Some toxic Impact of Digoxin in mice

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Abstract. The present study was designed to evaluate the toxic effect of digoxin on liver in biochemically by measuring liver transaminase enzymes level in the serum, gross lesion and Toxic signs in mice . The toxicity study was carried out on Three treatment groups, each consist of 10 mice divided according to daily treatment with digoxin to T1, T2 & C representing dosing orally with 5, 10 mcg/kg respectively the three group act as control and treated with distilled water in 21 day. The result showed significant (p<0.01) elevation in their serum clinical enzyme Aspartate Aminotransferase(AST)and Alanine Aminotransferase (ALT) level T1 and T2 groups when compared with contral groups in positively proportional with the dose of digoxin. Yellow of spots was noticed in the liver in T2 groups at the end of experiment. Toxic signs were also noticed increase proportionally with the dose of treated group including: Depression, Weakness, Extended body, In-coordination, Anorexia . In conclusion this study revealed that digoxin of both dose 5mcg/kg and10 mcg/kg in caused some toxic signs and toxic effect on liver tissue effect propotinal with the dose and period of expouser even at the therapeutic dose that indicate the precaution in using such drug.

Keywords: Digoxin, Some toxic, mice

1. Introduction

The Digoxin belongs to a class of medications called cardiac glycosides. It works by affecting have in common specific effects on the myocardium [1]. Digoxin is a purified cardiac glycoside extracted from the leaves of the foxglove plant (Digitalis purpurea) [2]. General uses for Digoxin include the following; prevention of Atrial Fibrillation, and Paroxysomal Atrial Tachycardia. Of these Digoxin has two effects with two different mechanisms of action. The first is the cholinergic effect or vagomimetic action, which slows the heart rate, this is responsible for the effects of Digoxin on the Sinoatrial and Atrioventricular nodes. This is all accomplished through a central mechanism that increases cholinergic stimulation to the heart. This causes a decrease in conduction through the SA node. Since the nerve which provides the heart with cholinergic activity is the Vagal nerve, this is often referred to as an increase in Vagal tone. The next is the positive inotropic effect. This effect results from competition between potassium and digoxin. These compete for binding sites on an enzyme, referred to as potassium-ATPase. So digoxin is a potassium blocker or antagonist. By blocking potassium from binding to the enzyme, Digoxin causes the heart muscle to be exposed to calcium for a longer period of time resulting in the heart contracting more forcefully [3,4]. Digoxin is large distributed used medicine to treat heart failure and atrial fibrillation, atrial flutter, and paroxysomal atrial tachycardia. Because digoxin is metabolized, eliminated by hepatocytes and has narrow therapeutic range [5]. Therefore, the study was design to determine the toxic effects therapeutic dose and double dose administration of digoxin on liver and recorded any toxic sign.
2. Materials & Methods:

2.1 Animals:
Thirty albino Swiss mice weighing 25-30g of either 10 were used. The animals were grouped and kept in cage housed at standard condition of light & ventilation & have freely access to standard rodent diet (commercial feed pellets) and tap water. The animal was kept for a week for adaptation. The animal work has agreed by animal ethics committee of Baghdad university college of veterinary medicine.

2.2 Experimental Design
In this study, 30 mice equally grouped at 3 groups according to digoxin dose as(T1, T2, & C) were used, each group consist of 10 mice given orally different doses of Digoxin at 5,10 microg/kg and distilled water respectively in all treated group, the animals were dosed daily for 21 days.

2.3 Parameter:
The following parameters were studied in all groups:

1. Estimation of serum enzyme activity for AST, ALT [6] by taking the blood from direct the heart after anesthetized mice.

   At the end of the experiment of each group (T1, T2, and Control) were anesthetized by a piece of gauze which was saturated with enough amount of chloroform and put in a suitable closed chamber and left for 1-2 minutes for saturation to achieve a good muscular relaxation then the animal was put inside the chamber till the signs of anesthesia achieved. The animal was then held for blood collection The anesthetized [7]. Mice were fixed by ligation on a board (35×20) cm with bandages. Using disposable insulin syringes of was inserted through the third intercostals space to puncture the heart and collect blood. It is favorable to palpate the heart and find the heart beats by the tip of fingers before inserting the needle [8].

