Studies of Anti-arrhythmic and Hypercholesterolemic Activities of Ayurvedic Preparation 'Lauhasab' in Rat Model

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ABSTRACT: Lauhasab, an Ayurvedic preparation, is widely used in anemia and cardiovascular diseases. Despite its claim as a cardio-tonic there is paucity of studies on pharmacological activities and toxicities. In this study, the anti-arrhythmic effect and impact on lipid profile were evaluated. Rats were pretreated with 0.28 and 2.8 ml/kg body weight of Lauhasab for 35 days and electrocardiographic tracings were recorded and analyzed to determine heart rate and occurrence of arrhythmia. Electrocardiogram recorded before digoxin administration showed significant decrease in mean heart rate along with longer duration of bradycardia than in *digoxin control* group after 35 days of chronic pretreatment with both doses of Lauhasab. In animal experiments, various arrhythmias were observed after intraperitoneal injection of digoxin. Lauhasab decreased the duration and delayed onset of time of various arrhythmias. It showed significant increase in cholesterol and triglyceride levels in a dose dependent manner. It can be concluded that Lauhasab possesses significant anti-arrhythmic activity against digoxin-induced arrhythmia. It also revealed hyperlipidemic effects.

Key words: ECG, digoxin, anti-arrhythmic, lipid, herbal remedy.

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death globally.[1] According to the World Health Organization (WHO), cardiovascular disease was the leading cause of death of non-communicable disease (NCD) in 2012 and was to blame for 17.5 million deaths, or 46% of NCD deaths.[2] Due to the poor economic condition in Bangladesh, a large number of the population relies on herbal medicines, especially Ayurvedic system of medicine. The major issue with these herbal medicines is that very little scientific data is available on their safety and efficacy. Also it is very challenging to try and standardize polyherbal medicines using conventional array of toxicological and analytical methods since a large number of active ingredients are found in a single preparation.[3] The present field of research has a very strong correlation with the health care system of Bangladesh. The demand for herbal drugs is increasing day by day for its cost effectiveness and perceived pharmacological responses and fewer side effects. For this, Lauhasab has been selected as very few reports have been published for the therapeutic efficacy and safety levels of this herbal preparation. It is a widely used remedy for anemia and a cardio-tonic. It is a preparation of various medicinally important plants and roasted iron (*lauha bhasma*). According to a study by Jagetia *et al.*[4], a mixture of *Terminalia beleracia, Emblica officinalis* and *Terminalia chebula* has shown anti-oxidant activity. According to Ghani[5], *Piper nigrum* has stimulant and carminative actions and it can be used in asthma, cholera, dyspepsia etc. *Zingiber officinale* has been used in dyspepsia, vomiting, spasm etc.[6] The hypoglycemic and anti-fertility activities of *Embelia ribes* are mentioned by Ghani[5] and *Trachyspermum ammi* has been used for its anti-spasmodic activity.[6] Although, the pharmacological activities of individual ingredients are well established, no study

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was found on the cardiovascular effects of Lauhasab preparation. Although Lauhasab has label claim to be cardio-tonic, there is no established evidence to support it. This study was performed to evaluate the safety of this drug and to investigate any pharmacological effects on heart. The main objectives of this work were to screen the drug for anti-arrhythmic effect in digoxin-induced arrhythmia, to investigate the effects on heart rate and to observe changes in lipid profile of rat model, if any.

MATERIALS AND METHODS

Drug and instruments. Marketed preparation of Lauhasab. Digoxin powder (API) was a kind gift from Aristopharma Ltd. Edan VET-300 Veterinary (China) ECG machine was used to record ECG in rats. Centrifuge machine and diagnostic kits for the lipid profile (with the exception of low density lipoprotein-cholesterol, LDL-C) were purchased from Exim GmbH® (Germany).

Experimental animals. Sixteen healthy Sprague-Dawley rats (90-120 gm) were obtained from the Animal Unit, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. The rats were individually housed in stainless steel cages at room temperature with sufficient ventilation in Animal House, Institute of Nutrition and Food Sciences, University of Dhaka. Additional rats were used to determine arrhythmogenic dose of digoxin in rats.

Animal feeding. The rats (n = 16) were allowed to acclimatize for four days prior to diet treatment. They were randomly divided into four groups (n = 4 rats/group). Distilled water was the only source of fluid, along with liquid drug in pretreatment groups. Fluid and feeds were provided *ad libitum* for the duration of the study (35 days). At the end of the study, each rat was re-weighed before being anaesthetized with ketamine hydrochloride, I.P. injection.

Experiment protocol. Animals were grouped into following 4 groups of 4 animals each: Group 1: rats were given normal food and water thrice daily for 35 days. This group of rats is referred as *Control* rats. Group 2: rats were given normal food and water thrice daily for 35 days. At 36th day digoxin was administered (20 mg/kg body weight, I.P.). This group of rats is referred as *Digoxin control* rats. Group 3: rats were given normal food and water with Lauhasab low dose (0.28 ml/kg bw) for 35 days. At 36th day digoxin was administered (20 mg/kg bw, I.P.). This group is referred to as *Lauhasab low dose pretreated rats*. Group 4: rats were given normal food and water with Lauhasab high dose (2.8 ml/kg body weight) for 35 days. At 36th day digoxin was administered (20 mg/kg bw, I.P.). This group of rats is referred as *Lauhasab high dose pretreated rats*.

