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Post-ingestional effects of red chilli powder containing capsaicin in stomach of house rat, *Rattus rattus*: histomorphological and histoenzymic studies

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ABSTRACT

Present study reports post-ingestional effects of red chilli in stomach of house rat, *Rattus rattus*. Three different groups of female rats (5 per group) were fed on bait containing 1 and 2% red chilli powder and plain bait, respectively. Histomorphological study revealed cellular degeneration of gastric region, sloughing and distortion of superficial layer of epithelium and lymphocytic infiltration in the treated groups of rats. Histoenzymic study revealed an increase in AKPase and decrease in LDH, SDH, G-6-PD and GLD activities in glandular stomach of treated groups of rats. The present study revealed that the ingestion of red chilli powder causes gastric lesions and variation in the activity of alkaline phosphatase and oxidoreductases.

Key words: Histoenzymic, Histomorphological, Rats, Red chilli.

Abbreviations: AKPase: Alkaline phosphatase, G-6-PD: Glucose-6-phosphate dehydrogenase, GLD: Glutamate dehydrogenase, LDH: Lactate dehydrogenase, SDH: Succinic dehydrogenase

INTRODUCTION

Red chillies show a number of physiological and pharmacological properties similar to the classes of drugs that are capable of inducing tissue damage (Srinivasan, 2005). Capsaicin, a homovanillic acid derivative is the principal pungent component in the chillies of plants of genus *Capsicum*. Fayaz and Ramachandran (2014) reported decrease in level of total lipids in rats receiving red pepper or capsaicin containing diets, without any change in the phospholipid or cholesterol content. Johnson (2007) reported that excessive consumption of capsaicin for longer period of time causes chronic gastritis, damage to kidney, liver and neurotoxic effects.

Effects of any drug on the absorptive processes in the stomach are effectively understood by various histological characteristics such as absorptive surface, its area and structure and the presence and localization of various enzyme systems. The histoenzymic studies on stomach of rats are negligible. Therefore, the aim of the present study was to study the histological and histoenzymic effects after ingestion of red chilli powder containing capsaicin in stomach of house rat, *Rattus rattus*.

MATERIALS AND METHODS

Red chilli powder of a pungent variety (*Capsicum annum* var. *Punjab Lal*) was used in present studies. Capsaicin content in red chilli powder was estimated using colorimetric method (Reddy and Sasikala, 2013).

Collection and maintenance of animals: For present study, the female house rat, *R. rattus* were live trapped from poultry farms in Ludhiana. In the laboratory, rats were acclimatized individually in cages for 15-20 days before the commencement of experiment. Food (loose mixture of cracked wheat, powdered sugar and ground nut oil (WSO bait) in ratio 96: 2: 2) and water were provided *ad libitum*. After acclimatization, healthy and mature rats were weighed and grouped for experimentation. Animals were maintained as per the guidelines of Institutional Animal Ethics Committee.

Treatment: Rats were divided into three groups of five animals each. Rats of group I were fed on WSO bait containing 1per cent red chilli powder, group II on 2 per cent and that of group III on plain WSO bait for 14 days. Food consumption was recorded daily after every 24 h and from total amount of food consumed, the dose (mg/kg bwt) of capsaicin ingested by each rat was determined.

Histomorphological studies: At the end of experiment, rats of both the treated and untreated groups were sacrificed and tissue samples were collected from both non-glandular and glandular parts of stomach. Tissues were fixed in 10 per cent neutral buffered formalin and processed for paraffin block preparation by acetone benzene schedule (Luna, 1968). The paraffin sections were obtained on glass slides with the help of rotary microtome and the stains mentioned in Table 1 were used for histological studies.

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Table 1: Histoenzymic techniques used on cryostat sections.

