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Effect of Novel Anti-Anginal Drug Ranolazine on Liver Enzymes in Albino Rats.

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ABSTRACT

To evaluate the effect of novel anti-anginal drug ranolazine on liver enzymes & liver histopathology of normal rat,ethanol induced hepatotoxic rat and that of silymarin on ranolazine & ethanol treated rats. For this study, 72 healthy albino rats were selected and divided into 12 equal groups. Rats of group 1 received distilled water while groups 2, 3 and 4 received Ranolazine in doses of 100, 200, 400mg/Kg respectively once a day for 3 weeks orally. Group 5,6,7 and 8 were pretreated with Ethanol 7.5 gm/kg orally once daily 1hr before administration of Dist. water and Ranolazine in the above 3 doses for 3 weeks. Groups 9 and 10 were pretreated with Silymarin 100mg/ kg orally once daily 1hr prior to administration of Ranolazine 200 and 400mg/kg . Rats of groups 11 and 12 received a combination of Ethanol + Ranolazine 200mg/kg and Ethanol + Ranolazine 400mg/kg respectively, for 3 weeks. Blood samples were collected on1wk and 3rd wk, from orbital venous plexus for the assay of liver enzymes i.e Aspartate aminotransferase , Alanine aminotransferase & Alkaline phosphatase, total and direct bilirubin. At the end of the study animals were sacrificed for histopathological study. Ranolazine is found to produce hepatotoxicity in high dose. It was found to aggravate Ethanol induced hepatotoxicity in animal models, which was preventable by Silymarin.

Keywords: Ranolazine, angina, liver enzymes, histopathology, rats, bilirubin,

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INTRODUCTION

Chronic angina is one of the most common cardiovascular disorders known to impair quality of life and decrease life expectancy, in developing and developed countries. Though considerable progress has been made in the treatment of angina, many patients continue to experience the symptoms while a few remain refractory to conventional medications [1].

Conventional antianginal drugs mainly act through two mechanisms.

First mechanism decreases the mitochondrial ATP formation by reducing heart rate, blood pressure and cardiac inotropism. This mechanism is exploited by drugs such as β blockers and nitrates [2].

The second mechanism increases oxygen supply to the area of ischemia, which is done by vasodilators and revascularisation procedures. However, most of these antianginal agents cause haemodynamic changes such as coronary vasodilatation, negative inotropism etc, resulting in symptomatic hypotension, bradycardia and worsening of cardiac failure. Ranolazine a novel antianginal drug ,produces cardiac anti-ischemic effect without affecting hemodynamics [3]. Ranolazine (+)N-(2,6-dimethylphenyl) -4(2-hydroxy-3-(2-methoxyphenoxy)-propyl)-1-piperazine acetamide dihydrochloride]) is an active piperazine derivative [4]. It has molecular weight of 427.54 and its molecular formula is(C24H33N3O40).It is a white or slightly yellow crystalline powder with melting point about(120^{o}c) and is stable for at least 5 y at 25^{o}C.The free base of ranolazine is soluble in dichlormethane,sparingly soluble in methanol,slightly soluble in ethanol or acetonitrile and practically insoluble in water [5]. As the free base of ranolazine is insoluble in water,ranolazine hydrochloride which is soluble in water has been used in animal studies.

Myocardial ischemia is associated with sudden increase in fatty acid levels resulting in enhanced oxidation of long chain fatty acids. Oxidation of fatty acids needs more ATPs than oxidation of carbohydrates and also an increased oxygen demand [6].

Moreover this may lead to accumulation of free fatty acids and lactic acid, increasing the acidosis. These mechanisms have detrimental effects on the contractility and efficiency of the heart. Therapeutic interventions, which shift myocardial substrate utilisation to glucose metabolism, may provide benefits to ischemic patients. This can be achieved by drugs which suppress fatty acid oxidation. Ranolazine, a partial inhibitor of fatty acid oxidation, shifts ATP production from fatty acid to more oxygen efficient carbohydrate oxidation, especially when it is elevated as in ischemia. It has been shown to stimulate glucose oxidation during increased plasma free fatty acid levels associated with myocardial ischemia [7].

In pathologic states such as ischemia, oxidative stress, myocardial stretch, and left ventricular hypertrophy, the INa either fails to close or it reopens too late, leading to an abnormally high increase in intracellular sodium [8].

This sodium elevation causes intracellular calcium to increase through the sodium–calcium exchanger. The increase can alter downstream pathways and lead to increased diastolic stiffness, diastolic coronary vascular compression, and poor oxygen demand–supply matching [9-11].

