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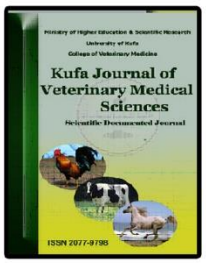

Study on Neurotoxic effects of Digoxin different doses in mice

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Abstract:

The present study was designed to evaluate neurobehavioral effects of two doses of digoxin(therapeutic and double dose) in mice.Two treatment groups(T_1, T_2), each consist of 10 mice divided according to daily oral treatment with Digoxin representing dosing orally with 5 ,10microgram/kg respectively the third group considered as control and treated with distilled water.

Neurobehavioral results showed that digioxin in T2 caused significant decrease proportional with the dose in autonomic and central nervous system activity manifested by in decrease urination frequency and fecal boluses, disturbance of vestibular system ,increase of time needed for 180° turning upward to avoid slope, and defect in degree of cognitive function of exploration , and neuromuscular compatibility with disturbance of locomotor activity(proportional with dose and period of exposure) .

While T1 showed significant effect in disturbance of vestibular system ,increase of time needed for 180° turning upward to avoid slope,and righting reflex , and neuromuscular compatibility with disturbance of locomotor activity(proportional with dose and period of exposure) ,while there were no significant change in other parameters above at that period.

In conclusion this study revealed that digoxin of both dose 5mcg/kg and10 mcg/kg in caused abnormal neurobehaviord effect propotinal with the dose and period of expouser even at the therapeutic dose that indicate the precaution in using such drug.

دراسة تأثير العصبي السمي لجرع مختلفة من الديجوكسين في الفئران

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ألخلاصة/

صممت هذه الدراسة لمعرفة تقيم التأثير السلوك العصبي السمي لجرعتين من عقار الديجوكسين (الجرعة العلاجية ومضاعفاتها)على الفئران . إذ احتوت كل مجموعة10 فئران جرعت فمويا وقسمت حسب نوع المعاملة اليومية لعقار الديجوكسين إلىT1,T2جرع الآتية / 5-10مايكروغرام /كغم من وزن الجسم ومجموعة ثالثة تمثلت بمجموعه السيطرة أعطيت ماء مقطر. أظهرت النتائج السلوك العصبي لمجموعة العلاجية T2 لعقار الديجوكسين انخفاضًا معنويًا في تثبيط الجهاز العصبي الذاتي والمركزي (قلة التبول والتبرز) واضطرابًا في عمل الجهاز ألدهليزي (زيادة الوقت اللازم للاستدارة 180° لتجنب الانحدار) و الحركة الموضعية وقابلية الحيوانات على استكشاف المحيط الخارجي وقلة الوعي واليقظة, وتثبيط التوافق العصبي العضلي (قلة فترة بقاء الحيوان متوازنًا على العصا الدوارة) إذ (تناسبت تناسبا طرديا مع الجرعة وفترة

التعرض). بينما أظهرت النتائج مجموعة العلاجية T1 تاثيراً معنويًا سبب اضطرابًا في عمل الجهاز ألدهليزي (زيادة الوقت اللازم للاستدارة 180° لتجنب الانحدار) و الحركة الموضعية, وتثبيط التوافق العصبي العضلي (قلة فترة بقاء الحيوان متوازنًا على العصا الدوارة). واضطرابا على القابلية الحركية وقدرتها على إظهار المنعكس الطبيعي لتصحيح وضع الجسم . إذ (تناسبت تناسبا طرديا متناسبًا مع الجرعة وفترة التعرض). بينما لم يكن هناك تاثيرا معنويا خلال الاختبارات المذكورة أعلاه في هذه الفترة.

أظهرت نتائج الدراسة بان عقار الديجوكسين سبب سلوكا عصبي غير طبيعي في كلا الجرعتين الجرعة 1T =5مايكروغرام/كغم و كذلك جرعة2T= و10مايكروغرام /كغم تناسب طردي مع الجرعة وفترة التعرض أي حتى الجرعة العلاجية ومما يجعله غير امن, وبسبب أهميه استعمال الدواء في الطب والعلاج لذا يجب الحذر عند استعماله.

Introduction:

Digoxin belongs to a class of medications called cardiac glycosides. It works by affecting sodium and potassium levels inside heart cells. This reduces strain on the heart and helps it maintain a normal, steady, and strong heartbeat. (1) Digoxin. is used to treat heart failure, usually along with other medications. It is also used to treat a certain type heart disease as chronic atrial fibrillation. Treating heart failure may help to maintain your ability to walk and exercise and may improve the strength of cadiac contractability. Treating an irregular heartbeat can decrease the risk for blood clots, that may reduce the risk for a heart attack or stroke.