Drug dose determination. Low dose pretreatment groups received recommended dose of drug (in bottle label), which was calculated for a 70 kg adult human being and re-estimated for rats to 0.28 ml/kg bw. High dose refers to ten times of the recommended dose, 2.8 ml/kg bw.

Electrocardiographic studies. Edan VET-300 Veterinary ECG machine was used to record ECG in rats.

Preparation of digoxin solution. Digoxin powder (API), kindly donated by Aristopharma Ltd., was dissolved in aqueous ethanol and ultrasonicated for 20 minutes to obtain a clear solution. Each 1 mL solution contained 4 mg of digoxin.

Preparatory stage. Rat was taken from the animal house and brought to the laboratory. After giving the rat around an hour to acclimatize, it was weighed by electronic balance for calculating dose of ketamine hydrochloride and digoxin. The rat was then anesthetized with ketamine (50 mg/kg bw). After the rat was sufficiently anesthetized it was placed on dissecting board filled with wax and pinned to it by small pins. Then electrodes, dipped in electrode gel, were connected to the designated positions, e.g. two forelimbs, two hind limbs and chest.

Induction of arrhythmia. Digoxin has been chosen to induce arrhythmia for this study. Digoxin arrhythmogenic dose (AD_{50}) in adult rats 13 ± 1 mg/kg have been proposed by Weinhouse *et al*.[7] This was taken as a reference point to start screening for

arrhythmogenic dose of digoxin for the current studies. Doses of 8, 10, 13, 15, 20 and 22 mg/kg bw were administered intraperitoneally in ketamine hydrochloride anaesthetized rats and electrocardiogram was monitored continuously for 60 minutes. Auto (all leads) and rhythm (lead II) were recorded to observe any characteristic arrhythmic changes in the heart beats. A concentration of 20 mg/kg bw was chosen which induced arrhythmia without causing death within 60 minutes.

***In vivo* ECG and cardiovascular parameter measurements.** ECGs recordings were performed after 20 min of intraperitoneal injection of 50 mg/kg bw ketamine hydrochloride and for a period of 30 minutes before and 60 minutes after intraperitoneal injection of digoxin (20 mg/kg bw). Arrhythmias were assessed by identifying and quantifying the different arrhythmias and changes in heart rate during the 60 min recording period. Figure 1 showed the protocol of ECG measurement timings. The electrocardiogram was recorded as lead I, II, III, aVR, aVL, aVF and V (chest lead). The recording apparatus was EDAN VET-300. For this study only lead II was analyzed. This procedure was repeated for every rat.[8]

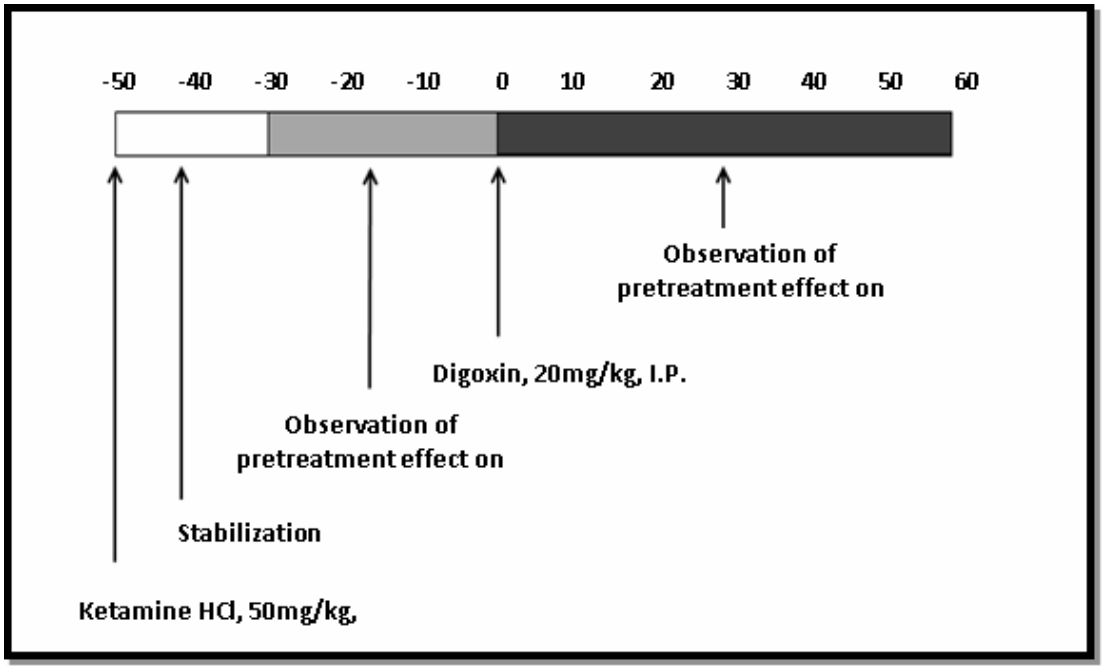

Figure 1. Diagram of the experimental protocol used. Protocol was used in the whole animal experiments with pre-treatment effect. The numbers above the bar indicate time (min).