Another mechanism is selective inhibition of late INa currents, resulting in decrease at the calcium overload on the cardiac fibres. The inhibition of INaL currents decreases the intracellular sodium concentrations, especially in ischemic myocytes where the current is amplified. Inhibition of sodium influx reduces calcium overload and decreases left ventricular wall tension, resulting in decreased myocardial oxygen demand.

The most common adverse effect of Ranolazine seen at higher plasma concentrations (eg, >8000 ng/mL), nausea, vomiting, dizziness, vertigo, abnormal vision, confusion, postural hypotension, and syncope [12,13].

Ranolazine is known to increase the QT interval on the electrocardiogram [14]. Extended QT intervals increase the risk of sudden cardiac death [15].

Because its effect on the QT interval is increased in the setting of liver dysfunction, it is contraindicated in persons with mild to severe liver disease [16]. Ranolazine is extensively metabolized in the liver and intestine by CYP3A4 and to a lesser extent by CYP2D6 enzyme [17]. It is both a substrate for and an inhibitor of these microsomal enzymes.

In a small pharmacokinetic study, it was observed that moderate hepatic impairment was associated with a 76% increase in AUC and 51% increase in Cmax and more than doubling of trough concentration of Ranolazine, compared to healthy subjects [18].

There is insufficient data regarding direct effect of Ranolazine on liver enzymes. Many patients with ischaemic heart disease are also alcoholic and the effect of Ranolazine on alcoholic hepatitis needs to be studied. It is a unique experimental study, conducted in Department of Pharmacology of a tertiary care hospital of Odisha. This study is to see the effect of Ranolazine which is a novel antianginal drug on liver enzymes of normal rat,ethanol induced hepatotoxic rat and that of silymarin treated rats.Patients of angina taking Ranolazine may be alcoholic or with deranged liver enzymes.So this research work focus regarding the use of Ranolazine in hepatic impairment conditions.

Hence the aims and objectives of this study is to evaluate the effect of ranolazine on liver enzymes of normal rat and of ethanol induced hepatotoxic rats . Also the effect of Silymarin on ranolazine treated rats and Silymarin and Ranolazine on ethanol treated rats is observed.

MATERIALS AND METHOD

Drugs and Chemicals

- Ranolazine (Sun pharma) 100,200,400mg/kg/po/OD
- Ethanol (Analytical grade) 7.5gm/kg/po/OD,
- Silymarin (Micro lab) 100mg/kg/po/OD,

The study was conducted in a tertiary care hospital in Eastern India, more specifically in Odisha.

Animals

Seventy two healthy albino rats of either sex weighing between 150-200gms were selected and randomly divided into 12 equal groups with six rats in each group. They were housed in the Departmental Animal House 12 : 12 light dark cycle. The animals had free access to food and water [19].

All animal experiments were approved by the Institutional Animal Ethical Committee

Ranolazine (Sun Pharma) tablet of 500mg strength was triturated in mortar & pestel, dissolved in distilled water from which it was administered in doses of 100, 200, 400mg/kg to different groups of animals. Ethanol (Analytical grade) in the dose of 7.5gm/kg and Silymarin (Micro lab) 100mg/kg were used for the experiment. Drugs and chemicals were administered for three weeks, once daily, intra-gastrically, in the following manner as mentioned in the plan of study.

PLAN OF STUDY:

NORMAL RATS	ETHANOL TREATED RATS	SILYMARIN TREATED RATS
GR 1 - Distilled water 200	GR 5 - Ethanol 7.5 + Dist. water	GR 9 -Silymarin 100+Ranolazine
GR 2 - Ranolazine 100 400	GR 6 - Ethanol 7.5 + Ranolazine 100	GR 10-Silymarin 100+Ranolazine
GR 3 - Ranolazine 200 Ethanol 7.5	GR 7 - Ethanol 7.5 + Ranolazine 200	GR 11-Sily 100+Rano 200 +
GR 4 - Ranolazine 400 Ethanol 7.5	GR 8 - Ethanol 7.5 + Ranolazine 400	GR 12-Sily 100+Rano 400 +

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Blood samples were collected before drug administration and after 1wk and 3wk of drug administration from orbital venous plexus after light ether anaesthesia to assay the liver enzymes i.e aspartate aminotransferase, Alanine aminotransferase [20] & Alkaline phosphatase [21] total and direct bilirubin [22].

