Effect of calcium hypochlorite and chloramine on blood biochemistry and sodium pentobarbital induced sleeping time in mice

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Abstract: Disinfectants are chemical agents used to eradicate, deactivate or kill microorganisms. Chemical disinfectants especially chlorine compound are extensively used for water sanitization. Among these calcium hypochlorite and chloramines are commonly used now a day. Large number of chemical compounds, drugs and endogenous substances are metabolized by hepatic enzymes known as cytochrome P450 enzyme system. Many chemicals are capable of enzyme induction. Enzyme induction may change the metabolism of other drugs and endogenous substances which may alter the plasma concentration of these chemicals. To evaluate the enzyme inducing ability of calcium hypochlorite and chloramine, sleeping time induced by sodium pentobarbital was noted in mice. Normal saline was taken as negative control. Rifampicin, chloramphenicol and grapefruit juice were taken as positive control group. On completion of dosing after 4 weeks, alteration in sleep induction and recovery times was noted and compared. Histological evaluation of liver was observed. Asignificant decrease in sleeping time was observed in calcium hypochlorite and chloramine treated groups. Both calcium hypochlorite and chloramine caused a significant change in liver enzymes and in the values of complete blood count.In histological evaluation both caused fat deposition in the hepatocytes. It was concluded from the study that both calcium hypochlorite and chloramine were hepatic microsomal enzyme inducer.

Keywords: Cytochrome P450, calcium hypochlorite, chloramine, enzyme induction.

INTRODUCTION

Water is used for drinking, washing, food processing, irrigation and fish farming. Use of water for all such purposes may cause health hazards due to presence of harmful chemicals in it (Dijkstra and Husman, 2014). The pollution of water bodies is, nowadays, a main environmental problem, affecting human health, water resources, and ecosystems (Tarazona, 2014). Water contains deleterious substances that are hazardous to the health. Water purification is done by various means. Chemical disinfection is one of the processes used for the purification of water bodies (Bianchi *et al*., 2014).

Calcium hypochlorite, $Ca(OCl)_2$, belongs to chemical class of halogenoxyacid salts (Su *et al*., 2009). Calcium hypochlorite of excellent quality is used in swimming pools for sanitization of water. It is suitable source of chlorine for general sanitation and disinfection purposes (Dychdala and Custer, 1974). When calcium hypochlorite is dissolved in water, it exhibits good disinfecting and bleaching properties (Brown *et al*., 1959).

Before human consumption last treatment step for wastewater and drinking water is chemical sanitization. Due to the less production of halo acetic acids (HAAs) and trihalomethanes (THMs) chloramines have become popular as a substitute for water disinfection and longer residual time in the water supply system. For the water treatment when chlorine and ammonia are co-administered chloramines are formed that show maximum antimicrobial activity with minimum formation of disinfection byproducts (Mckay *et al*., 2013).

Enzyme induction has the capability to change the pharmacologic effects of drugs. 'Sleep-time' is one of the parameters to evaluate the level of enzyme induction (Okey, 1990). Sleep is induced by the activation of γ-aminobutyric acid (GABA)-ergic neurons present in the CNS (Liu *et al*., 2013).

Elevation of serum g-GT is considered as an index of hepaticenzyme induction and the activity of this enzyme is also known to increase inhepatobiliary diseases (Farombi *et al*., 1999).

Calcium hypochlorite and Chloramine may change the level of other drugs due to their enzyme inducing property. Due to this pharmacokinetic interaction dose adjustment may be required to obtain desirable therapeutic response.

MATERIALS AND METHODS

Experimental animals

For this project 60 Albino mice of five to six weeks old were purchased from Veterinary Research Institute (VRI) Lahore and were kept in cages in animal shed of

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Department of Pharmacology and Toxicology UVAS, Lahore under possible hygienic conditions. Mice were provided with standard pellet diet and water during whole experimental period. All the ethical issues according to the institutional guide-lines regarding the experimental use of mice were kept in consideration.