2. Clinical signs development in all groups through the period of experiment (21 day) and recorded in 7 days, 14 day, and 21 days.

3. Gross lesion in organ & tissue at the end of treatment period, all the animal were sacrificed after withdrawing of blood the organ involved in study (Liver) was dissected & cleaned for gross lesion examination.

3. Statistics analysis
Results were expressed as means ± standard error that subjected to statistical analysis using one-way analysis of variance (ANOVA) and LSD. The significance level considered was (p < 0.01).

4. Result and Discussion

4.1 Estimation of serum enzyme activity for AST, ALT:
The table 1. showed a significant (P<0.01) increase of serum AST and ALT level in T2 and T1 treated groups when compared with Control group.

| Group n=10 mice | AST (I/U) after 21 days | ALT (I/U) after 21 days |
|----------------|------------------------|------------------------|
| C= control group | 50.90±0.5A | 12.25±0.85A |

Table 1. Effect of Digoxin on the enzyme activity (ALT, AST)
In this study, the activity of these enzymes in serum T1&T2 groups were higher than the control group may reflect hepato cellular damage according proportionally with the increases in digoxin doses in mice. These results confirmed the finding of [9,10] The amino transferases (enzymes) involved in this reaction AST and ALT are present in hepatocytes and leak into blood with liver cell damage. They found that digoxin or cardiac glycoside administration associated with a significant increase in serum level of AST and ALT enzyme when compared with control group. This may be due to the damage and hydrolysis in the hepatocytes that occur as a toxic effect of digoxin on liver cells directly and this is corresponding [11].

It was reported that chronic toxicity study of digoxin in adult rats received different doses for 90 days causes increase in serum AST & ALT level [12].

4.2 Gross Lesion
Effect of digoxin on the liver:

Liver of T2 group showed yellowish spots on the surface increase the with increased in dose of drug while T1 show no change on the surface of liver appear nearly control group as that show clear glistening surface. As shown in figure (1).

|          | Therapeutic dose of digoxin | AST | ALT |
|----------|----------------------------|-----|-----|
| T1       | 5mcg/kg.BW                 | 63.20±1.31B | 20.15±0.99B |
| T2       | double of therapeutic dose of digoxin 10 mcg/kg .BW | 89.00±0.77C | 32.70±0.84C |

M±SE=mean+ standard error
-Different capital letters represent significant differences between groups(P<0.01)

|          |       |
|----------|-------|
| C        | T1    | T2    |

Figure 1. Effect of digoxin on mice liver

n=10

c=control group

T1= Treated groups with 5 mcg/kg digoxin
T2= Treated groups with 10 mcg/kg digoxin.
Arrow = presence yellowish spots on the liver of treated in T2 digoxin in mice.

This result due to toxic effects of digoxin might be responsible for mice as reported by\textsuperscript{[9]} on the liver damage cells presented as degeneration, necrosis, minimal to sever periportal fibrosis and septal fibrosis it seem that the toxicity observed in this study might be due to the damage to the liver\textsuperscript{[12]} This results T1 in may be because the doses of digoxin were not enough to cause gross lesion effect on liver.

4.3 Toxic Signs (Clinical Signs)

Toxic sign were also noticed dose treated group: including: Depression, Weakness, Extended body, Incoordination, Anorexia.in T1, T2 comparison with control group in different period in Table 2.

| Table 2. Effect of Digoxin on the Clinical Signs (Toxic Signs) in different period |
|-------------------------------------------------|
| **Group**                                      | **Day of treatment** | 7\textsuperscript{th} | 14\textsuperscript{th} | 21\textsuperscript{th} |
| n=10 mice                                      |                     |                      |                      |                      |
| Control= D.W                                    |                          | -                     | -                     | -                     |
| T1=received the therapeutic dose of             |                          | Weakness              | -                     | -                     |
| digoxin 5 mcg/kg B.W                            |                          | Depression            | ++                    | +++                   |
| Extended body                                  |                          | -                     | -                     | +                     |
| In-coordination                                |                          | Anorexia              | -                     | +                     |
| T2=received the double dose of digoxin          |                          | Weakness              | ++                    | +++                   |
| 10 mcg/kg B.W                                   |                          | Depression            | +                     | ++                    |
| Extended body                                  |                          | -                     | ++                    | +++                   |
| In-coordination                                |                          | Anorexia              | +                     | ++                    |