On 22nd day 2 rats from each group were sacrificed by ether over dose.A portion of liver tissue of each rat was preserved in 10% formaldehyde solution and sent for histopathological study to department of pathology.Haematoxylline and eosin were used for staining and histopathological slides of the liver cells were photographed for further analysis [23,24]. Data expressed as Mean ± SEM and statistical difference between means was determined by ANOVA followed by Tukey kramer's post-hoc test.

OBSERVATION:

Table I: Effect of Drugs on Serum Liver Enzymes of Rats

GR	DRUG DOSE	DUR	A S T	A L T	A L K	D BIL	T BIL
1	D. WATER	1 WK 3 WK	125 ± 11.2 131 ± 14.4	30 ± 4.8 35 ± 6.4	630 ± 22.2 592 ± 18.4	0.18 ± .8 0.16 ± .4	0.33 ± .9 0.36 ± .5
2	RANO 100	1 WK 3 WK	220 ± 16.6* 149 ± 19.4	49 ± 8.8* 45 ± 4.4	777 ±24.8* 654 ±16.9*	0.17 ± .7 0.16 ± .3	0.32 ±.2 0.34 ± .6
3	RANO 200	1 WK 3 WK	271 ± 15.2* 264 ± 18.6*	55 ± 5.1* 49 ± 7.4*	854 ± 1.8* 763 ± 9.4*	0.18 ± .5 0.17 ± .4	0.34 ± .9 0.36 ± .4
4	RANO 400	1 WK 3 WK	298 ± 20.6* 251 ± 16.8*	57 ± 9.8* 52 ± 6.4*	1115±36.8* 962 ± 20.4*	0.16 ± .6 0.16 ± .5	0.33 ± .3 0.37 ± .6
5	E THANOL + D.WATER	1 WK 3 WK	145 ± 14.2 269 ± 11.9*	48 ± 4.9 69 ± 9.4*	800 ± 14.8* 1342±16.4*	0.15 ± .2 0.17 ± .4	0.36 ± .9 0.37 ± .8
6	E THANOL +RANO 100	1 WK 3 WK	225 ± 20.2* 291 ± 18.4*	50 ± 4.8* 65 ± 9.4*	830 ± 16.8* 1352±26.2*	0.16 ± .9 0.17 ± .3	0.37 ± .7 0.38 ± .6
7	ETHANOL +RANO 200	1 WK 3 WK	264 ± 22.2* *288* ± 20.8*	55 ± 4.8* 66 ± 6.4*	845 ± 15.5* 1376 ± 22.4*	0.18 ± .9 0.16 ± .5	0.36 ± .5 0.39 ± .6
8	ETHANOL +RANO 400	1 WK 3 WK	322 ± 22.2* 310 ± 14.4*	60 ± 5.5* 65 ± 6.4*	894 ± 17.6* 1394 ± 16.4*	0.14 ± .8 0.15 ± .2	0.35 ± .9 0.39 ± .7
9	SILYMARIN +RANO 200	1 WK 3 WK	129 ± 14.8 139 ± 14.9	40 ± 4.6 42 ± 6.4	638 ± 13.8 662 ± 16.9	0.17 ± .2 0.16 ± .4	0.33 ± .4 0.35 ± .5
10	SILY MARIN +RANO 400	1 WK 3 WK	133 ± 12.6 140 ± 14.4	38 ± 4.8 35 ± 5.2	595 ± 14.0 642 ± 19.2	0.15 ± .7 0.17 ± .4	0.33 ± .9 0.36 ± .6
11	SILY + ETH +RANO 200	1 WK 3 WK	144 ± 18.2 140 ± 15.2	30 ± 5.5 35 ± 6.6	600 ± 12.3 592 ± 15.9	0.18 ± .8 0.17 ± .6	0.35 ± .8 0.36 ± .4
12	SILY + ETH +RANO 400	1 WK 3 WK	148 ± 11.2 152 ± 14.4	47 ± 4.9 50 ± 6.2	640 ± 14.8 662 ± 16.7	0.18 ± .7 0.16 ± .4	0.34 ± .9 0.36 ± .6

