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TOXIC EFFECTS OF ERGOTAMINE ON THE LIVER AND BLOOD OF ALBINO RATS

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ABSTRACT

Male albino rats were orally administrated with three dosages of ergotamine. The highest dose of ergotamine (0.4167 mg/ml), caused severe damage to the liver tissue. At 0.06 mg/ml, the liver showed minor changes and normal structural pattern was intact. At the highest dose, the changes were very severe and normal pattern of hepatocytes and architecture of the liver tissue totally DISAPPEARED. Minor changes on hematological parameters were observed, no bacteremia was detected and fecal samples were normal. *In vitro* studies, ergotamine slowly induces well-sustained vasoconstriction that can not be eliminated with drug washout. It appears that, ergotamine tends to produce a pattern of subjective effects that varies as a function of dose.

Key-words: ergotamine, Albino rats, liver, histopathology, hematology, fecal examination.

INTRODUCTION

The term "ergot" is generally used to describe species of the fungus genus *Claveceps*, the sclerotia formed by the fungi specifically *Claviceps purpurea*, and the wide range of unique alkaloids produced by the fungi following infestation of grain. The ergot alkaloids are derivatives of the tetra cyclic compound 6-methlyergoline (Berde and Sturmer, 1978). There simply is no such single entity as ergot from the pharmacologic, toxicological, biological, or clinical point (Berde, 1978). The "classic" natural ergot alkaloid is ergotamine. It is continually used as a treatment of vascular headache (Rall, 1990), producing constriction of both veins and arteries (Berde and Sturmer, 1978; Miller-Schweinitzer and Weidmann, 1978), and extensively metabolized in the liver. Reduction in liver function may lead to accumulation and sequesteration in various tissues that probably accounts for its long-lasting effects.

Contraindications to the use of ergotamine tartrate include renal or hepatic failure; coronary, cerebral, and peripheral vascular disease; and sepsis. Based upon several reports (Andersson, 1975, 1988), detoxification frequently carried out through rapid discontinuance rather than slow, gradual reduction. *In vitro* studies show that ergotamine slowly induces well-sustained vasoconstriction that can not be eliminated with drug washout (Mikkelsen *et al.*, 1981; Ostergaard *et al.*, 1981). The aim of the present study was to observe the histopathological and hematological effects of acute and chronic doses of ergotamine on liver and blood of male albino rat.

MATERIALS AND METHODS

Animal Models:

Male albino rats kept in the animal house of Department of Zoology for a period of two weeks to examine any abnormalities or any pathological symptoms and provided food and water *ad labitum*. Only those rats were used in the study which showed no symptoms of any illness. All animals employed in this study were of the same age (12 weeks) and body weight (250 g).The fed animals were monitored continuously throughout the test period (90 days), with one day interval**.**

Drug and Dosage:

Three different dosages of ergotamine tartrate (Novartis Pharma) were administered to the male albino rats. The dosages were calculated so as to match the dosages given to the human beings according to their weights. The dosages were given via oral route through gastric feeding needle with a ball tip to prevent introduction of the needle into the trachea.

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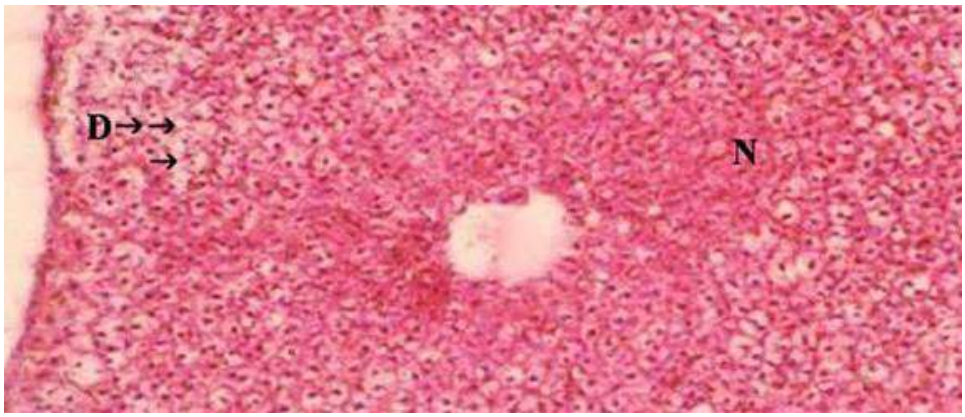

Fig..1. Transverse section of the liver tissue of orally treated albino rat with 0.06 mg/ml of ergotamine, showing necrosis (N) of the hepatic cells and degeneration (D) of the cytoplasm while the nucleus is normal. (X50). (H&E stain).

Fig.2. Transverse section of the liver tissue of orally treated albino rat with 0.4167 mg/ml of ergotamine, (acute dose), showing intensive reaction of ergotamine. The individual liver cells cannot be seen, central venule is abnormal and shows thrombosis (T). Large vacuolated areas are seen around the central venule (V). (X50). (H&E stain).

