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THE EFFECT OF BISOPROLOI AS FAST DISSOLVE SUBLINGUAL TABLET ON THE HEART RATE OF STRESSED RABBITS

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ABSTRACT

The hormone adrenaline, also known as epinephrine, is produced by the adrenal glands and is essential for the body's "fight or flight" response. Studies show that the β -blocker drug Bisoprolol lowers the sympathetic nervous system's activation brought on by stress by blocking the release of adrenaline via the mediate route. The study investigates the impact of selective β_1 blockers (Bisoprolol fast dissolve sublingual tablets, 10 mg) on rabbits' heart rates while they are subjected to physical stress. 18 rabbits were individually subjected to five minutes of physical stress in a lab setting. In a randomized controlled crossover study, each rabbit received one of three treatments: no stress exposure and no treatment (control group- treatment-1); stress exposure for five minutes and no treatment (treatment-2); and stress exposure for five minutes and treatment with bisoprolol fast dissolve tablets (treatment-3). Before and after being exposed to stress, the heart rate was recorded. Treatment 3 demonstrated a substantial reduction in rabbits' heart rates following treatment ($p = 0.5$) when compared to the control group. Based on results obtained, Bisoprolol fast-dissolving sublingual tablets, can prevent hearts from beating faster when they stressed or exercised.

KEYWORDS: Heart Rate Variability, Super disintegrant, Fast Dissolve Sublingual Tablet, Heart Rate, Autonomic Nerves System, Bisoprolol Fumarate.

1. INTRODUCTIONS

Fear is physical a stressful experience for Rabbits, which can lead to changes in their heart rate and blood pressure due to the activation of the autonomic nervous system.[1-4] These physiological indicators are valuable because they can be measured non-invasively, without affecting the animal's well-being. Rabbits are particularly prone to physical stress and may hide their symptoms, making it important to study the effects of fear-induced stress, especially in pet Rabbits.[5-8] Stress is a mental or physical imbalance brought on by negative stimuli that can result in abnormalities of the body, mind, and behavior. A non-invasive electrocardiographic technique called heart rate variability (HRV) examines the variation in the time between heartbeats and illustrates how well the heart can react to physiological and environmental stimuli. Studies have revealed that physical stress influences HRV, which can be utilized as an objective indicator of psychological well-being and stress.[1,9] The adrenal glands create the hormone adrenaline, also known as epinephrine, which is crucial for the body's "fight or flight" response. According to studies, the β_1-blocker medicine Bisoprolol reduces the sympathetic nervous system's activation due to stress by preventing the release of adrenaline through the mediated pathway. The physiological responses brought on by the release of adrenaline, such as an increase in heart rate and blood pressure, may therefore be lessened by Bisoprolol. This demonstrates that bisoprolol may be helpful in reducing the tachycardia caused by stress, fear, and exercise.[10-13] A selective β1-blocker called bisoprolol lowers heart rate both at rest and during activity. This is because of its β_1-selectivity, which prevents tachycardia brought on by stress, fear, and exercise. According to studies, the β_1-blocker medicine, bisoprolol, can prevent healthy volunteers' hearts from beating faster when they exercise.[5,7,14]

Based upon this literature, we wanted to investigate further the effect of bisoprolol as fast dissolve sublingual tablets 10 mg on the heart rate of rabbits after exposure to physical stress for five minutes. Each rabbit was subjected to 3 treatments: no exposure to physical stress or treatment (treatment 1, control); physical stress exposure for five minutes without treatment (treatment 2); and physical stress exposure for five minutes plus treatment with bisoprolol as fast dissolve sublingual tablets (treatment 3) in a randomized controlled crossover study design. The difference between treatments was measured using the heart rate as a clinical parameter.

As suggestion of the treatment, the heart rates of the Rabbits considerably lowered and returned to normal, proving that Bisoprolol fast dissolve sublingual tablet may be helpful in assisting Rabbits in recovering from stressful situations and can be used in veterinary medicines.

2. MATERIALS AND METHODS

a. Study limits

i. Study place

All research methodology of formulations performed at Yemen Egyptian Pharmaceutical Company, **(YEPHCO R & D LAB),** at Bani-Matter, Sanaa governorate, Republic of Yemen.

All research clinical studies on rabbits performed at faculty of veterinary medicine, Sanaa University, republic of Yemen.

ii. Study period

01-03-2023 to 30-05-2023. About three months.