Sr.No	Enzyme	Substrate	Method	Incubation Time	Reference
(i)	Alkaline phosphatase (AKPase)	Naphthol AS-MX phosphate disodium salt in combination with Fast Blue R.R.	Simultaneous coupling azo dye method using substituted naphthols	30 min	Barka and Anderson (1963)
(ii)	Lactate dehydrogenase (LDH)	Na-DL-lactate	Standard method of bound enzyme by Nitro BT method	30 min	Pearse (1972)
(iii)	Succinic dehydrogenase (SDH)	Di—succinate	Standard method of bound enzyme by Nitro BT method	15 min	Pearse (1972)
(iv)	Glutamic dehydrogenase (GLD)	Na-L-glutamate	Standard method of bound enzyme by Nitro BT method	15 min 30 min	Pearse (1972) Pearse (1972)
(v)	Glucose-6-phosphate dehydrogenase (G-6-PD)	Di-Na-Glucose-6-Phosphate	Standard method of bound enzyme by Nitro BT method		
(vi)	Reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase)	Co-enzyme (NADPH)	Standard method of bound enzyme by Nitro BT method	35 min	Pearse (1972)
(vii)	Reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase)	Co-enzyme (NADH)	Standard method of bound enzyme by Nitro BT method	35 min	Pearse (1972)

Histoenzymic studies: The fresh unfixed tissues from different parts of stomach were collected, placed in OCT (optimal cutting temperature) compound and frozen in liquid nitrogen. Cryostat sections (10 µm thick) were obtained on clean glass slides and stored at -20°C in deep freezer until they were incubated for different enzyme substrates as given in Table 1. The enzyme activities were expressed semi quantitatively from weak (+) to intense (+++++) reaction according to Pearse (1972). The positive and negative controls were carried out wherever possible.

RESULTS AND DISCUSSION

The amount of capsaicin content in red chilli powder was found to be 0.77 mg/g of red chilli powder. The actual dose of capsaicin ingested by rats fed on bait containing 1 and 2 per cent red chilli powder was 0.78±0.10 and 1.20±0.39 mg/kg bwt, respectively.

Histomorphological changes in stomach: The consumption of bait containing red chilli powder caused degenerative changes in different layers of stomach of rats of both the treated groups. No histological changes were seen in the stomach of rats of control group. The mucosa of non glandular part of stomach was highly folded and was lined by keratinized stratified squamous epithelium in control group. However, there was sloughing and distortion of outer keratinized layer in treated groups I and II (Fig 1). At 1 per cent concentration, the degenerative changes were observed in all the layers of stomach. It was also noticed that the sloughing of keratinized layer occurred in a peculiar manner starting from luminal to adluminal side. The severity of changes increased with the increase in the concentration of red chilli powder as there was complete sloughing of this layer at some places in rats of treated group II (Fig 2). It was also observed that the thickness of tunica mucosa decreased with the increase in concentration of red chilli powder. The decreased thickness of the keratinized layer may be due to more sloughing with the increased concentration of red chilli powder.

At 1 per cent concentration of red chilli powder, there were found degenerative changes and vacuolization in mucosa layer leading to decreased number of mucous cells (Fig 3). The number of chief cells and parietal cells were found increased in the treated animals (Fig 3). The submucosa layer showed lymphocytic infiltration and congestion of blood vessels along with the presence of an exudate at some places in rats treated with 1 per cent red chilli powder (Fig 4). There were also found increased number of parietal cells and aggregates of lymphocytic infiltration in lamina propria of rats treated with 2 per cent red chilli powder (Fig 5).

The histopathological changes caused by ingestion of red chilli powder in the present study corroborate well with the observations of Arora *et al*. (2011) and Chukwu (2006) who have reported that capsaicin causes histopathological changes after oral ingestion. It may be due to the increased secretion of gastric juice caused by burning sensation produced by capsaicin when contacting the mucous membranes (Buchheim, 1873). In general, it was also observed that all the histological changes in different parts of stomach occurred from luminal to adluminal side *viz.*, from tunica mucosa to tunica serosa layer.

Histoenzymic changes: The histoenzymic alterations in the stomach of rats are summarised in Tables 2 and 3. There is no previous histoenzymic study on the stomach of rats after treatment with red chilli powder to compare with present study.