- (NON), +(SLIGHT), ++(OBVIOUS), +++(MODERATE), ++++(SEVER)

The toxic effects show of Digoxin result from action CNS neurotransmitters disturbance responsible for toxic effect in mice as agree reported by Kobayashi\textsuperscript{[13]}

This sign might be attributed to two reasons, the first one is central nervous system(CNS) depression, resulted possibly from Inhibitory CNS are either caused by neurotransmission opposite to dopamine,serotonin, epinephrine or due to barin damage .In the same study, the finding was that high digoxin concentration promotes release of dopamine by exocytic and carrier mediated process in rat brain\textsuperscript{[14]} and the other sign maybe related, the second factor is due to neuromuscular blocking action resulted from anticholinesterase activity accumulation of acetylcholine which cause adepolarizing neuromuscular action\textsuperscript{[15]}.

The toxic digoxin It has been postlated however, the same study, the finding was that revealed that digoxin of both dose 5 mcg/kg and 10 mcg/kg in caused abnormal neurobehavioral effect proportional with the dose and period of exposure even at the therapeutic dose\textsuperscript{[16]}.
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References

[1] Virgadamo, S.; Charnigo, R.; Darrat, Y.; Morales, G.; and Elayi, CS. 2015 Digoxin: A systematic review in atrial fibrillation, congestive heart failure and post myocardial infarction. World J Cardiol., 7(11):Pp:808-816.

[2] Roberts, DM.; Gallapatthy, G.; Dunuwille, A.; and Chan BS. 2015 Pharmacological treatment of cardiac glycoside poisoning. British Journal of Clinical Pharmacology, 81(3):Pp: 488–495.

[3] Kurian, M. 2015 The effect of digitalis on the heart- an update. J. Pharm. Sci. & Res., 7(10):Pp: 861-863.

[4] Pincus, M. 2016 Management of digoxin toxicity. Australian prescriber, 39:Pp: 18–20

[5] Ehle, M.; Patel, C.; Giugliano, RP. 2011. Digoxin: clinical highlights: a review of digoxin and its use in contemporary medicine. Crit Pathw Cardiol. , 10(2):P: 93-8.

[6] Donald, D. Holmes, 1984. Clinical Laboratory Animal Medicine 1st ed. printed by Iowa State University Press, P.50010.

[7] Hafez, E.S.E. 1970 Reproductive and breeding. Techniques For Laboratory animals. Lea and Fibges,philadilphia.

[8] Titez, NW. 1999 Text book of clinical chemistry, 3RD ED C.A. Burtis, E.R.Ashwood, W.B.Saunders. Pp: 652-1245.

[9] Salih, RA.; and Alkhayyat, AA.2016 Toxic effect of Nerium Oleander leaves extract on biochemical parameters in rabbit serum. Al-Anbar J. Vet. Sci., 9(2):Pp: 1-8.

[10] Ragab, AR.; Al-Mazroua1, MK.; Abdel-Rahman, RH. 2012 Clinical utility of serum digoxin level in cardiac patients for diagnosis of chronic digitalis toxicity. Journal of Clinical Toxicology, 2(9):Pp: 1-5.

[11] Abbed, AM. 2017 The effect of digoxin on the enzymatic activity of glutamate oxaloacetate transaminase. Journal University of kerbala, 15(1):Pp: 155-160.

[12] Abbas A. Khadhair and Saleh K. Majeed 2017 Study the toxicity of digoxin on liver and their transaminase enzymes level in Journal University of kufa medical sciences , 8(2):Pp: 73-80.

[13] Kobayashi, K. 2001 Role of Catecholamine Signaling in Brain and Nervous System Functions: New Insights from Mouse Molecular Genetic Study. Journal of Investigative Dermatology Symposium Proceedings, 6, 115–121.

[14] Arbutnott G W ,fairbrother t S & Butcher S P, 1990 Dopamine release and metabolism in rat striatum—an analysis by in vivo brain microdialysis .pharmacol ther, 48:281.

[15] Mohammed,F.K.andOmer,V.E.V.1982 Modification of Michels of Electrometric Method for Rapid Measurementof Blood Cholinesterase Activityin Animal:Amini-review. Veterinary-Human Toxicology,24:119-121.
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