Biochemical studies

Collection of blood, serum sample preparation and measurement of serum lipid profile. Rats were fasted overnight before collection of blood. Blood samples were withdrawn from heart aorta puncture using 5 ml hypodermic syringe and dispensed into 1.5 ml microcentrifuge Eppendorf tubes. The samples were allowed to stand for 30 minutes at room temperature to clot. Serum for the assays was thereafter separated from the clot by centrifugation at 4000 rpm for 5 min and biochemical determinations were carried out immediately after separation of the serum from the clot.

The assays were performed according to the manufacturer's instruction. LDL-C concentrations were estimated using the method of Friedewald[9] as shown below:

Test includes: Cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol (calculated), LDL-C: HDL-C and cholesterol : HDL-C

Methodology: Spectrophotometric (Colorimetric)

Data analysis. Data were expressed as mean ± S.E.M. Differences in mean values between experimental groups were analyzed by one way ANOVA (analysis of variance) followed by Dunnet's multiple comparison test where applicable. Differences in mean values between experimental groups were analyzed by two tailed student's t-test. A probability value less than 0.05 ($p < 0.05$) was defined to be significant and probability value less than 0.001 ($p < 0.001$) was defined to be highly significant.

RESULTS AND DISCUSSION

Observation of body weight. Body weight was taken initially on day 1 and later on day 36 to note changes (Table 1). It showed a significant ($p < 0.05$) difference in bw observed between the Lauhasab 0.28 and 2.8 ml/kg pretreated group and the control group by five weeks after initiating oral administration.

Low dose pretreatment = 0.28 mL/kg; high dose pretreatment = 2.8 mL/kg. n=5. Values are expressed in grams (g) and presented as mean±SEM, ns = not significant, *p<0.05 when compared to control group.

Electrocardiographic studies

Determination of arrhythmogenic dose of digoxin. Doses of 8, 10, 13, 15, 20, 22 mg/kg bw of digoxin etc. were administered and 20 mg/kg bw has been selected as optimal dose for inducing arrhythmia (Figure 2).

Table 1. Effect of drugs in body weight (g) after pretreatment.

Treatment		Initial weight (g)	Final weight (g)	Body weight gain (g)
Control		108.75±4.27	130±2.04	21.25±3.15
Lauhasab	Low dose	103.75±5.54	145±6.45	41.25±1.25*
	High dose	105±6.45	176.25±5.54	71.25±3.15*

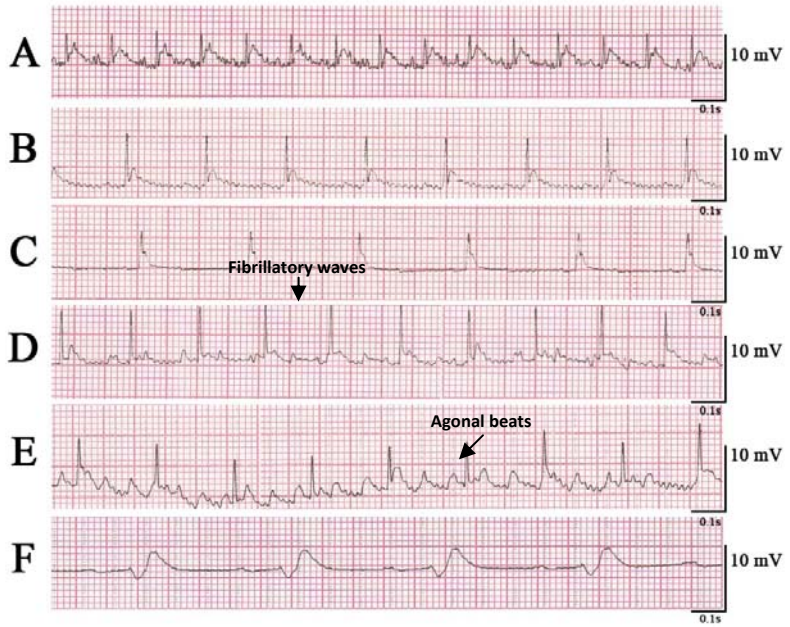

Figure 2. Arrhythmogenic dose (AD) determination by administering varying doses of digoxin (selected tracings: A. 8 mg/kg body weight; B. 10 mg/kg body weight; C. 13 mg/kg body weight; D. 15 mg/kg body weight; E. 20 mg/kg body weight ; F. 22 mg/kg body weight).