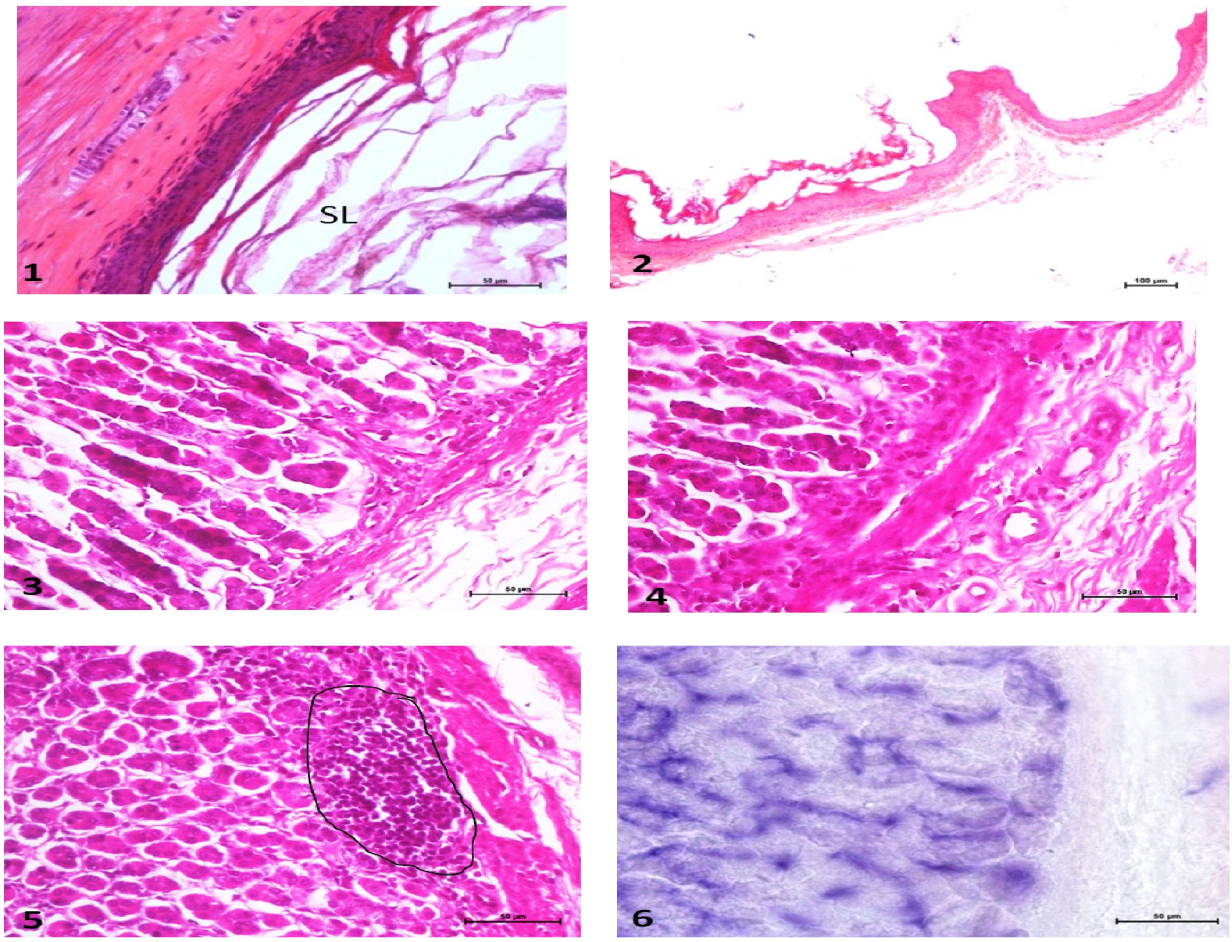


Figure 1. Photomicrograph of non glandular stomach showing sloughing of keratinized epithelium in layers in treated rats (1 per cent red chilli powder). H & E x400

Figure 2. Photomicrograph of non glandular stomach showing more sloughing of keratinized epithelium in treated rats (2 per cent red chilli powder). H & E x100

