

## CHANGES IN LEVELS OF SUBSTRATES AND ENZYMES IN SOME ORGANS OF FEMALE RATS IN RESPONSE TO PLANT EXTRACT

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**ABSTRACT:** Levels of glycogen, lactate, pyruvate, total proteins, free amino acids and enzymes (LDH & SDH) have been recorded in brain, heart, kidney and liver of rats in response to treatment with crude drug combination. The study indicates increase in the levels of glycogen, pyruvate, total proteins, LDH and SDH and decrease in levels of lactate and free amino acids.

### INTRODUCTION

Antifertility activity of many crude plant extracts has been studied to discover safe, effective and cheap contraceptive preparations. The use of plant extracts as antifertility compounds has been claimed to cause minimal or no side effects when compared to conventional contraceptives (1). However, Mathur *et al.* (1982) (2) have studied antifertility and histopathological effects of extracts of *Carica papaya* seed on female albino rats and reported significant tissue damage. In the present paper, an attempt is made to study the alterations in the levels of substrates and enzymes in brain, heart, kidney and liver of rats treated with crude drug combinations A & B.

### MATERIALS AND METHODS

Preparation of crude drug combinations has been dealt elsewhere (3).

**Combination A:** Pet. Ether (60 – 80°C) extracts of levels, bark, and stem of *Azadirachta indica* in equal proportion; Pet. Ether (60 – 80°C) extract of fruits of

*Piper longum* and Pet. Ether extract of berries of *Embelica officinalis* (2 : 1 : 1).

**Combination B:** Aqueous extract of leaves and stem of *A. indica* in equal proportion; aqueous extract of fruits of *P. longum*, aqueous extract of berries of *E. officinalis* and aqueous extract of seeds of *Gossypium indicum* (1 : 1 : 1 : 1).

### Experimental

The female albino rats (Wistar strain) about 2 – 3 months old, of proven fertility were maintained at laboratory conditions for about two weeks and estrous cycle were studied. These rats were paired with male rats in a ratio of 3 : 1 and subsequently vaginal smears were observed for presence of spermatozoa. The rats showing spermatozoa in their vaginal smears were scored as mated. The mated rats were divided into 3 groups of eight animals each. First two groups of rats were orally administered A & B crude drug combination in the form of suspension with 1%

Carboxymethyl cellulose (CMC) at a dose of 200 mg/kg for a period of seven days from first day of pregnancy. Third group of rats served as control and was administered 1% CMC at the same dose.

On the 21<sup>st</sup> day, after parturition the rats were sacrificed. Their brain, heart, kidney and liver were processed for the estimation of glycogen by the method of Klicepera **et al.** (4). Lactate was estimated by the method of Barker and Summerson (5) as modified by Huckabee (6). Pyruvate was estimated by the method of Freedmann and Haugen

(7). Total proteins were estimated following Biuret method of Goronall **et al.** (8). Free amino acids were estimated by the method of Moore and Stein (9). LDH and SDH levels were estimated following method of Prameelamma **et al.** (10).

## RESULTS AND DISCUSSION

The levels of substrates and enzymes in the brain, heart, kidney, and liver of control and combinations A & B treated female albino rats are given in Table 1.

**TABLE 1**

**Changes in levels of substrates and enzymes in female albino rats treated with crude drug combinations.**

	<b>Brain</b>	<b>Heart</b>	<b>Kidney</b>	<b>Liver</b>
Glycogen <sup>1</sup>				
A	62.64 ± 2.50	42.40 ± 1.77	84.46 ± 2.28	1649.49 ± 17.04
B	120.96 ± 2.85	67.12 ± 1.42	94.22 ± 1.90	1409.59 ± 16.22
C	80.32 ± 3.22	62.12 ± 1.30	69.91 ± 1.60	1531.86 ± 16.87
Pyruvate <sup>1</sup>				
A	114.15 ± 1.34	104.03 ± 1.80	98.61 ± 1.26	114.87 ± 1.75
B	92.75 ± 2.37	108.72 ± 1.79	121.56 ± 1.56	123.16 ± 1.58
C	75.74 ± 1.75	85.73 ± 2.00	113.92 ± 1.63	120.59 ± 1.80
Lactate <sup>1</sup>				
A	75.36 ± 1.21	121.00 ± 1.72	95.14 ± 1.67	136.52 ± 1.63
B	68.96 ± 2.16	105.17 ± 2.12	79.54 ± 2.78	108.03 ± 2.03
C	99.74 ± 1.75	159.25 ± 1.81	119.48 ± 1.28	179.79 ± 2.10
Total Proteins <sup>2</sup>				
A	10.00 ± 0.50	13.03 ± 0.63	14.05 ± 0.41	20.24 ± 0.94
B	13.39 ± 0.58	19.16 ± 0.63	17.66 ± 0.41	25.00 ± 0.75
C	8.19 ± 0.59	15.15 ± 0.70	12.70 ± 0.53	17.27 ± 0.67
Free amino acids <sup>1</sup>				
A	635.98 ± 4.90	586.29 ± 3.83	676.06 ± 6.38	630.74 ± 2.80
B	675.68 ± 3.97	590.56 ± 4.71	650.23 ± 5.58	510.68 ± 2.34
C	764.15 ± 4.62	675.66 ± 4.36	735.98 ± 6.15	587.84 ± 2.93