Due to the differenaces in pharmacokinietic profile of digoxin (such bioavailability and gastrointestinal absorbtion, distrbution and excretion), there has a tendency to overlook its intoxication.(2).

In addition, it has likewise appeared little therapeutic window which could increase the hazard factors of harmfulness in patients being treated with digoxin treatment with a proportion of 5 to 35 % in hospitalized patients (3,4).

This difference is partly due to the preferential concentration of the glycosides in the myocardium; the levels reached in the CNS are much lower. neurologic side effects as do occur are more or less subjective . Present Study amied to evaluate some neurobehavioral effect in mice exposed to different doses of digoxin.

Materials and Methods: Animals:

Thirty (30) albino Swiss mice weighing 25-30g of either sexes were used .The animals were grouped and kept in cage housed at standard condition of light and ventilation and have freely access to standard rodent diet (commercial feed pellets) and tap water. the animal were kept for a week for acclimatization in optimum conditions of breeding at 22±3 ºC with about (14/10) Hours (Light/Dark) cycle standard ,pilliets ,and water ..provided ..ad libitum (5).

Experimental Design:

in this study ,30 mice equally grouped at 3 groups according to digoxin dose as(T_1,T_2&C)were used, each group consist of10mice given orally different doses of Digoxin at 5,10microg/kg and distilled water respectively in all treated group,the animals were dosed daily for 20 days.

Parameter:

1. **Open Field Method / 3 Minutes (6, 7):**

The test evaluates the general locomotor activity, exploration (squares crossed by four legs of animal forward and backward), rearing and also including frequency of defecation and urination (Autonomic nervous impressed).We placed the mouse in the center of arena of open field apparatus and counted the number of squares crossed, rearing, fecal boluses and urine pools during (3) minutes. Arena was cleaned after each test animal.

2. **Negative Geotaxis**

The mouse is placed on a45degree slope with its head pointing down the incline, it normally turns around and crawls

up the slope,This test reflects vestibular function,the maximum time allowed is 60 seconds (8).

3. **Head Pocking Test**

This test determines the degree of cognitive function of animal exploration of environment. In this test registration of the frequency of head entrance into pores during (3) minutes (9).

4**.** 5. **Righting Reflex Test**

This test evaluates the motor activity of mice, when the mouse is placed on its back, it should immediately right themselves to an upright position on all four paws . The time needed for adjusting posture was recorded.

5**. Cleft Avoidance Test**

This test is useful for detection of the proper reflexes,This test putting mice close to cleft of table with height more than (9) cm, the time needed by mice to turn off was recorded (10).

6.Stand on Rota Road Test.

This test reflects neuromuscular coordination or ataxia ,The time for animal to achieve coordination on the Rota road without falling down is recorded (9).

Statistics analysis:

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

Results and Discussion:

1**. Open field test/3 minutes**

It consists of four elements:

The result of open field test(crossed squares) showed that at 10and20 days of treatment, T1 and T2 groups showed a significant decrease in number of crossed squares ($P<0.01$) in comparison with the control group.

Frequency of fecal bolus/3 minutes :

Also significant reduction in frequency of fecal bolus in comparison with the control group.

Treatment T2 groups showed a significant($P<0.01$)decrease of fecal bolus in comparison with control group in all exposure period

while **T1** showed non a significant effect in comparison with the control group.

Urine frequency / three minutes:

The results of T2 treatment group showed significant decrease in urine frequency ($P\leq0.01$) after in all exposure period comparison with with the control group while T1 showed non a significant effect in comparison with the control group at exposure period.

Rearing / 3 minutes:

The results of T1and T2 treatment group showed significant decrease in Rearing ($P\leq0.01$) in all exposure period comparison with with the control group Table (1).