Chemicals

Research grade Calcium hypochlorite, Chloramine, Rifampicin, Chloramphenicol, Sodium pentobarbital and Grapefruit were purchased from the local market.

Experimental protocol

Sixty mice were divided into six groups having 10 mice in each. Group A was taken as negative control and was administered Normal Saline orally for a period of 4 weeks. Group B was taken as first positive control and was administered Rifampicin at the dose of 50mg/kg intraperitoneal for a period of 4 weeks. Group C was taken as second positive control and was administered Chloramphenicol at the dose of 100mg/kg intraperitoneal for a period of 4 weeks. Group D was taken as third positive control and was administered Grapefruit juice at the dose of 0.2ml/dayorally for a period of 4 weeks. (Rifampicin is hepatic microsomal enzyme inducer and Chloramphenicol and Grapefruit juice is hepatic microsomal enzyme inhibitor.) Group E was administered with water containing Calcium Hypochlorite at the dose of 10mg/L orally for a period of 4 weeks. Group F was administered water containing Chloramine at the dose of 10mg/L orally for a period of 4 weeks.

Evaluation of effect on sodium pentobarbital induced sleeping time in mice

On the next day at the culmination of 4 weeks Sodium Pentobarbital was administered at a dose of 40mg/kg intraperitoneal to all the groups and sleeping time induced by the Sodium Pentobarbital was evaluated and compared with the control groups by using parameters onset of hypnosis, rightening reflex, pain senses and time of recovery.

Evaluation of hepatic enzymes and blood biochemistry

Upon successful recovery of mice of all the groups from sleep blood samples were collected by heart puncture. Blood was centrifuged and serum was separated. Serum was analyzed for change in liver enzymes and blood biochemistry by performing complete blood count and liver function test.

Histological evaluation

The mice were sacrificed by using chloroform. The mice were dissected and the liver of the animals were removed and preserved in 10% formalin solution. Slides were prepared and observed under light microscope.

RESULTS

Effect on sodium pentobarbital induced sleeping time in mice

Pretreatment with calcium hypochlorite decreased sleeping time, time duration for absence of rightening reflex and time duration for absence of pain senses in comparison to normal saline treated group. Pretreatment with chloramines also caused a significant decrease in sleeping time, time duration for absence of rightening reflex and time duration for absence of pain senses in comparison to normal saline treated group. These results were similar to rifampicin (enzyme inducer) treated group. Chloramphenicol and Grapefruit juice (enzyme inhibitor) increased the sleeping time, time duration for absence of rightening reflex and time duration for absence of pain (table 1).

Effect on liver enzymes

Pretreatment with calcium hypochlorite caused a significant elevation in serum total bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate transaminase level in comparison to normal saline treated group. The value of serum albumin was decreased significantly. Similarly chloramines also caused a significant elevation in total serum bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate transaminase levels. The serum albumin level was decreased significantly in comparison to normal saline treated group. Rifampicin increased the level of total serum bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate transaminase while serum albumin level was slightly decreased in rifampicin treated group in comparison to normal saline treated group. Chloramphenicol and grapefruit juice decreased the level of serum total bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate transaminase but increased the level of serum albumin in comparison to normal saline treated group (table 2).

Effect on complete blood count

When the results of CBC of calcium hypochlorite treated group were compared with normal saline treated group there was an increase in white blood cells, lymphocytes and granulocytes, red blood cells, hemoglobin, hematocrit value, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and platelets count. Mean corpuscular volume was similar to that of untreated group. When the results of CBC of chloramine treated group were compared with normal saline treated group there was an increase in white blood cells, lymphocytes and granulocytes, red blood cells, hematocrit value, mean corpuscular hemoglobin and platelets count. The values of hemoglobin and mean corpuscular hemoglobin concentration were less than the normal saline treated group. The value of mean corpuscular volume was similar to that of normal saline treated group (table 3).