In figure 2, panel A showed no changes in heartbeat. Panel B-C showed bradycardia, panel D showed unstable atrial fibrillation, panel E displayed stable induction of arrhythmia, atrial flutter (AF), and panel F demonstrated agonal beats or dying beats indicating severe digitalis intoxication. The therapeutic and toxic level of digoxin are more than 100 times higher in rodents than in humans.[10] The dose of digoxin used in this study (20 mg/kg bw) has been found out, by trial and error based approach on control rats, to be arrhythmogenic (Figure 2). This dose is in the lower limit of toxicity in the rat heart, a species with extremely low sensitivity to digoxin.[11]

Effect of Lauhasab on arrhythmia. In the whole animal experiments with rats, the injection of digoxin (20 mg/kg bw, i.p. bolus) induced various types of atrial tachyarrhythmias such as atrial fibrillations (AFib), atrial flutters. It also induced ventricular bigeminy, junctional rhythm (inversed p wave), idioventricular rhythm. Additionally it showed digitalis effect characterized by ST-sagging (Figure 3).

Figure 3 showed typical ECG tracings of digoxin control group showing NSR (A) and changes after digoxin (20 mg/kg bw, i.p.) administration (B-F). Panel (A) shows normal sinus rhythm (NSR), (B) AFib, (C) AF 5:1, (D) bigeminy, (E) junctional rhythm, (F) digitalis effect. The recording speed from panel A-E was 50 mm/sec. ECG tracings were chosen from one of the four (n=4) similar and representative control experiments. NSR: normal sinus rhythm, AFib = Atrial Fibrillation, AF=Atrial Flutter.

Effects of drugs (pre-treatment, 0.28 and 2.8 ml/kg) on each arrhythmia and changes in heart rhythm are shown in Figures 4 and 5.

Figure 4 showed the ECG tracings of Lauhasab (pre-treatment, 0.28 ml/kg) indicating NSR (A) and changes after digoxin (20 mg/kg bw i.p.) administration (B-D). Panel (A) shows control rhythm, (B) junctional rhythm, (C) AF 4:1, (D) idioventricular rhythm. The recording speed from panel A-D was 50 mm/sec. ECG tracings were chosen from one of the four (n = 4) similar and representative control experiments. NSR: normal sinus rhythm, AFib: atrial fibrillation, AF: atrial flutter. No atrial fibrillation, bigeminy rhythm and digitalis effect was observed in this group.

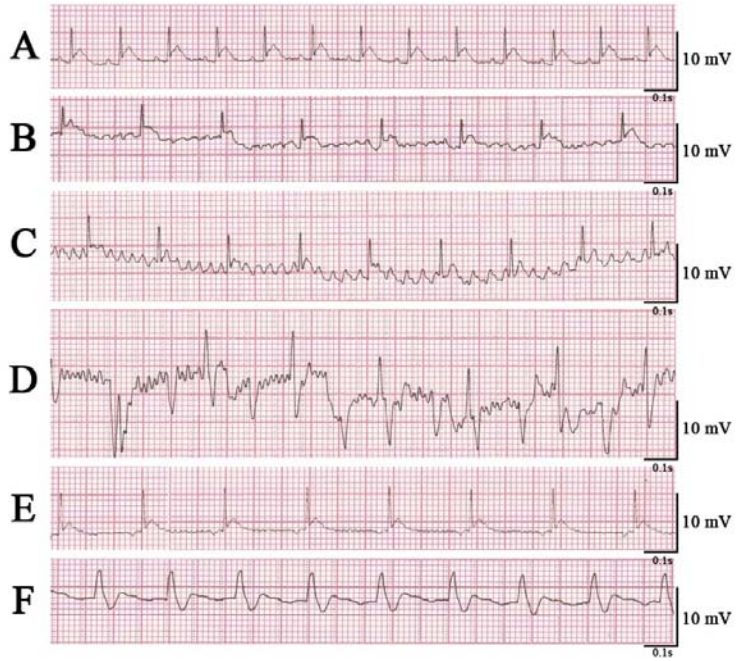

Figure 3. Changes in electrocardiogram in control group before and after digoxin administration.

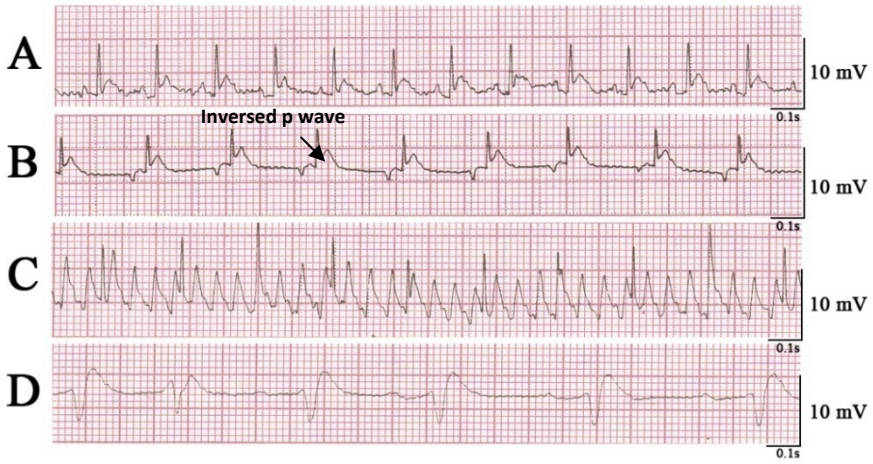

Figure 4. Changes in electrocardiogram in low dose group of Lauhasab before and after digoxin administration.