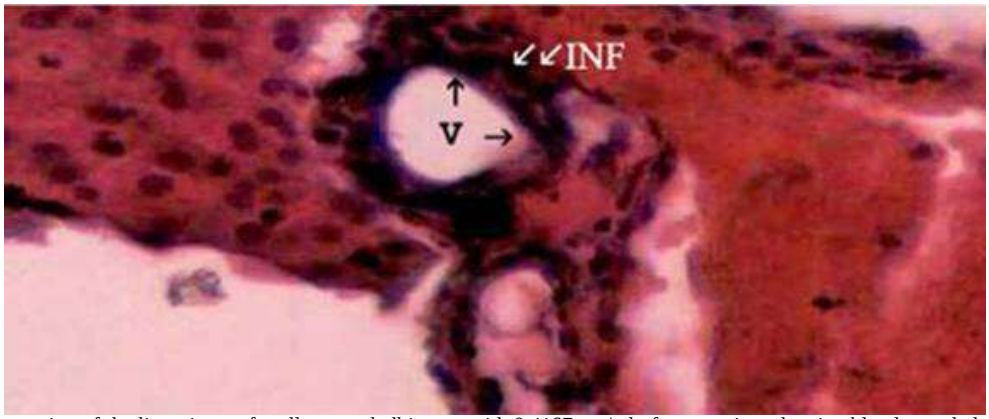

Fig.3. Transverse section of the liver tissue of orally treated albino rat with 0.4167 mg/ml of ergotamine, showing blood vessel abnormality (V), infiltration of microphages and other inflammatory cells (INF). (X 200). (H&E stain).

Histopathological Evaluation:

The rats were anesthetized with chloroform. The blood was drawn via heart puncture using sterile syringe from these animals. The blood was used for hematological and microbiological tests. After collection of the blood an incision was made in the abdomen and portion of the liver was fixed in 10% formalin and processed for histological studies.

Hematological Evaluation

Following hematological parameters were evaluated:

1. Hemoglobin…….(Hb)
2. Total Red Blood Cells Count……...(RBC)
3. Erythrocyte Sedimentation Rate ……..(ESR)
4. P.C. V…….. ……..(Hct)
5. Total White Blood Cells Count…….(WBC)
6. Differential Leucocytes Count ……...(DLC)
7. Platelet Count (according to Dacie and Lewis. 1995)

Microbiological Evaluation:

For microbiological studies, blood and fecal samples were cultured according to methods given by Wistreich and Lechtman (1984).

RESULTS AND DISCUSSION

The dosed animals were monitored continuously throughout the test period (90 days with one day interval) and during this period none of them showed any signs of illness. However, the animals which were given acute doses of ergotamine (0.4167 mg/ml) showed marked changes. Gross examination of the organs revealed hemorrhage in the liver. Histopathology showed that the higher the dose of ergotamine, the severe was the damage to the liver. At 0.06 mg/ml of ergotamine, atrophy of hepatocytes started with shrinkage of the cytoplasm, necrosis at some places, and condensation of hepatocytes around the central vein. Slugging was also seen in the central vein but the architectural condition was still identifiable (Fig. **1**). At higher dose, prominent changes in hepatocytes, central veins and portal tract areas occurred. At the highest dose (0.4167 mg/ml), the changes were very severe and normal pattern of hepatocytes and architecture of the liver tissue was totally lost, dislocation of liver tissue from the portal tract area was obvious producing large spaces around this area. Slugging and complete occlusion of the large vein was produced. A small artery and bile duct section was also seen in its vicinity. (Fig. **2**), vessel abnormality and infiltration of macrophages and other inflammatory cells were observed (Fig. **3**).

TableI. Mean followed by SD of hematological values of treated rats with different doses of ergotamine.

Test / Animal Group (Standard Value)	Hb g/dL	Total RBC Count 10^6/mm^3	ESR mm/Hr	PCV Vol%	Platelet Count 10^3/mm^3	Total WBC Count 10^3/mm^3	Differential Leucocytes Count					
							N %	L %	M %	E %	B %	Abnormal Immature Cells
	11.5-16.1	6.76-9.75	3-7	37.6-50.6	150-460	6.6-12.6	17-38	47-91	1-4	3-8	0-3	
***E1**	14.9±2.08	4.9±0.43	06±1	45±5	220±28.6	10.2±2.25	48±5.29	49±9.00	01±0	02±1	00	**NIL**
***E2**	16.4±0.1	5.2±0. 2	05±1	49±0.5	196±2	9.0±1.73	23±4.58	75±2.64	01±0	01±0	00	**NIL**
***E3**	13.9±0.43	4.6±0.34	04±0	42±5.5	195±27	9.7±1.3	45±6.08	52±2.0	02±0	01±0	00	**NIL**
***EA**	10.9±0.62	5.9±0.1	04±1.73	37±6.08	160±8.54	14.2±0.55	20±1.00	75±3.46	01±0	02±0	00	**NIL**
***C**	15.2±0.17	7.2±0.78	04±2.64	48±2	250±6.24	11±1.00	33±3.60	65±21.9	01±0	01±0	00	**NIL**

Hb=Hemoglobin; **B=**Basophile; **RBC=**Red Blood Cells; **E=**Eosinophile; **PCV=**Packed Cell Volume;
E1= The animal group given 0.03 mg/0.5 ml of ergotamine =0.06 mg/ml; **WBC=**White Blood Cells;
E2=The animal group given 0.06 mg/1.5 ml of ergotamine =0.04 mg/ml; **N=**Neutrophile; **E3=** The animal group given 0.09 mg/3 ml of ergotamine. =0.03 mg/ml; **EA=**The animal group has given acute dose of 1.25 mg/3 ml of ergotamine. =0.4167 mg/ml**; L=**Lymphocytes; **C=**Control; **M=**Monocyte;
* There were three replicates animals for each observation.