Table 1: Effect of drugs on Sodium Pentobarbital induced sleeping time in mice

Group No.	Absence of Rightening Reflex (min)	Reappearance of Rightening Reflex (min)	Onset of Hypnosis (min)	Duration of Sleep (min)	Absence of Pain Senses (min)	Reappearance of Pain Sense (min)
Group A	2.66±0.49	26.3±1.76	3.9±0.87	21.02±1.72	1.6±0.51	27.9±1.85
Group B	2±1.52	0.57±2.08	0.18±2.82	0.47±2.12	0.16±1.52	0.63±2.51
Group C	2.6±1.42	49.7±3.2	3.27±1.46	47.65±3.2	1.65±0.81	51.35±3.4
Group D	2.5±0.52	59.1±3.3	4±0.66	56.2±3.28	1.4±0.51	60.7±3.48
Group E	2.5±0.97	16±2.8	3.1±1.01	13.2±2.59	1.5±0.66	17.7±2.67
Group F	3.3±1.11	8.8±2.38	3.45±0.73	7.1±2.03	2±0.81	10.7±3.8

Table 2: Effect of drugs on various enzymes of liver mice

Group No.	Serum Total Bilirubin (0-0.9) mg/dl	Alkaline Phosphatase (35-96) U/L	Alanine Aminotransferase (17-77) U/L	Aspartate Transaminase (54-298) U/L	Serum Albumin (2.5-3) mg/dl
Group A	0.8±0.19	89.3±4.24	55.8±4.68	124±6.37	2.91±0.52
Group B	1.68±0.22	155.3±5.47	91.6±4.83	330.3±3.33	2.16±0.46
Group C	0.24±0.04	19.4±2.79	11.5±2.17	36.4±5.12	3.94±0.47
Group D	0.28±0.21	19.97±0.76	13.07±0.32	38.4±3.77	4.11±0.28
Group E	1.5±0.25	132±3.74	71.6±4.83	300.7±3.68	1.78±0.49
Group F	2.24±0.38	173±4.05	89.6±3.56	352±2.44	2.16±0.23

Table 3: Effect of drugs on various parameters of CBC

Group No.	WBC×1-3 /µl± SD	LYM×10-3/µl± SD	GRA×10-3 /µl ±SD	RBC×1-6 /µl ±SD	HGB g/dl± SD	HCT % ± SD	MCV fl ± SD	MCH pg ± SD	MCHC g/dl ± SD	PLT×10-3 /µl ± SD
Group A	7.24 ±0.94	4.66 ±0.76	0.55 ±0.05	7.79 ±0.08	13.04 ±1.09	37.85 ±0.73	52.3 ±2.66	11.76 ±0.66	28.17 ±0.73	332.8 ±8.74
Group B	19.33 ±1.65	10.2 ±0.81	5.58 ±0.64	5.47 ±0.16	7.8 ±0.51	32.46 ±0.58	52 ±1.33	12.43 ±0.79	17.98 ±0.86	550 ±6.6
Group C	0.72 ±0.08	0.46 ±0.27	0.24 ±0.03	4.79 ±0.08	7.9 ±1.10	28.15 ±0.77	34.3 ±2.83	9.7 ±0.18	18.4 ±0.86	73.9 ±3.07
Group D	5.94 ±0.60	4.36 ±1	0.49 ±0.05	7.01 ±0.92	11.54 ±0.63	32.5 ±1.06	42.5 ±2.36	10.85 ±0.62	18.17 ±0.73	194.8 ±7.9
Group E.	22.59 ±1.71	13.31 ±0.80	7.5 ±0.52	11.96 ±1.2	13.94 ±0.74	48.31 ±0.76	49 ±2.82	15.2 ±0.90	15.87 ±0.98	694.2 ±6.56
Group F	26.01 ±1.02	15.01 ±0.71	7.5 ±0.52	15.1 ±0.66	8.51 ±0.61	38.31 ±0.76	49 ±5.37	14± 2.78	14.18 ±0.70	512 ±6.96