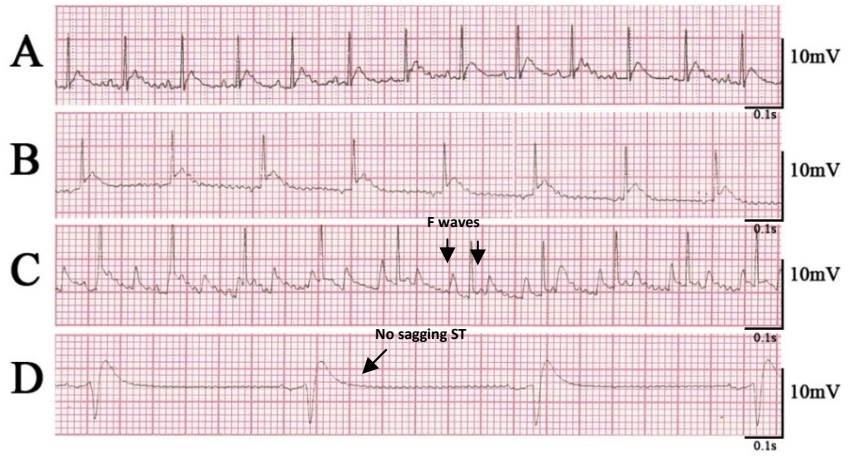

Figure 5. Changes in electrocardiogram in high dose group of Lauhasab before and after digoxin administration.

Figure 5 showed ECG tracings of Lauhasab (pre-treatment, 2.8 ml/kg) showing NSR (A) and changes after digoxin (20 mg/kg, i.p.) administration (B-D). Panel (A) showed control rhythm, (B) junctional rhythm, (C) AF 24:1, (D) idioventricular rhythm. The recording speed from panel A-D was 50 mm/sec. ECG tracings were chosen from one of the four (n = 4) similar and representative control experiments. NSR: normal sinus rhythm, AFib: atrial fibrillation, AF: atrial flutter. Again, no atrial fibrillation, bigeminy rhythm and digitalis effect was observed in this group.

In figure 6, panel A showed control (Group 2) (20 mg/kg bw digoxin, i.p.), panel B showed pre-treatment of Lauhasab (0.28 ml/kg bw) and panel C showed pre-treatment of Lauhasab (2.8 ml/kg bw). In all panels, arrow indicates intraperitoneal injection of digoxin. (n=4 for all groups). Time count was -30 minute after 20 minutes of stabilization after anesthesia (ketamine HCl, 50 mg/kg bw) and 0 minute after digoxin administration.

In both doses (Figures 4, 5 and 6) Lauhasab revealed several changes in heart beat compared to digoxin control group. Both groups suppressed atrial fibrillation and bigeminy rhythms. But more was still prone to atrial flutter were changed. Both dose reversed sagging ST of digitalis toxicity.

Figure 7 showed that in the control condition, i.e. in presence of digoxin, the duration of normal sinus

rhythm (NSR), sinus Bradycardia (SB), atrial fibrillation (AFib), atrial Flutter (AF), bigeminy rhythm (BR), junctional rhythm (JR), and digoxin effect (DE) were 8, 23, 2.25, 13.5, 2.75, 6.5and 2.75 (min), respectively.