n = 6; ANOVA with Post hoc Tukey Kramer test; * = p < 0.05

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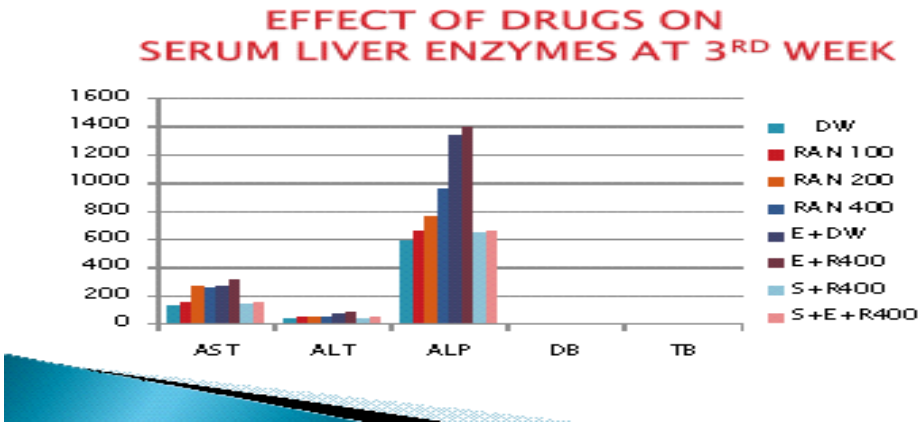