Abbreviations A. Normal Saline, B. Rifampicin, C. Chloramphenicol, D. Grapefruit juice, E. Calciumhypochlorite, F. Chloramine

Histological evaluation

Normal Saline caused no change in cell structure. All cells were of normal size and shape. Normal sinusoids were present. No change in cell integrity (fig. 1A). Rifampicin caused degeneration of hepatocytes. Damaged cells were visible. Sinusoids were enlarged. There was also accumulation of fats in the hepatocytes (fig. 1B). In chloramphenicol treated group cell size was increased. Globular shape large cells were present. Damaged hepatocytes were visible with large accumulation of fat (fig. 1C). Grapefruit juice did not produce any change in structure of hepatocytes. Cells were of normal shape and normal size. Slightly enlarged sinusoids were present (fig. 1D). In calcium hypochlorite treated group cell size was increased. There was deposition of fats in large amount. Cell appearance was just like adipose cell. Hepatocytes were damaged (fig. 1E). Chloramine caused mild to moderate deposition of fats in the hepatocytes. Cell size was increased. Sinusoids were enlarged. Cell integrity was maintained (fig. 1F).

DISCUSSION

Chlorine compounds are being used for sanitization of drinking water and swimming pools for many years. Calcium hypochlorite and chloramines are commonly used chlorine compounds for this purpose (Hidalgo and Dominguez, 2000). Chlorinated benzene compounds were potent hepatic microsomal enzyme inducer. They decreased hexobarbital induced sleeping time in mice (Carlson and Tardiff, 1976).

According to results of this study when sodium pentobarbital was used with normal saline there was an average sleeping time of 21.02 minutes. Rightening reflex was remained absent for 26.3 minutes and pain senses remained absent for 27.9 minutes. When sodium pentobarbital was used with calcium hypochlorite, sleeping time was reduced to 17.1 minutes, rightening reflex was remained absent for 19.1 minutes and pain senses remained absent for 20.6 minutes. Similarly when chloramine was administered with sodium pentobarbital sleeping time was reduced to 7.9 minutes, rightening reflex was remained absent for9.7 minutes and pain senses remained absent for 11.7 minutes. The reduction in sleeping time, absences of rightening reflex and absences of pain senses was higher in chloramines treated group than the calcium hypochlorite treated group. The similar effects were observed in Japanese quails (Cecil *et al.*, 1975). These results were according to the study conducted to evaluate that Polycarbonated biphenyls increased liver weight and decreased sleeping time (Sanders and Kirkpatrick, 1975).

As rifampicin is established hepatic enzyme inducer. When results of our study were compared with rifampicin it showed that enzyme inhibiting effect of chloramines was somewhat similar to enzyme inhibiting effect of rifampicin. Calcium hypochlorite also induced enzyme inhibition but when it was compared with rifampicin, enzyme inhibition induced by calcium hypochlorite was less than rifapmicin. Chloramphenicol and grapefruit juice both are hepatic microsomal enzyme inhibitors. Chloramphenicol and grapefruit juice increased the sleeping time, duration of absence of rightening reflex and duration of absence of pain senses. When results of calcium hypochlorite and chloramines were compared with these there was marked differences in the results.

The second phase of this study was to evaluate the effect of calcium hypochlorite and chloramines on hepatic enzymes and blood biochemistry. Polychlorinated biphenyls had the ability to alter the hepatic enzyme levels and plasma steroid levels in fish (Sivarajah *et al.*, 1978). For this purpose liver function test and complete blood count was performed respectively. When the results of LFT of calcium hypochlorite were compared with the normal saline treated group it was evaluated that the value of serum total bilirubin, alkaline phosphatase, aminotransferase and aspartate transaminase in saline treated group was 0.8mg/dl, 89.3U/L, 55.8U/L and 124 U/L respectively which was increased to 1.5mg/dl and 132U/L, 71.6U/L and 300.7U/L respectively. The value of serum albumin was decreased in calcium hypochlorite treated group significantly. Its value was 2.91mg/dl in normal saline treated group, which was reduced to 1.78 mg/dl in calcium hypochlorite treated group.