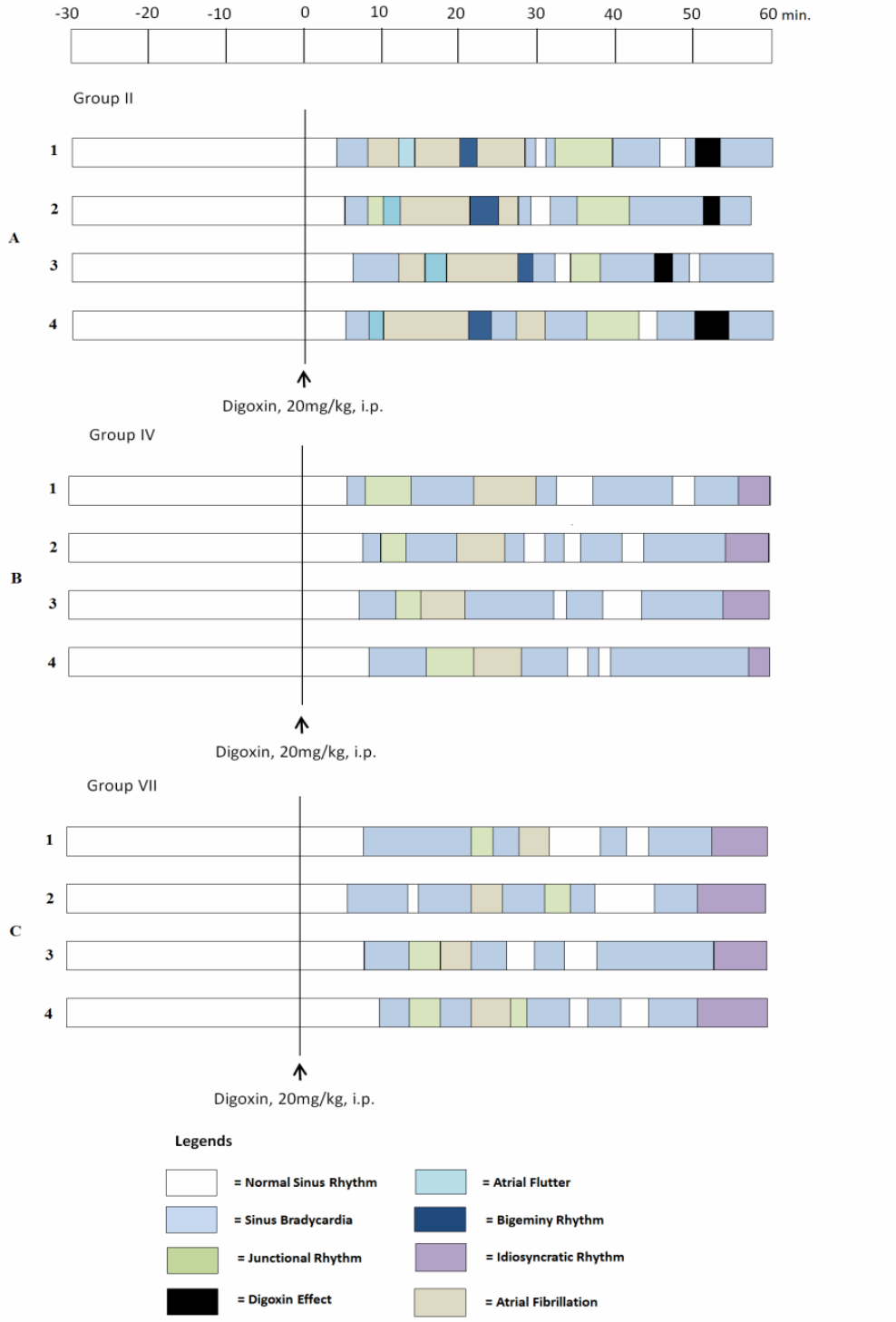

Figure 6. Effects of pre-treatment of Lauhasab on digoxin-induced arrhythmias.

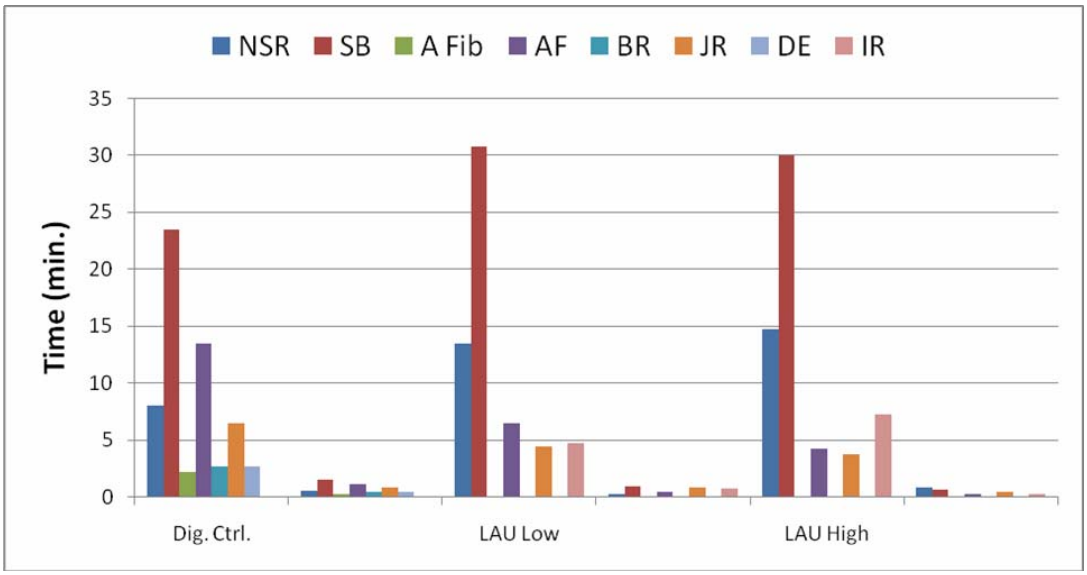

Figure 7. Duration of different changes in ECG.