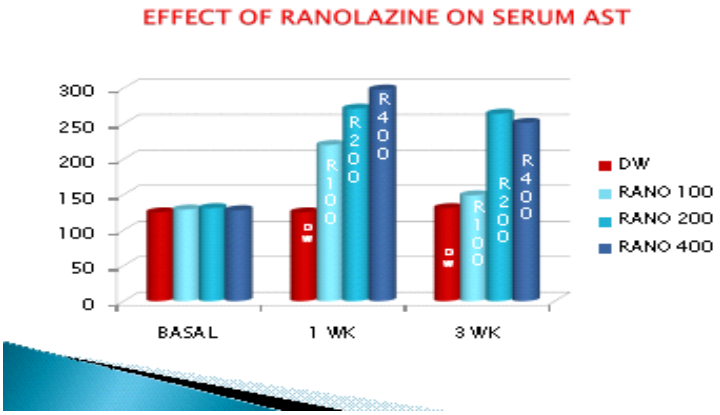

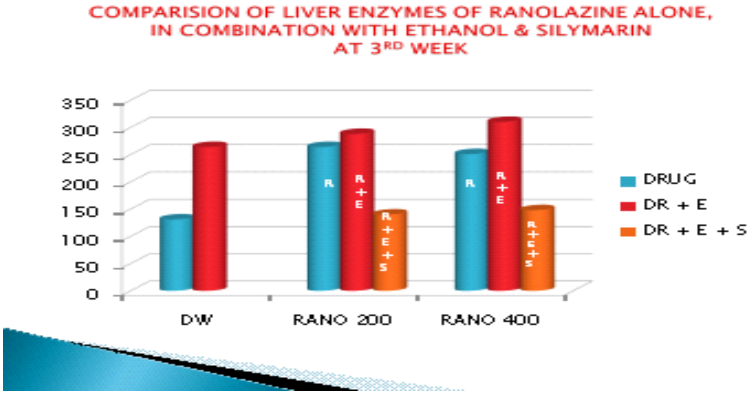

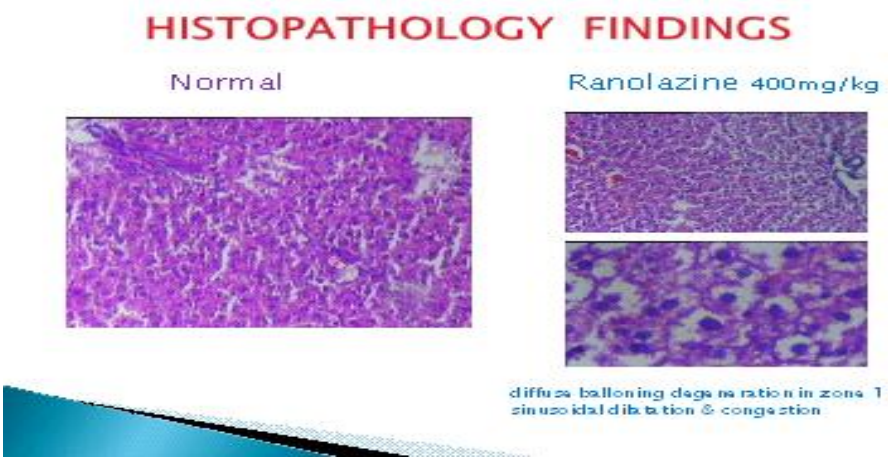

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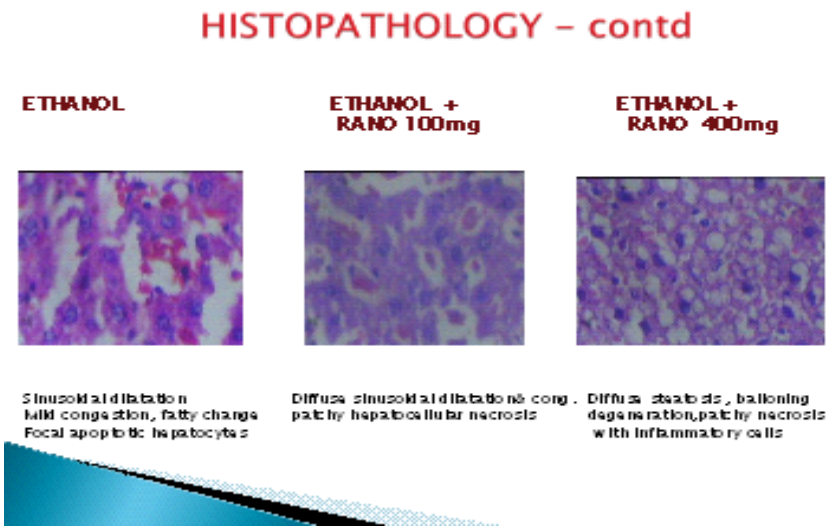

RESULTS

Ranolazine increased the serum level of AST, ALT&ALK significantly in a dose dependent manner after one week of treatment (Group 2,3,4 rats) which indicates hepatic damage.The level of the same enzymes also remained elevated significantly at the end of 3wks but the peak were comparatively less than that after 1wk of treatment .No significant change in direct and total bilirubin level was noticed. Ethanol a known hepatotoxic agent produced a significant rise in serum level of all liver enzymes the peak rise being higher at 3wk interval (322 ± 22.2*).But serum bilirubin values both direct and total remained unchanged,combination of ranolazine and ethanol was found to produce significant rise in the level of all liver enzymes,the peak values being more than that with ethaanoltreatment alone indicating exhibition of an additive effect.Rat of groups 9 & 10 receiving silymarin + Ranolazine 200mg & 400mg/kg doses respectively exhibited a significant fall in the level of all liver enzymes in comparision to ranolazine treated groups alone. Sylimarine treatment to rats of groups 11 & 12 receiving both ethanol and ranolazine together also revealed a significant fall in serum level of all liver enzymes in a similar fashion as that of group 9 & 10.

Examination of histopathology of liver slices of rats of different groups revealed that ranolazine (400mg) treatment for 3 weeks produces pathological changes in form of balloning degeneration in zone 1, sinusoidal dilatation and congestion in similar pattern as the hepatotoxic agent ethanol. Moreover when ranolazine was administered along with ethanol the hepatic damage was more pronounced in dose dependent manner viz. diffuse steatosis balloning degeneration,patchy necrosis with infiltration of inflamatory cells.

DISCUSSION

Ranolazine is reported to be contraindicated in patients of liver diseases [25].Ethanol is known to produce hepatic injury and incidence of CAD is more in alcoholic.Hence CAD patients with alcoholic habbit wheather should be prescribed with ranolazine is detectable.Therefore high doses of ranolazine have been administered for 3 weeks to albinorats to test any adverse effect of the drug alone as well as attempts have also been made to assess the effect of ranolazine along with ethanol to draw a definite conclusion as regards its safe use in alchoholics.

Ranolazine has the potential not only to raise the liver enzymes,but also can induce and aggravate hepatic damage.In ethanol treated rats,even in the low dose it can cause direct hepatotoxic effect.Ethanol produces decrease in hepatic content of glutathion (GSH),which is an important biomolecule that affords protection against chemically-induced cytotoxicity [26,27]. Wang et al., showed the protective effect of silymarine against ethanol-induced changes in these parameters. Ranolazine on liver enzymes of rat. This research is unique and novel because there was not so much data available on this topic.This reasearch provides a path regarding use of unique drug Ranolazine in patients who are refractory to conventional antianginal drug and are prone to hepatic damage

Moreover Ranolazine plasma conc is increased in patients with hepatic functional impairment.Hence Ranolazine should be avoided in patients having hepatic impairment,as well as in chronic alchoholics patients of IHD who are already prone to hepatic damage.

CONCLUSION

The data presented inthis study showed that Ranolazine causes transient rise in liver enzymes which peaks at 1wk and declined thereafter. Ranolazine also potentiates ethanol induced hepatotoxicity. Silymarine protects against Ranolazine & Ethanol induced hepatotoxicity.

Ranolazine should be avoided in patients having hepatic impairment,as well as in chronic alchoholics patients of IHD who are already prone to hepatic damage. Hence Liver function should be monitored in patients on ranolazine therapy.

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