Figure 3. Photomicrograph showing increased number of parietal cells and decreased mucous cells in treated rats (1 per cent red chilli powder). H & E x400

Figure 4. Photomicrograph showing increased number of parietal cells, lymphocytic infiltration in lamina propria and exudate (Ex) in submucosa layer in treated rats (1 per cent red chilli powder). H & E x400

Figure 5. Photomicrograph showing increased number of parietal cells and aggregates of lymphocytic infiltration (circle) in lamina propria in treated rats (2 per cent red chilli powder). H & E x400

Figure 6. Photomicrograph showing increase in AKPase activity in parietal cells and chief cells (CC) in treated group (2 per cent red chilli powder). Azo dye method x 400

Activity of alkaline phosphatase (AKPase): In non glandular stomach and limiting ridge, the activity of AKPase was found to be negligible to weak in the keratinized layer and moderate in non keratinized layer of control group. There was no change in activity of AKPase in treated groups in non glandular stomach and limiting ridge. There was an increase in AKPase activity from moderate to strong in parietal cells and chief cells in the group treated with 2 per cent red chilli powder as compared to the control group (Fig 6), however, the other layers showed similar activity. As AKPase enzyme plays an important role in cellular proliferation and transport of substances and metabolites across the cell membrane (Gavosto and Pileri, 1958).

Activity of oxidoreductases: *Lactate dehydrogenase (LDH).* In non glandular stomach and limiting ridge, keratinized layer showed weak to moderate LDH activity which increased from moderate to strong in non keratinized layer in control group. The non glandular stomach and limiting ridge showed a marked loss of LDH activity in non keratinized layer in treated groups. In the group treated with 2 per cent red chilli powder, glandular stomach showed a general loss of LDH activity as parietal cells, chief cells and

Table 2: Histoenzymic distribution of different enzymes in various tunics of rat non-glandular stomach and limiting ridge in control group and red chilli powder (RCP) treated group.

Enzymes	Non glandular stomach				Limiting ridge			
	Keratinized layer		Non keratinized layer		Keratinized layer		Non keratinized layer	
	Control	RCP	Control	RCP	Control	RCP	Control	RCP
AKPase	0/+	0/+	++	++	0/+	0/+	++	++
LDH	+/++	+/++	++/+++	+	+/++	+/++	++/+++	+
SDH	+/++	+	+	+	+/++	+	+	+
GLD	+/++	+	+/++	+/++	+/++	+	+/++	+/++
G-6-PD	+/++	+	++/+++	++/+++	++	+	+/++	+/++
NADH	+/++	+	++/+++	+/++	+/++	+	++/+++	+/++
NADPH	+/++	0	++/+++	+/++	+/++	+	++/+++	+/++

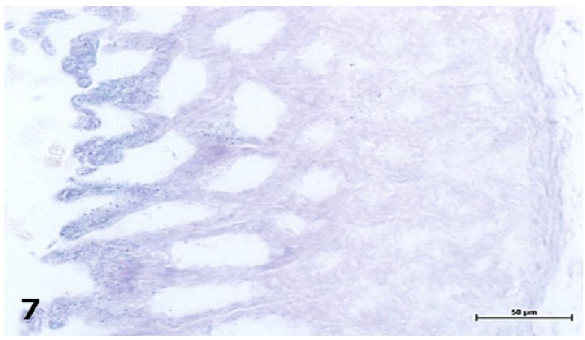
0-neglegible, +-weak, ++-moderate, +++-strong, ++++-intense

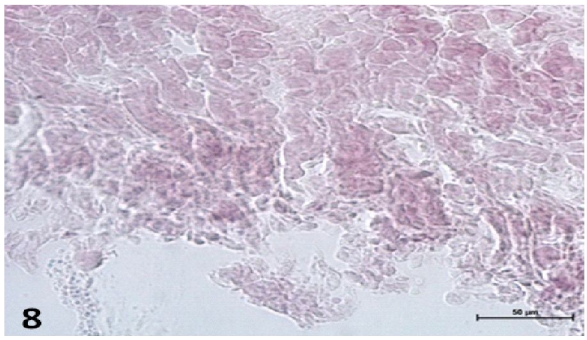
tunica submucosa showed negligible activity which was found to be weak in tunica muscularis and tunica serosa.