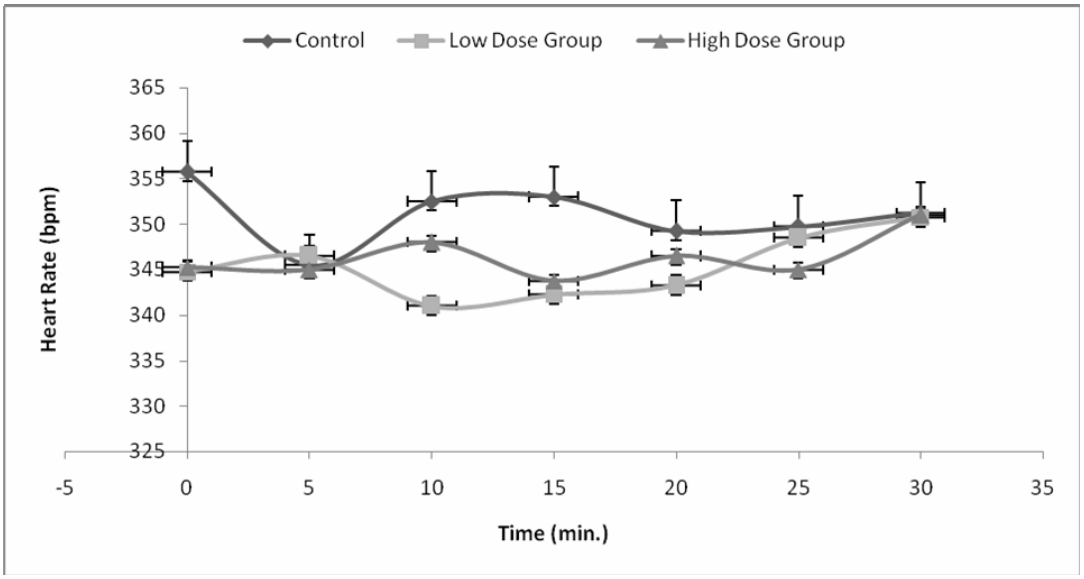

Figure 8. Effect of Lauhasab pretreatment on heart rate.

According to figures 6 and 7 showed, the duration (mean, n=4) of normal sinus rhythm (NSR), sinus bradycardia (SB), atrial flutter (AF), junctional rhythm (JR), and idioventricular rhythm (IR) in the presence of Lauhasab (pre-treatment, 0.28 ml/kg bw) were 13.5, 30.75, 6.5, 4.5 and 4.75 (min), respectively. During pre-treatment of Lauhasab (2.8 ml/kg bw) these durations (mean, n=4) were 14.75, 30.0, 4.25, 3.75 and 7.25 (min) and from figures 6 and 7 there was no occurrence of atrial fibrillation, bigeminy and digitalis effect. Lauhasab also delayed onset of atrial flutter from 10 min.(Group 2) to 19.5 min. in low doses and 23.25 min. in higher doses. Thus, pre-treatments with Lauhasab proved to be effective in shortening the duration of atrial flutter considerably, and delaying arrhythmia dose dependently in digoxin-induced arrhythmias in the whole rodent model.

Effect on heart rate. Heart rate changes before and after induction of digoxin toxicity was noted in control (Groups 1 and 2) and chronic drug pretreated groups and shown in figures 8 and 9.

Figure 8 showed the effect on heart rate of rodents pretreated with Lauhasab (Low dose = 0.28 ml/kg, High dose = 2.8 ml/kg) before digoxin injection (20 mg/kg, i.p.), n=4. Decrease on heart rate was significant ($p < 0.05$) in Lauhasab pretreated groups.

Figure 9 displayed the effect on heart rate of rodents pretreated with Lauhasab (Low dose = 0.28 ml/kg bw, High dose = 2.8 ml/kg bw) after digoxin Injection (20 mg/kg bw, i.p.). Lauhasab, in either doses, shows no significant effect on heart rate ($p > 0.05$).

Study of serum lipid profile. Serum total cholesterol, triglyceride, high density lipoprotein-cholesterol and low density lipoprotein-cholesterol were analyzed by using spectrometric assay and shown in figure 10 and table 2.

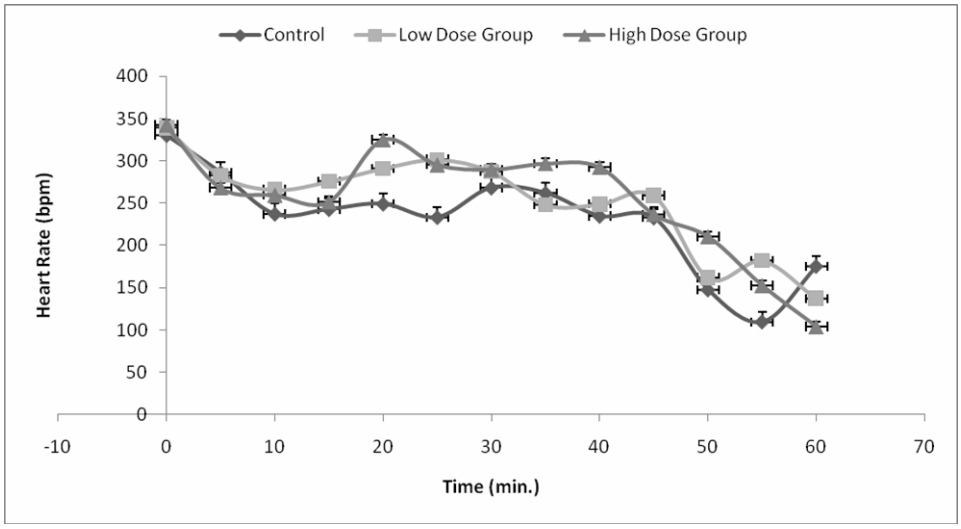

Figure 9. Effect of Lauhasab on digoxin-induced arrhythmia.