Active pharmaceutical ingredient and Excipients were shown in table No. (1).

Table 1: Active Pharmaceutical Ingredient and Excipients Used.

NO	Name of materials	Uses in formulation
1	Bisoprolol Fumarate	API
2	Avicel PH 102	Diluents, lubricant, & disintegrant.
3	D (-) Mannitol	Diluent, sweetener's, tonic agent.
5	Crospevidone	SD
7	Magnesium stearate	Lubricants, Glidants, & anti-adherent.
8	Aerosol 200	Lubricants, Glidants, & anti-adherent.

iii. Equipment

As shown in table No. (2)

Table 2: Equipment's Used.

NO	Instruments used	Manufacturing company
1	Pediatrics' stethoscope	Japan
2	Animal balance	China
3	Animal cages	Local mad
4	Stopwatch	China
5	Fighting inducer	Local mad
6	Animal feeding syringes	China

b. Study design

Each rabbit was subjected to each of the three treatments in a randomized controlled and crossover study design. The Rabbits were divided into three groups (A, B, and C), with respecting the homogeneity of the rabbit population in the 3 groups, see table No. (3). The difference in heart rate between the groups was measured to determine the effect and times needed for the Bisoprolol fast dissolve sublingual tablet to normalize heart rate of the physical stressed Rabbits. The Experiment was carried out at the College of Veterinary Medicine, Sanaa University for 3 different time periods (phases), 7^{th}-9^{th} Jun (phase-1), 13^{th}-15^{th} Jun (phase-2), and 19^{th}-21 Jun (phase-3), with a time difference of 3 days between each phase, after the acclimatization process, which lasted 14 days from 24 May to 6 Jun 2023 G.C.

Table 3: Study Design Plan.

May 24 – Jun 6 acclimatization period (pre-test)	Phase (1) 7^{th}-9^{th} Jun	Phase (2) 13^{th}-15^{th} Jun	Phase (3) 19^{th}-21 Jun
Group-A (No=6), Group-B (No=6), and Group-C(No=6) Individual Observations	Group-A (No=6): Treatment 1	Group-C (No=6): Treatment 2	Group-B (No=6): Treatment 3
	Group-B (No=6): Treatment 2	Group- A (No=6): Treatment 3	Group-C (No=6): Treatment 1
	Group-C (No=6): Treatment 3	Group-B (No=6): Treatment 1	Group-A (No=6): Treatment 2

c. Animals and housing conditions

Eighteen healthy local domestic Rabbits (male sex), weighing (1.4 -2 kg) were used in this study. These animals were acclimatized for 14 days (pre-test period) in individual cages (humidity 20 %) at room temperature (25°C) in a 12 hrs. dark and 12 hrs. light cycle and given feed and water at regular time. The study was performed in accordance with the Animal Ethical Guidelines rules and regulations by a high academic and qualified worker, as shown in Fig (1).

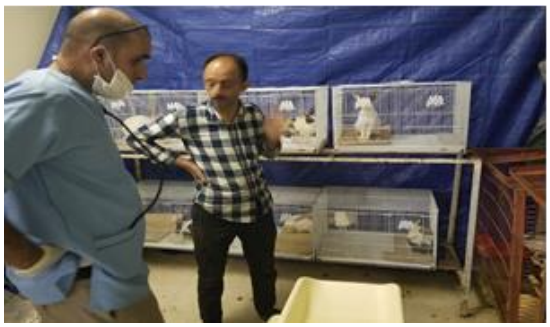

Figure 1: Animals and Housing Conditions.

d. Bisoprolol as fast dissolve sublingual tablets formula

- Label claimed: 110 mg.
- Batch size: 1180 tablets for each formula.
- Punch size: tableting on 5X8 mm oblong shape punch with a mid-groove on one side.

This formula of Bisoprolol as fast dissolve sublingual tablets prepared by direct compression methods. The constituents of formula used in this work were shown in table No. (4).

Table 4: Constituents of Bisoprolol as Fast Dissolve Sublingual Tablets Formula.