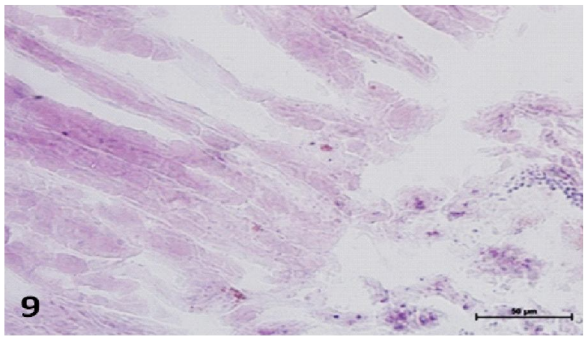
Succinic dehydrogenase (SDH): The non glandular stomach and limiting ridge showed weak to moderate activity in the keratinized layer and weak in non keratinized layer of surface epithelium in control group. But in the group treated with 2 per cent red chilli powder, the keratinized layer showed weak activity in non glandular stomach and limiting ridge. In the group treated with 2 per cent red chilli powder, glandular stomach showed a general loss of SDH activity. The surface epithelium, tunica muscularis and tunica serosa layer showed weak SDH activity, but the parietal cells, chief cells and tunica submucosa showed negligible activity in treated groups (Fig 7).

Glutamate dehydrogenase (GLD): The keratinized and non keratinized layers of non glandular stomach and limiting ridge showed a weak to moderate GLD activity in control group. In the group treated with 2 per cent red chilli powder, keratinized layer showed a loss of activity. In the same group, the activity of GLD was negligible in surface epithelium and tunica submucosa layer whereas mucous cells, parietal cells, chief cells, tunica muscularis and serosa layer showed weak activity as in control group (Fig. 8).

Glucose-6-phosphate dehydrogenase (G-6-PD). In non glandular stomach, the activity of G-6-PD was found to be weak to moderate in keratinized layer and increased from moderate to strong in non keratinized layer and limiting ridge







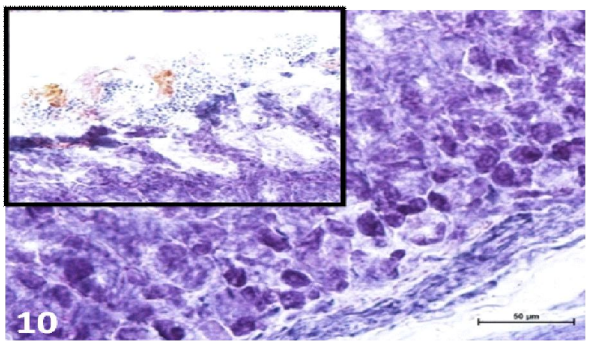


Figure 7. Photomicrograph showing loss of SDH activity in surface epithelium (E), parietal cells (PC), chief cells and submucosa layer in treated group (2 per cent red chilli powder). Nitro BT method x 400

Figure 8. Loss of GLD activity in surface epithelium (E) in treated group (2 per cent red chilli powder).Nitro BT method x 400

Figure 9. Photomicrograph showing loss of G-6-PD activity in surface epithelium, weak to moderate activity in mucous cells, moderate in parietal cells and chief cells in treated group 2 per cent red chilli powder. Nitro BT method x 400

Figure 10. Photomicrograph showing loss of NADH diaphorase activity in surface epithelium in treated group (2 per cent red chilli powder). Nitro BT method x 400

Table 3: Histoenzymic distribution of different enzymes in various tunics of rat glandular stomach in control group and red chilli powder (RCP) treated group.