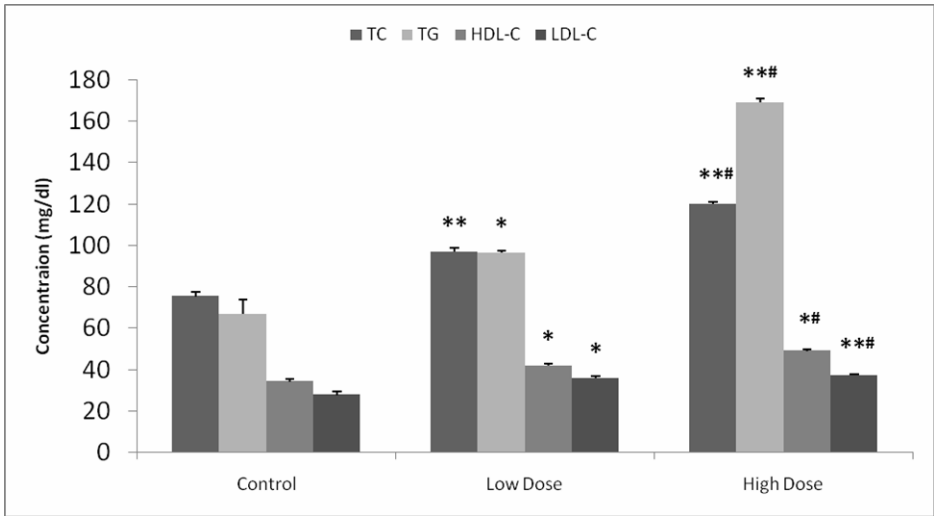

Figure 10. Lipid profile after chronic treatment with Lauhasab.*$p<0.05$**, $p<0.001$ when compared to control group;#$p<0.05$when compared to low dose. Here, TC= Total Cholesterol, TG= Triglyceride.

Table 2. Serum lipid parameters of Lauhasab.

Parameter	Control	Lauhasab	
		Low Dose Pretreatment	High Dose Pretreatment
Ratio; LDL-C:HDL-C	1.24	1.17	1.32
Ratio; TC:HDL-C	2.72	2.71	3.23

Figure 10 presented that, after pretreatment with prescribed dose (0.28 ml/kg bw b.i.d.) of Lauhasab for 35 days highly significant increase in total cholesterol ($p<0.001$), LDL-C ($p<0.05$) and HDL-C ($p<0.05$), and moderate increase in triglyceride ($p<0.05$) was noted in groups pretreated with the prescribed dose. Again, rodents pretreated with higher dose (2.8 ml/kg bw b.i.d.) of Lauhasab showed highly significant increase in weight along with total cholesterol ($p<0.001$), triglyceride ($p<0.001$), LDL-C ($p<0.001$), and moderately significant increase in HDL-C ($p<0.05$). Increase in triglyceride ($p<0.001$) and total cholesterol ($p<0.001$) in high dose pretreated rats is highly significant and increase in HDL-C ($p<0.05$) and decrease in LDL-C ($p<0.001$) were significant compared to the lower dose pretreated groups. So, increase in TC and TG was higher in high dose pretreated group.

This dose dependent increase in cholesterol, triglyceride and body weight due to Lauhasab pretreatment results in obesity in mice and may be attributed to stimulation of peroxisome proliferator-activated receptor-γ (PPARγ), which was implicated in causing obesity and hyperlipidemia.[12] One or more compounds in Lauhasab may work as PPARγ antagonist and decrease lipid metabolism in adipose cells. Further study on PPARγ expression may shed some light on this.

Table 2 showed, considerable decrease in LDL-C/HDL-C ratio and considerable increase in cholesterol ratio after high dose pretreatment. Dangerously high level of TG associating with a high TC/HDL-C ratio usually indicates increased risk of coronary heart disease.[13,14] So, Lauhasab in high doses may increase atherosclerosis risk.

CONCLUSIONS

Lauhasab demonstrated marked increase in bodyweight, cholesterol and triglyceride levels. It also showed negative chronotropic effect after chronic administration in rats. Lauhasab preparation, proved to be effective in digoxin-induced arrhythmia by decreasing duration and delaying onset of various arrhythmias. Still there are plenty of scopes to establish toxicity profile of this preparation in liver and other organs and exploring mechanisms of hyperlipidemic activity of Lauhasab in rodent model by investigating 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HGM-CoA reductase) and peroxisome proliferator-activated receptor-γ (PPARγ) expression, and mechanism of its protective action on digoxin-induced arrhythmia. So, considering the potential bioactivity, these drugs can be further screened for their unexplored efficacy and hepatic and renal toxicity to rationalize its medicinal uses.

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