In comparison to rifampicin the values of all the parameters of LFT of calcium hypochlorite were less than rifampicin values. There was significant difference found in the values of alkaline phosphatase, alanine aminotransferase and aspartate transaminase. There was no significant difference found in the values of total serum bilirubin and serum albumin levels of calcium hypochlorite. The values of serum total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate transaminase and serum albumin was 1.68mg/dl, 155.3 U/L, 91.6U/L, 330.3U/L and 2.16mg/dl respectively in rifampicin treated group. The values of serum total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate transaminase and serum albumin was 1.68 mg/dl, 155.3U/L, 91.6U/L, 330.3U/L and 2.16mg/dl respectively in rifampicin treated group. In calcium hypochlorite treated group the values of serum total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate transaminase and serum albumin was found to be 1.5mg/dl, 132U/L, 71.6U/L, 300.7U/L and 1.78mg/dl respectively.

When the values of LFT of calcium hypochlorite were compared with the LFT values of chloramphenicol there was vast difference in the values. The values of serum total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate transaminase and serum albumin was 0.24mg/dl, 19.4U/L, 11.5U/L, 36.4U/L and 3.94mg/dl respectively in chloramphenicol treated group. In calcium hypochlorite treated group the values of serum total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate transaminase and serum albumin was found to be 1.5mg/dl, 132U/L, 71.6U/L, 300.7U/L and 1.78mg/dl respectively.

The values of LFT of calcium hypochlorite when compared with the LFT values of grapefruit juice there was vast difference in the values. The values of serum total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate transaminase and serum albumin was 0.28mg/dl, 19.97U/L, 13.07U/L, 38.4U/L and 4.11 mg/dl respectively in grapefruit juice treated group. In calcium hypochlorite treated group the values of serum total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate transaminase and serum albumin was found to be 1.5mg/dl, 132U/L, 71.6U/L, 300.7U/L and 1.78mg/dl respectively. Similar effects were produced by polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs), well known chlorine compounds that have potent inductive effect on liver microsomal mixed-function oxidases (Yoshimura *et al.*, 1985).

In comparison to normal saline the value of serum total bilirubin, alkaline phosphatase, aminotransferase and aspartate transaminase was increased in chloramine treated group from 0.8mg/dl to 2.24mg/dl, 89.3U/L to 173U/L, 55.8U/L to 89.6U/L and 124U/L to 352U/L respectively. The value of serum albumin was decreased in chloramine treated group from 2.91mg/dl to 2.16mg/dl.

There was a vast difference found in the values of LFT of chloramine and the LFT values of chloramphenicol. In comparison to chloramphenicol the value of serum total bilirubin, alkaline phosphatase, aminotransferase and aspartate transaminase was increased in chloramine treated group from 0.24mg/dl to 2.24mg/dl, 19.4U/L to 173U/L, 11.5U/L to 89.6U/L and 36.4U/L to 352U/L respectively. The value of serum albumin was decreased in chloramine treated group from 3.94mg/dl to 2.16mg/dl.

When the values of LFT of chloramine were compared with the LFT values of grapefruit juice there was vast difference in the values. There was an increase in the values of serum total bilirubin, alkaline phosphatase, aminotransferase and aspartate transaminase in chloramine treated group from 0.28 mg/dl to 2.24mg/dl, 19.97U/L to 173U/L, 13.07U/L to 89.6U/L and 38.4U/L to 352 U/L respectively. The value of serum albumin was decreased in chloramine treated group from 4.11mg/dl to 2.16mg/dl. These results were similar to the findings that polychlorinated biphenyls had an ability to increase the microsomal hepatic enzymes (Litterst *et al*., 1972). Such results were similar to the findings that enzyme inducing activity was increased as the degree of chlorination enhanced (Johnstone *et al*., 1974).