CNS neurotransmitters disturbance responsible for nuerotoxic effect.in mice as agree reported by Kobayashi The results can attributed to the digoxin effect on dopamine and/or another catecholamine and CNS neurotransmitters in dose dependent manner Catecholamines (such as dopamine and nor epinephrine) are the principle neurotransmitter which can mediate a wide range of the central nervous system functions. Such as motor control, cognition, emotion, memory, processing and endocrine modulations.(11).It was noticed that defects in catecholamine's transmission are implicated in range of neurologic and neuropsychiatric disorders. Generally, increase in dopaminergic neurotransmissions can cause an increase in locomotor activity and the inhibition of neurotransmission leads to suppress the locomotor activity. However, different receptor subtypes and terminal regions do not have contribution in the same way(12). Inhibitory CNS are either

caused by neurotransmission opposite to dopamine, serotonin, epinephrine or due to barin damage that seen more in higher doses of digoxin in mice brain. In the same study, the finding was that high digoxin concentration promotes release of dopamine by exocytic and carrier mediated process in rat brain(13)

Table (1): Open field test/3 minutes of mice with two different doses of digoxin during 10 day and 20 day period.

Period / Group / n=10 mice	befor treatment M±SE	10 days of treatment M±SE	20days of treatment M±SE
Number of crossed squares/3 minutes			
C D.W	49±0.60 A a	48±0.50 A a	48.5±0.45 A a
T1(5 mcg/kg)	48±0.70 A a	42±0.20 B b	40±0.40 B b
T2(10 mcg/kg)	47.5±0.55 A a	33±0.30 C b	22±0.5 C c
Frequency of fecal bolus/3 minutes			
C D.W	3.10±0.50 A a	3.60±0.60 A a	3.90± 0.90 A a
T1 (5 mcg/kg)	3.50±0.50 A a	3.00±0.50 A a	2.80±0.80 A a
T2(10 mcg/kg)	3.80±0.30 A a	2.00±0.15 B b	1.60±0.25 B b
Frequency of urination			
C D.W	0.55±0.40 A a	0.60±0.20 A a	0.66±0.60 A a
T1(5 mcg/kg)	0.58±0.30 A a	0.60±0.30 A a	0.58±0.34 A a
T2(10 mcg/kg)	0.60±0.20	0.40±1.10	0.20±1.30

	A a	B b	B b
Rearing / 3 minutes			
C D.W	3.30±0.30 a A	2.80±0.40 a A	3.00±0.35 A a
T1 (5 mcg/kg)	2.80±0.20 a A	1.50±0.60 b B	1.00±0.45 B b
T2(10 mcg/kg))	3.00±0.20 a A	1.30±0.40 b B	0.90±0.20 b B

M±SE=mean+ standard error

-Different small letters represent significant differences within groups(P<0.01) –

-Different capital letters represent significant differences between groups(P<0.01)

Negative geotaxis test/60seconds :

The results of negative geotaxis test showed that at 10day and20day treatment. T1and T2 showed significant increase in time of negative geotaxis in a significant increase (P<0.01) at in comparison with the control group (table2)

This result dueto the cerebellum modulation and coordination of muscular activity are fundamental in skilled voluntary movement in addition to the movement and posture equilibrium. Cerebellum is responsible for smoothly coordinated movement. (14).

Reported found of presence projections from multiple sources as well as that project from vestibular nerve, cortical structure, cerebellar structure are ended in vestibular neuclie. As a result any damage to one or more of these regions leads to sending impaired signals to the vestibular neuclei which can affect the coordination and posture. Moreover, more cerebellum dysfunctions can delay movement initiation (15)

,so any damage to one or more of these regions send impaired signals to vestibular neuclei that might affect the coordination and posture, Further more , cerebellum dysfunction leads to delay intiation of movement(16).

Table (2): Negative geotaxis test/60 seconds of mice two dosed with two different doses of digoxin during 10 day and 20 day period.

Period / Group n=10 mice	befor treatment M±SE	10 day of treatment M±SE	20 day 0f treatment M±SE
C D.W	2.00±0.6 A a	2.2±0.4 A a	2.4±0.3 A a
T1(5 mcg/kg)	2.1±0.30 A a	3.00±0.22 B b	3.40±0.6 B b

T2(10mcg/kg)	2.4±0.32 A a	3.7±0.3 B b	4.00±0.5 B b

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

3.Head pocking test/3minutes:

The results of head pocking test showed that at 10 dayand20 days of treatment T1 showed non a significant change in head pocking (P<0.01) in comparison with the control group. While T2 groups showed a significant decrease positively proportional with the dose amount and exposure period in head pocking in comparison with control group . (table 3).it seem that nuerotoxic effect observed in this study might due to

The defects in the regulation of neurotransmitters release and/or abnormalities in the level of extracellular neurotransmitters which are considered as a core component of hypothesis on neuronal foundation of behavior and cognitive disorders and the symptoms of neuropyschatric and neurodegenerative disorders.