Enzymes	Tunica mucosa								Tunica submucosa		Tunica muscularis		Tunica serosa	
	Surface epithelium		Mucous cells		Parietal cells		Chief cells							
	Control	RCP	Control	RCP	Control	RCP	Control	RCP	Control	RCP	Control	RCP	Control	RCP
AKPase	0/+	0/+	0/+	0/+	++	++/+++	++	++/+++	0	0	0	0	0	0
LDH	+/++	+	+/++	+/++	+	0	+	0	+	0	+++	+	+	+
SDH	++/+++	+	+/++	+/++	+	0	+	0	+	0	+	+	+	+
GLD	+	0	+	+	+	+	+	+	+	0	+	+	+	+
G-6-PD	++/+++	+/++	++/+++	++/+++	+++	++	+++	++	+	0	+	+	+	+
NADH	+++	+	++	++	+++	+/++	+++	+/++	+	+	+++	+/++	++	+/++
NADPH	+++	+	+/++	+/++	+++	+/++	+++	+/++	+	+	+++	+/++	++	+/++

0-negligible, +-weak, ++-moderate, +++-strong, ++++-intense

showed moderate activity in keratinized layer and weak to moderate in non keratinized layer in control group. In the group treated with 2 per cent red chilli powder, there was a loss of G-6-PD activity in keratinized layer of limiting ridge and non glandular stomach. There was loss of G-6-PD activity in surface epithelium, parietal cells and chief cells and tunica submucosa layer in the group treated with 2 per cent red chilli powder as compared to control group (Fig. 9). Parietal cells and chief cells showed moderate activity, while the activity in tunica submucosa, muscularis and serosa layer varied from negligible to weak.

There was a loss of activity of LDH and SDH in treated rats which might be due to damage of mitochondria in degenerative cells caused by capsaicin. Similar findings have been observed by Gill (2000) on different organs of buffalo after selenium toxicity. The GLD catalyzed deamination of glutamic acid to alpha ketoglutaric acid and reverse reaction. The decrease of GLD activity in the glandular and non glandular stomach of treated rats may be due to the toxic effect of red chilli powder. The presence of G-6-PD indicates its role in the glucose metabolism, so less activity in present study denotes reduced functional activity of gastric mucosa in the treated animals.

Activity of diaphorases

Reduced nicotinamide adenine dinucleotide diaphorase (NADH): In non glandular stomach and limiting ridge, the activity of NADH diaphorase was found to be weak to moderate in keratinized layer and moderate to strong in non keratinized layer in control group. In the group treated with 2 per cent red chilli powder, keratinized and non keratinized layers showed a loss of activity as keratinized layer showed weak activity and non keratinized layer showed weak to moderate activity of NADH diaphorase. In the group treated with 2 per cent red chilli powder, there was a loss of NADH diaphorase activity as compared to control. The activity was found to be weak in surface epithelium and strong activity was found in chief cells and parietal cells, tunica submucosa showed weak activity which was found to be weak to moderate in tunica muscularis and tunica serosa (Fig. 10).

Reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH*).* The non glandular stomach showed weak to moderate NADPH diaphorase activity in keratinized layer and moderate to strong in non keratinized layer in control group. But in the group treated with 2 per cent red chilli powder, there was a loss of activity as compared to control group, as keratinized layer showed negligible NADPH diaphorase activity and non keratinized layer showed weak to moderate. The limiting ridge also showed weak to moderate NADPH diaphorase activity in keratinized layer and moderate to strong in non keratinized layer in control group. But in the group treated with 2 per cent red chilli powder, there was a loss of activity as compared to control group. There was a

loss of NADPH diaphorase activity in parietal cells and chief cells in the group treated with 2 per cent red chilli powder as compared to control group. The granular type reaction of NADPH diaphorase was observed in tunica muscularis layer in treated group.

NADH diaphorase and NADPH diaphorase are a coenzyme dehydrogenase and is an indicator of metabolic activity of the cells (Shrader and Zeman, 1972). In the present study, there was a loss of NADH and NADPH activity in treated rats due to capsaicin toxicity. Similarly, Gill (2000) reported decrease in the NADPH activity in different organs of buffalo after selenium toxicity.

Present study thus reveals the post-ingestional histological and histoenzymic changes of red chilli powder containing capsaicin in the stomach of rats.

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