Chlorinated compounds increased the number of white blood cells (Exon and Koller, 1983). When the results of CBC of calcium hypochlorite treated group were compared with normal saline treated group the values of white blood cells, lymphocytes and granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin and platelets were increased from 7.24×10-3/µl to 22.59×10-3/µl, 4.66×10-3/µl to 13.31×10-3/µl, 0.55×10-3/µl to7.5×10-3/µl, 7.79×10-6/µl to11.96×10-6/µl, 13.04 g/dl to 13.94g/dl and 37.85% to 48.31%, 11.76 pg to 15.2 pg and 332.8×10-3/µl to 694.2×10-3/µl respectively while the values of mean corpuscular volume and mean corpuscular hemoglobin concentration were reduced from 52.3 fl to 49 fl and 28.17g/dl to 15.87g/dlin calcium hypochlorite treated group (Jensen and Jorgensen, 1983).

In comparison to rifampicin treated group the values of white blood cells, lymphocytes and granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin and platelets were increased from 19.33 × 10 -3/µl to 22.59 × 10-3/µl, 10.2× 10-3/µl to 13.31 × 10-3/µl, 5.58 × 10-3/µl to7.5 × 10-3/µl, 5.47 × 10 -6/µl to11.96 × 10-6/µl, 7.8g/dl to 13.94g/dl, 32.46% to 48.31%, 12.43 pg to 15.2 pg and 550 ×10-3/µl to 694.2 × 10-3/µl respectively while the values of mean corpuscular volume and mean corpuscular hemoglobin concentration were reduced from 52 fl to 49 fl and 17.98 g/dl to 15.87g/dl in calcium hypochlorite treated group respectively.

In comparison to chloramphenicol treated group there was a significant difference found in all the values of CBC of calcium hypochlorite treated group. The values of white blood cells, lymphocytes and granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and platelets were increased from 0.72 × 10-3/µl to 22.59 × 10-3/µl, 0.46 × 10-3/µl to 13.31 × 10-3/µl, 0.24 × 10-3/µl to7.5 × 10-3/µl, 4.79 × 10-6/µl to11.96 × 10-6/µl, 7.9g/dl to 13.94g/dl, 28.15% to 48.31%, 34.3 fl to 49 fl, 9.7 pg to 15.2 pg and 73.9 ×10 -3/µl to 694.2 × 10-3/µl respectively while the value of mean corpuscular hemoglobin concentration was decreased from 18.4g/dl to 15.87g/dl in calcium hypochlorite treated group respectively.

In comparison to grapefruit juice treated group there was a significant difference found in all the values of CBC of calcium hypochlorite treated group. The values of white blood cells, lymphocytes and granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and platelets were increased from 5.94 × 10-3/µl to 22.59 × 10-3/µl, 4.36 × 10-3/µl to 13.31 × 10-3/µl, 0.49 × 10-3/µl to 7.5 × 10-3/µl, 7.01 × 10-6/µl to 11.96 × 10-6/µl, 11.15g/dl to 13.94 g/dl, 32.5 % to 48.31 %, 42.5 fl to 49 fl, 10.85 pg to 15.2 pg and 194.8×10-3/µl to 694.2 × 10-3/µl respectively while the value of mean corpuscular hemoglobin concentration was decreased from 18.17 g/dl to 15.87 g/dl in calcium hypochlorite treated group respectively.

When the results of CBC of chloramine treated group were compared with normal saline treated group the values of white blood cells, lymphocytes and granulocytes, red blood cells, hematocrit, mean corpuscular hemoglobin and platelets were increased from 7.24 × 10-3/µl to 26.01 × 10-3/µl, 4.66× 10-3/µl to 15.01 × 10-3/µl, 0.55 × 10 -3/µl to7.5 × 10-3/µl, 7.79× 10-6/µl to15.1 × 10-6/µl, 37.85% to 38.31%, 11.76 pg to 14 pg and 332.8×10 -3/µl to 512 × 10-3/µl respectively while the values of hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration were reduced from13.04g/dl to 8.51 g/dl, 52.3 fl to 49 fl and 28.17 g/dl to 14.18g/dl in chloramine treated group respectively.