These findings are in consistent with previous study shown that inhibition of serotonin uptake by mouse brain synaptosomes.(17)or may beas result of increased binding of digoxin to Na+-K+ATPase that could lead to altrered sodium-calcium and intercellular calcium accumulation leading to cell destruction.(18)

Table (3):Head pocking /3minutes test of mice two doses of digoxin during 10 day and 20 day period.

Period / Group N=10mice	befor treatment M±SE	10 day of treatment M±SE	20 day 0f treatment M±SE
C D.W	9.8±0.3 A a	10.2±0.30 A a	9.8±0.2 A a
T1 (5 mcg/kg)	9.9±0.3 A a	10.2±0.35 A a	9.5±0.5 A a
T2 (10 mcg/kg)	10.2 ±0.5 A a	8. 5±0.5 B b	7.00±0.2 B c

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

4.Righting.reflex.test/parts,of.seconds(Seconds=100 Parts) :

The results of righting reflex test showed that at 10 dayand20day of treatment T1 & T2 groups showed significant increase in time of positively proportional with the dose amount and exposure period righting reflex in comparison with the control group. table (4) .

Digoxin as same as glycoside have been previously reported to affect adrenergic and noradrenergic transmission in the synaptic ganglia (19)

Who agree with reported that administration of digoxin at dose of1.0mg for dog under anesthesia cause on effect CNS of digoxin occur in depended of direct stimulation of receptor in either the peripheral afferent or efferent autonomic nerves system.

Table(4) :Righting reflex /parts of seconds of mice two doses of digoxin during 10 day and 20 day period.

Period / Group n=10mice	befor treatment M±SE	10 day of treatment M±SE	20 day of treatment M±SE
C D.W	1.32 ±0.22 A a	1.35±0.15 A a	1.36±0.20 A a
T1 (5 mcg/kg)	1.30±0.20 A a	2.20±0.30 B b	2.50±0.40 B b
T2 (10 mcg/kg)	1.30±0.30 A a	2.6±0.22 B b	2.90±0.30 B b

M±SE=mean+ standard error

-Different small letters represent significant differences within groups(P<0.01)

-Different capital letters represent significant differences between groups(P<0.01)

5 .Cleft avoidance test/seconds :

The results of cleft avoidance showed that at 10 day and 20day of treatment**, T1** group showed non a significant increase in time of cleft avoidance , while T2 group showed a significant increase(P<0.01) in time positively proportional with exposure period of cleft avoidance in comparison with the control group (table.5). This finding can be attributed to the neurodegenerative changes and the changes in the level of cellalar neurotransmitters (16).

Table (5): Cleft avoidance test/seconds of Mice two doses of digoxin during 10 day and 20 day period.

Period / Group n=10mice	befory treatment M±SE	10 day of treatment M±SE	20 day oftreatment M±SE
C D.W	2.20 ±0.22 A a	1.90 ±0.30 A a	1. 98 ±0.20 A a
T1 (5 mcg/kg))	2.08 ±0.3 A a	1.75 ±0.20 A a	1.60 ±0.40 A a
T2 (10 mcg/kg)	2.00 ±0.20 A a	1.00 ±0.16 B b	0. 70 ±0.10 B b

M±SE=mean+ standard error.

Different small letters represent significant differences within groups(P<0.01)

-Different capital letters represent significant differences between groups(P<0.01)

6.Stand on Rota Rod:

The results of Stand on Rota Rod showed that T1 & T2 groups at showed significant decrease in time($P \leq 0.01$) positively proportional with the dose amount in comparison with the control group. table (6).

neuromuscular compatibility (decrease of stability on Rota road) observed in this study might be due to the damge in cerebellum, The cerebellum modulation and muscle coordination activity have a key role in skilled voluntary movement in addition to the posture equilibrium. Cerebellum is responsible for smoothly coordinating movement (14).

Table (6): Stand on Rota rod test/seconds ofmice two different doses of digoxin during 10 day and 20 day period.

Period / Group n=10mice	befory treatment M±SE	10 day of treatment M±SE	20 day oftreatment M±SE
C D.W	3.16±0. 30 A a	3.40±0.40 A a	3.30±0.15 A a
T1 (5 mcg/kg))	3.00±0.50 A a	2.40±0.20 B b	1.20±0.32 B c
T2 (10 mcg/kg)	2.40±0.20 A a	2.20±0.30 B b	1.00±0.80 C c

-Different small letters represent significant differences within groups($P<0.01$)
-Different capital letters represent significant differences between groups($P<0.01$)

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