2}) the ferric form (Fe^{+3}) which is incapable of binding O_2 [10], either directly or by oxidizing agent form during their metabolism[11] . A decrease had been shown in haemoglobin concentration in beef cattle after one day treatment with burdizzo castration following Lidocaine HCl local anesthetisia, which is a result agreed with ours [12] , while the decline in haemoglobin concentration in group B may be result from using Diclofenac as another researcher provid that patient talking $NSAID_s$ show mean decrease in haemoglobin concentration over 4 – 12 weeks assessment [13] , and there were a laboratory adverse events which were worse with oral Diclofenac , include haemoglobin changes [14] . An earlier study on a portion of a population taking $NSAID_s$ found that over one half of patients who received NSAIDs for three to six months experienced a significant decrease in hemoglobin [15]. From the above reasons we can justify that hemoglobin decreasing in group C may result from the injection of the mixture these two hemoglobin decreasing drugs which made the adverse effects of

them greater . As a reverse effect of using Diclofenac combination with other drugs it had been shown that their was a significant less of haemoglobin decline in patient taking diclofenac/misprostol than patients receiving Diclofenac alone , that reverse effect to ours may be explained by that the diclofenac/misoprostol combination is unique in possessing an active component and misoprostol used to prevent NSAID from inducing gastrointestinal damage, Ulcer damage and associated serious complications and clinically significant side effects associated with the use of NSAID [13].

It had been shown an increase number of WBC_s count (leukocytosis) in the treated groups (A ,B and C which showed the highest number) and that may be result from the administration of anesthetic agent [16] and that agreed with the results which showed a great increase in total WBC_s numbers in beef cattle groups treated with Lidocaine combination with other drugs [12] .

The differential WBC count the results showed an increase in both eosinophils and basophils numbers in the treated groups (A ,B and C) basophils play a role in allergic reaction because they have molecules on their surface which allow binding to other cells and to glycoprotein on the external surface and also contain histamine and other substance which cause the adverse symptoms of allergy [17] this increase may explained in one hand by the use of lidocaine , one of the amide type drug, that made a marked drop in allergic reaction , the amide group of the drug may contain the preservation methylparaben , a chemical similar in structure to paraminobenzoic acid , and hence cause allergic reaction[18] , in another hand it may result from administration of drugs which may coupled to body component and thereby undergo conversion from a hapten to a full antigen which will sensitize certain individuals and causing hypersensitivity [19] it had been reported that the use of $NSAID_s$ including Diclofenac induce respiratory disease associated with eosinophilia [20] at last, the decline in neutrophils number in group B (Neutropenia) may result from the Diclofenac administration because the anti-inflammatory drugs are one of the causes of Neutropenia [21].

CONCLUSION

Injection of a mixture of lidocaine and Diclofenac caused decline in haemoglobin concentration, an increase in both total WBC_s count and the percentage of eosinophils and basophils which mean an increase in allergic reaction .

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تأثير الليدوكايين و الدايكلوفيناك و مزيجهما على بعض معايير الدم في الفئران المختبرية

شيرين جواد كاظم العلي
قسم علوم الحياة ، كلية العلوم ، جامعة البصرة،البصرة،العراق

الخـــــلاصـــــة

تضمنت الدراسة تقييم التأثيرات الدموية الناتجة عن الحقن بالعضلة لـ (١ ملغم/كغم) من الليدوكايين (المجموعة A) و الديكلوفيناك (المجموعة B) و مزيج من الليدوكايين و الديكلوفيناك (المجموعة C) في الفئران المختبرية . أظهرت النتائج انخفاض معنوياً في تركيز الهيموغلوبين و وصلت اقل قيمة له في المجموعة C و بلغت (٧,٠٤ غم/ ١٠٠مل) كما أظهرت النتائج زيادة معنوية في كل من العد الكلي لخلايا الدم البيضاء و أعداد الحمضات و بلغت أعلى قيمة لها في المجموعة C (٩,٨٨ × $١٠^{٣}$ /ملم^{٣} ، ١١,٢ %) على التوالي ، في حين إن أعلى قيمة لأعداد القعدات كانت في المجموعة B و بلغت (٨ %) كما أظهرت نفس المجموعة انخفاضاً معنوياً في أعداد العدلات بلغ (١٧,٦ %) . و لم تشهد أعداد خلايا الدم البيضاء غير الحبيبية (اللمفاوية و الخلايا وحيدة النواة) أي تغير في أعدادها في جميع المجاميع .

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