In comparison to rifampicin treated group the values of white blood cells, lymphocytes and granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin and platelets were increased from 19.33 × 10 -3/µl to 26.01×10 -3/µl, 10.2 × 10-3/µl to 15.01 × 10 -3/µl, 5.58 × 10-3/µl to7.5 × 10-3/µl, 5.47 × 10-6/µl to15.1 × 10-6/µl, 7.8g/dl to 8.51 g/dl, 32. 46% to 38.31%, 12.43

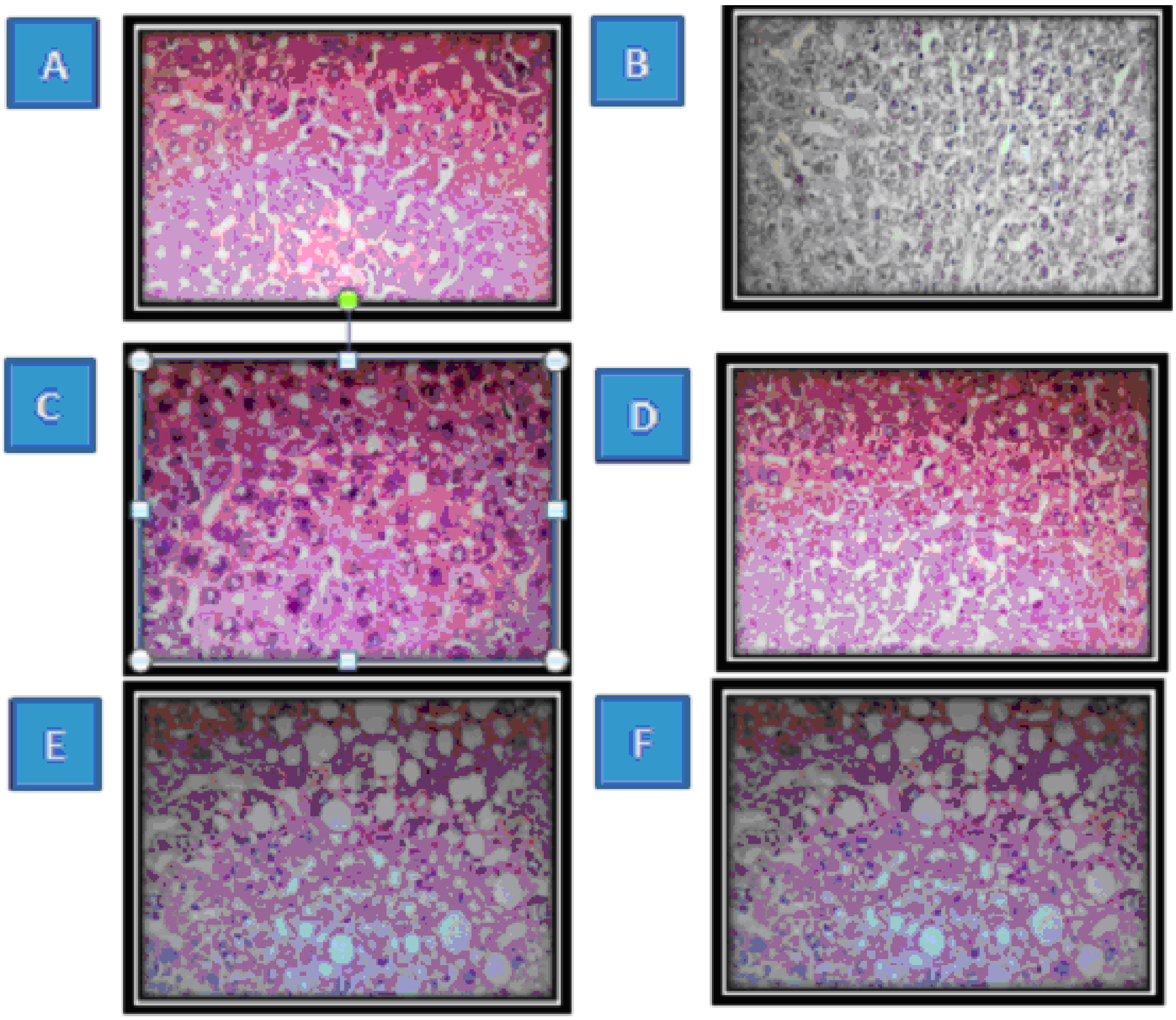

Fig. 1: Transverse section of the liver of albino mice after treatment with test drugs. (original magnification ×100)

A. Normal Saline no change in cell structure
B. Rifampicin caused degeneration of hepatocytes
C. Chloramphenicol Globular shape large cells were present along with fatty change
D. Grapefruit juice Cells were of normal shape and normal size
E. Calcium hypochlorite cell size increased with fatty deposition.
F. Chloramine caused mild to moderate deposition of fats in the hepatocytes. Sinusoids were enlarged. Cell integrity was maintained.

pg to 14 pg and 550×10-3/µl to 512 × 10-3/µl respectively while the values of mean corpuscular volume and mean corpuscular hemoglobin concentration were reduced from 52 fl to 49 fl and 17.98g/dl to 14.18g/dl in chloramine treated group respectively.

The values of CBC of chloramine treated group were higher than the chloramphenicol-treated group. In comparison to chloramphenicol treated group the values of white blood cells, lymphocytes and granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and platelets were increased from 0.72 × 10-3/µl to 26.01 × 10-3/µl, 0.46 × 10-3/µl to 15.01 × 10-3/µl, 0.24 × 10-3/µl to7.5 × 10-3/µl, 4.79×10-6/µl to15.1×10-6/µl, 7.9g/dl to 8.51g/dl, 28.15% to 38.31%, 34.3 fl to 49 fl, 9.7pg to 14 pg and 73.9×10-3/µl to 512×10-3/µl respectively while the value of mean corpuscular hemoglobin concentration was reduced from18.4g/dl to 14.18g/dl in chloramine treated group respectively.