LDH <sup>3</sup>				
A	534.12 ± 3.79	1120.38 ± 4.20	625.45 ± 5.41	995.83 ± 6.59
B	510.10 ± 3.12	1020.93 ± 3.87	579.18 ± 5.30	870.12 ± 6.73
C	404.16 ± 3.79	800.28 ± 3.41	454.16 ± 5.19	812.50 ± 6.20
SDH <sup>3</sup>				
A	630.13 ± 4.00	872.37 ± 4.63	679.42 ± 3.36	470.56 ± 2.84
B	583.41 ± 4.35	832.43 ± 4.54	837.41 ± 3.76	610.51 ± 2.58
C	431.66 ± 4.23	733.33 ± 4.44	633.33 ± 3.47	586.12 ± 2.24

1. µg | 100 mg we weight of tissue
2. mg | 100 mg wet weight of tissue
3. µ moles of formazan formed | g wet weight of tissue | hr

A = Combination A, B + Combination B, C = Control.

Anti-implantation studies carried out on the female rats after one week of drug treatment have revealed that combination A caused 48.13% inhibition of implantation and 41.40% inhibition of pregnancy, while combination B caused 36.08% inhibition of implantation and 17.75% inhibition of pregnancy (11). The same drug combinations have exhibited significant anti-ovulatory activity (12). However, in male rats treated with the same combinations no abnormal changes in the spermatogenic cycle or on sperm production was observed (3).

Glycogen levels in brain and heart of combination A treated rats was found decreased by 22.01% and 31.74% respectively, while glycogen levels kidney and liver increased by 20.81% and 7.71% respectively. In combination B – treated rats, except for liver tissue, all other organs recorded an increase in glycogen content compared to control. With the exception of kidney and liver of combination A treated rats, Pyruvate levels in all other organs

studied increased, their levels ranging between 2.13% in liver of combination B treated rats to 50.71% in brain of combination A treated rats. Lactate levels decreased in all treated organs by 19.53% in kidney of combination A treated to 39.9% in liver of combination A treated to 39.9% in liver of combination B treated rats.

With the exception of heart, in combination A treated rats, the total proteins in all the organs increased. The increase ranged between 10.63% to 63.49%. Moderate decrease in the levels of free amino acids was recorded in all the tissues studied, with the exception of liver of combination B – treated rats.

The levels of enzymes, LDH and SDH increased in all the organs of rats treated with combination B, their values ranged between 7.09% to 27.52% and 41.6% to 31.15% respectively. In case of rats treated with combination A, with the exception of liver, which recorded a decrease of 19.71% in SDH level compared to control, the

values of enzyme activity increased in the range of 22.56% to 40.00% in case of LDH and 7.28% to 46.02% in case of SDH.

The rise in the pyruvate levels with the concomitant decrease in lactate levels and the enhanced levels of enzyme activity clearly suggest increased oxidative metabolism in these organs. The enhanced metabolism might be to counter the ill effects of the drug combination and to metabolize it to safer constituents for effective excretion. The rise in protein levels and decrease in free amino acids may indicate protein synthetic activity.

Triterpenoids of **A. indica** are found to reduce proteolytic activity (13).

In contrast to the levels of pyruvate and lactate during estrous in rats treated with combinations A & B, the lactate levels are found decreasing with increase in pyruvate levels. The increase of glycogen levels and pyruvate levels along with decrease in lactate levels are indicative of enhanced oxidative metabolism in the treated organs in contrast to the controls. The values obtained are suggestive of possible lipid breakdown and glycogenesis. However this needs further elucidation.

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