Ingredients for one tablet	Concentration in (mg)	Pharmaceutical Function
Bisoprolol (API)	10	Active Pharmaceutical Ingredients
Crosspovidone (SD)	5	Super Disintegrant
Avicel PH 102	43.7	Pharmaceutical Filler & Binder
D (-) Mannitol	50	Pharmaceutical Binder, Sweetener & Disintegrant
Aerosil 200	0.30	Pharmaceutical Anti-Adherent
Magnesium Stearate	1.00	Pharmaceutical Lubricant
Total Weight (Mg)	110	

e. Experimental set-up

The experimental rabbits subjected to physical stress and exercise studies was performed by 3 high qualified, experience, and academic experimenters (AS, SS and IS). The experimental set-up involved induce physical stress and measuring the heart rate of rabbits at different times as:

- Before confused and subjected to stress,
- Exactly after confused and subjected to stress,
- After 1 hr. After confused and subjected to stress,
- After 2 hrs. After confused and subjected to stress.

And the data was collected by the same experimenter using a stethoscope, stopwatch, conductions of physical stress and fear.[5,15,16]

During the study, all controllable conditions were kept the same for all rabbits to ensure consistency. However, some variables such as weather, and minor noises were not controllable due to field conditions and may have not affected the results.[17,18]

f. Treatment

The Rabbits were subjected to three types of treatments:

1. Group A (n=6) normal rabbits not induced & not treated (treatment-1, control gr),
2. Group B (n=6) induced rabbits & not treated (treatment 2), and
3. Group C (n=6) induced rabbits treated with Bisoprolol (treatment 3).

Fear and subjection to physical stress continuous at least for 5 minutes.

g. Data collection

The heart rate as parameters was used to determine the effect of Bisoprolol 10 mg (FDSLTs) on these studied rabbits. The induction of heart rate in rabbits was done by physical stress.[1,19] The persons handling the animals were very experienced in working with experimental rabbits and the heart rate was measured with a standard stethoscope.[20] The measurements of the heart rate were taken by the same experimenter to guarantee homogeneity in counting. All the rabbits were always carried and held by the same experimenter and all controllable conditions were similar for all the rabbits during the study. The heart rate of the rabbits was measured by placing a stethoscope under the front legs and timed for 30 seconds on a stopwatch, then the number of heart beats counted was multiplied by two and documented.[5,14]

Rabbits (treatment 2, treatment 3) were subjected to physical stress for 5 minutes to stimulate their heart rates and then heart rates were measured immediately after the stressful event (Zero-times), then after 1 hour, and after 2 hours and the data were recorded.[5,21]

Fast dissolving Bisoprolol tablets were prepared by dissolving each tablet (10 mg) with 2.5 mL of tap water in an oral syringe.

The heart rates of the rabbits group A, (control) were measured simultaneously with the heart rates of group B and group C.[5,22]

These procedures were repeated in each of the three phases (phase 1, phase 2, and phase 3), taking into account the switching of groups[5,23], as shown in Fig. (2).

Figure 2: Data Collections.

h. Ethical note

The ethical note in this study confirms that the rabbits used in the experiment were not considered laboratory animals and were constantly monitored to ensure their welfare. The researchers did not conduct any invasive procedures on the Rabbits, and the experiment would have been stopped immediately if any rabbit had shown signs of distress or impaired welfare. The study was approved by Veterinary college in Sanaa University and followed ethical guidelines to ensure the well-being of the animals.

i. Statistical analysis

To determine the effect of Bisoprolol FDSLTs, A one-way ANOVA test was used for statistical analysis to obtain confident results.[5,24]

3. RESULTS AND DISCUSSION

Heartbeats (HB) or heart rate of Rabbits could be used as a clinical parameter to measure Bisoprolol effect, in phase 1 as appeared in table No. (5), there is no change in heart rate of rabbits at zero time, after 1h, and after 2hrs of measurements (group A), but the significant change appeared when Rabbits physically induced. The heart rate increased markedly to reached to 270.22 ± 1.2413, 261.67 ± 2.3381, and to 256.11 ± 1.6555 in (group B), at zero time, after 1hrs, and after 2hrs respectively. The effect of Bisoprolol as fast dissolved tablet clearly appeared in Rabbits physically induced, and gave them Bisoprolol (group C), in which heart rate returned to normal value of 269.44 ± 0.5018, 240.22 ± 1.0037, and 220.22 ± 0.8074 at zero time, after 1hrs. and after 2hrs. respectively as appeared in table No. (5). Generally, Bisoprolol successfully reduced heart rate of indued Rabbits where it returned to normal value after 2 hrs. (max effect of Bisoprolol reached after 2 hrs.). These results emphasize phase 2, and phase 3 as shown in table No. (5).