The values of CBC of chloramine treated group were higher than the grapefruit juice treated group. In comparison to grapefruit juice treated group the values of white blood cells, lymphocytes and granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and platelets were increased from 5.94×10-3/µl to 26.01×10-3/µl, 4.36×10-3/µl to 15.01×10-3/µl, 0.49×10-3/µl to7.5×10-3/µl, 7.01× 10-6/µl to 15.1×10-6/µl, 32.5% to 38.31%, 42.5 fl to 49 fl, 10.85 pg to 14 pg and194.8×10-3/µl to 512×10-3/µl respectively while the values of hemoglobin and mean corpuscular hemoglobin concentration were reduced from 11.54g/dl to 8.51g/dl and 18.17g/dl to 14.18g/dl in chloramine treated group respectively. These results were analyzed by one-way ANOVA, results were statistically significant at P value <0.05.

Histological evaluation was done to detect any change in the liver structure. There was no change found in normal saline treated group. In calcium hypochlorite treated group, hepatocytes were damaged due to fat deposition in the cell. Cell appearance was just like adipose cell. Similarly chloramine also caused mild to moderate fat deposition in the hepatocytes. Slight enlargement in sinusoids was visible. The same histopathological alterations and levels of hepatic microsomal enzymes were observed in the rats fed with Aroclor 1242.Histopathological examination revealed lipid vacuolation and proliferation of smooth endoplasmic reticulum in the hepatocytes of treated animals (Brukner *et al*; 1974).

CONCLUSION

The results of this study suggested that calcium hypochlorite and chloramines are hepatic microsomal enzyme inducer. As calcium hypochlorite and chloramines are used for sanitization of drinking water and swimming pools there are chances of interaction of these chemicals with the drugs, which are metabolized by hepatic microsomal enzymes. So in such cases there is need to increase the dose of drugs to obtain the required therapeutic results.

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