Hematological analysis of blood for control and treated animals are presented in (Table 1). Microbiological analysis of blood test for control and treated animals revealed no bacteremia (Table 2). Likewise, feces of the treated

animals did not show presence of any pathogenic bacteria (Table 3) and the microbial contents were the same as for the controls (untreated).

Table 2. Mean microbiological values of blood samples of rats treated with different doses of ergotamine.

Media	ANIMAL GROUPS ERGOTAMINE					ORGANISM ISOLATED
	*E1	*E2	*E3	*EA	*C	
NA	_	_	_	_	_	**NIL**
BA	_	_	_	_	_	**NIL**

E1: The animal group has given dose of 0.03 mg/0.5 ml of ergotamine. = 0.06 mg/ml; **E2:** The animal group has given dose of 0.06 mg/1.5 ml of ergotamine. = 0.04 mg/ml; **E3:** The animal group had given dose of 0.09 mg/3 ml of ergotamine. =0.03 mg/ml; **EA:** The animal group has given acute dose of 1.25 mg/3 ml ergotamine. = 0.4167 mg/ml; **C:** Control animal were given distilled water; **NA:** Nutrient Agar; **BA:** Blood Agar; - Negative, No growth; * There were three replicates animals for each observation.

Table 3. Mean microbiological values of fecal samples of rats treated with different doses of ergotamine.

Media	ANIMAL GROUPS ERGOTAMINE					MAJOR ORGANISM ISOLATED
	*E1	*E2	*E3	*EA	*C	
NA	+	+	+	+	+	***E. coli, Lactobacilli***
BA	+	+	+	+	+	*S. aureus*
Mac	+	+	+	+	+	*E. coli*
EMB	+	+	+	+	+	*E. coli*
BHI	+	+	+	+	+	*B. subtilis*
SDA	-	-	-	-	-	NIL
SSA	-	-	-	-	-	NIL
BSA	-	-	-	-	-	NIL
SMA	+	+	+	+	+	*Lactobacilli*

E1: The animal group has given dose of 0.03 mg/0.5 ml of ergotamine. = 0.06 mg/ml; **E2:** The animal group has given dose of 0.06 mg/1.5 ml of ergotamine. = 0.04 mg/ml; **E3:** The animal group has given dose of 0.09 mg/3 ml of ergotamine. =0.03 mg/ml; **EA:** The animal group has given dose of 1.25 mg/3 ml of ergotamine. = 0.4167 mg/ml; **C:** Control animal given distilled water; **NA:** Nutrient Agar; **BA:** Blood Agar;
Mac: MacConkey's Agar; **EMB** Eosin Methylene Blue Agar; **BHI:** Brain Heart Infusion Agar; **SDA:** Sabarose Dextrose Agar;
SSA: Salmonella Shigella Agar; **BSA:** Bismuth Sulphite Agar; **SMA:** Skimmed Milk Agar; - Negative, No growth; + Positive, Present;

- There were three replicates animals for each observation

In *vivo*, with the increase in dose of ergotamine the pathogenicity increased (Ghanem et *al.*, 2004). Long-term high dose of ergotamine can induce marked peripheral vasoconstriction and pain (Glazer *et al*., 1966). Ergotamine

causes temporary narrowing of blood vessels throughout the body. Ergotamine induced valvular disease were documented pathologically. It also reduces distensibility of the common carotid artery (Barenbrock *et al*., 1996). Human coronary artery is contracted by the action of ergotamine (Brink *et al*., 1998). Naturally, when blood supply is impaired and toxicity is present the functioning of the organs is badly affected which is the end result of the tissue damage which can lead to fatal consequences.

Ergotamine is extensively metabolized in the liver resulting in impaired normal hepatic metabolism and hepatic function (Perrin, 1985); in the present observation it was found that higher the dose, the more severe was the tissue damage. The manifestation described in this experiment could probably be due to poor circulation and high blood pressure cause by ergotamine.

It was observed, that the epithelium lining of central vein disappeared completely, necrosis and inflammation of hepatic tissue was obvious. Blood vessels were damaged with infiltration of macrophages and other inflammatory cells and occlusion of some of the vessels observed. (Figs.**1**-**3**). Sinusoids were also dilated. Necrosis was also observed in the vicinity of portal tract area with disintegration of hepatic cords and hepatocytes. The effect of ergotamine on brain and kidney will be reported in detail elsewhere.

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