Table 5: Statistical Analysis of Phase 1,2, and 3 Rabbits Grouping.

Phase 1	**0 Time HR**	**After 1 hour HR**	**After 2 hours HR**
Group A(Control)	219.89±0.98	220.78±1.42	220.67± 0.7303
Group B (Induced)	270.22±1.24	261.67±2.34	256.11± 1.6555
Group C (Induced + Bisoprolol)	269.44±0.50	240.22±1.00	220.22 ± 0.8074
Phase 2	**0 Time HR**	**After 1 hour HR**	**After 2 hours HR**
Group A(Control)	220± 0.8433	220.11±0.89	220.56±1.1483
Group B (Induced)	269.78±1.09	262.89±1.72	257.22±1.5444
Group C (Induced + Bisoprolol)	270.22±1.09	240.11±1.29	218.67±1.6329
Phase 3	**0 Time HR**	**After 1 hour HR**	**After 2 hours HR**
Group A(Control)	220 ± 0.8433	220.11±0.89	220.56±1.1483
Group B (Induced)	269.78±1.09	262.89±1.72	257.22±1.544
Group C (Induced + Bisoprolol)	270.22±1.09	240.11±1.29	218.67±1.633
Data represented as mean ± standard deviation (SD), HR = Heart Rate			

The term "autonomic cardiac control" refers to the control of heartbeats and rhythm by the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). The sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis are how the autonomic nervous system (ANS) reacts to psychological stress, causing physiological changes such the release of noradrenaline from the locus coeruleus. Increased sympathetic and decreased parasympathetic tone are related with dysregulation of the autonomic nerve control of the cardiovascular system, which is a major factor in coronary artery disease and the

development of potentially fatal ventricular arrhythmias. The autonomic nervous system's (ANS) sympathetic and parasympathetic functions can be measured with the help of the heart rate variability (HRV) technique. Since doctors first realized how important heart rhythms might be in the 19th century, HRV has been the subject of intensive research to better understand the physiological mechanisms and behavioral processes at play. Today, HRV is a common clinical parameter that incorporates geometric measurements, time-domain, and frequency-domain indices. Heart rate variability (HRV) can be used as a technique to reflect cardiac activity and general autonomic health in clinical conditions, according to the discussion part of the study. Studies have demonstrated that HRV variables alter in response to stress caused by diverse techniques because HRV is sensitive to changes in autonomic nervous system (ANS) activity associated with stress. When analyzing the connection between stress and HRV, it is crucial to consider the patient's medical and psychological history as well as the overall autonomic background.[1,25,26]

Based on the results obtained, group (B, and C) rabbits exposed to physical stress showed an increase in heart rate. According to the results, Bisoprolol FDSLTs significantly lowered the heart rate of induced rabbits' group (C), indicating that bisoprolol FDSLTs can assist rabbits in dealing with stress.

Based on results bisoprolol 10 mg as fast dissolve sublingual tablets lower heart rate both at rest and during activity. This is because of its β_1-selectivity, which prevents tachycardia brought on by exercise according to assumed hypothesis.[5, 27]

4. CONCLUSION

- According to the findings of the In Vivo study, stress and exercise cause a considerable increase in the heart rate of rabbits, which can be utilized as a clinical parameter rather than invasive procedures. Hence, the Heart Rate of rabbits under the influences was successfully and significantly lowered by Bisoprolol Fast Dissolve Sublingual Tablets, and it reverted to normal within two hours. (Bisoprolol's maximum effect is felt after two hours). These provide proof that Bisoprolol Fast Dissolve Sublingual Tablets can be used to treat tachycardia in animals and humans. So, our created Bisoprolol Fast Dissolve Sublingual Tablets formula, show rabid absorption and reach to maximum clinical effects after 2 hr. Accordingly, Bisoprolol Fast Dissolve Sublingual Tablets can be used as a pharmaceutical dosage form in animals.

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6. RECOMMENDATION

We advised further research to completely understand the specific amount of Bisoprolol FDSLTs that reduces heart rate and the effects of the medication on exposure to numerous fighting rabbits, as well as breeds and pets. In addition, these formulations may be used on humans.

7. **FUNDING:** NO.

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