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## ORIGINAL ARTICLE

# Effects of Contaminated Drinking Water with Microcystins Toxins on Cows Liver and toxins removal

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**ABSTRACT**
*General, Liver is an important organ that with the largest body part of animal, it works as a central role in processing of a metabolic biochemical reactions for survival and healthy life. Liver controls some important body functions such as carbohydrates, proteins and fat absorption in animals's body and also excrete the substances formed in the duration of body growth. Medical tests of Liver function are very important that may help to identification of hepatic disease in both animals and humans such as estimation of serum globulin, albumin, ALT, GGT, bilirubin and AST levels. However, there is little information in literature about these tests for hepatic problem in cows. The methodology was adopted as cows samples were collected as 50 but find 47 out of 50 (94%) of cows were suffered from liver diseases that were investigated properly. The swelling of Liver was confirmed by performing the LFTs medical and biochemical tests of liver that were followed by sign and symptoms. The final solution of this problem is the proper treatement of drinking water by using ferric chloride as coagulant that have a good capacity to removing the said toxins from drinking water.*
***Keywords:*** *liver function, Proteins, Blood testing, serum albumin*

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## INTRODUCTION

The cyanobacteria are blue - green algae that may exist as photosynthetic pigments seen inside cyanobacterial cells. Basically, a cyanobacterium is a chief group of bacteria that can be found all over the world. Cyanobacteria in freshwater is accumulate on the surface of water in the form of blooms as it have high concentration in form of the blue-green is known as scums. Some species of Cyanobacteria is produced toxins, these toxins are characterised into their type of action with other chemical agents like as 1.neurotoxins (e.g. anatoxins), 2.hepatotoxins (e.g. microcystins). Skin toxins are produced irritants as feeling on skins and other types of toxins are producing from related other sources. Neurotoxins and Hepatotoxins both are produced by cyanobacteria as normally that can found on the surface of water. They mostly found in the water supplies as drinking water resource [1].

The Microcystins toxins are the commonly known as hepatotoxin and it has the common dose observed as $LD_{50}$ . And it has 50.0 µg/kg values that are observed in mice by applying the intra peritoneal injection method. Microcystins has 200 times more poison and toxic than metal cyanide. These toxins have structural variations include amino acid substitutions and alterations like as demethylation and methylation. The Drinking water supplies were contaminated with Cyanobacteria toxins that were a main cause of a health risk for domestic animals, human beings. Cyanobacteria is produced both types of toxins like nodularin and microcystins that are known as hepatotoxins. They are powerful promoters of tumour and can bind to threonine and serine protein phosphatase enzymes as known a slow protein activity by chemical reaction mechanisms. The hepatotoxicity is cause by the entering ability of nodularin and microcystins that is started the hepatocytes process as making the strong cause of hyperphosphorylation of liver proteins, and that may cause of liver cells destruction [2].

Keeping this in view the present study has pictured with the objective of assessment of some liver function

tests for the diagnosis of hepatic insufficiency in clinical cases of infectious and non- infectious diseases in buffaloes and cows under over sampling regime [3].
In present study, we are investigating contaminated drinking water toxic effect that microcystins toxins on (cows) animal's basic health and find a possible a method for moving from drinking water.

## MATERIALS AND METHODS

### Collection of Drinking Water Samples

Three types of drinking water samples are collected as randomly given in list below,

- ground water
- canal water
- Upper water storage tanks

The (n= 116) frequency of samples were used and temperature was recorded as 27 $^0$C during samples collections area (Jahangir Town). All the samples were collected in sterilized PVC bottles that mostly were filled 100 % by volume. Water sampling is followed the standards methods of water sampling.

### Collection of Blood Samples

All the Blood sampling had been taking day time near 11:00 am after measuring their normal body blood pressure and temperature. After surveys and interviews we were selected cows blood samples as 60 cows from houses where infected persons were present.
The selected blood samples of cows were taken for toxins analysis for estimating the live functions. All blood samples were shifted to Chemical Biotech Lab for performing Biochemical and Microbiological analysis.

### Microcystins Toxin Testing Method

The ELISA is most perfect method for the rapid screening of blood samples for identification of microcystins. The sensitivity of this method is very clear with standard operational procedures. The ELISA method was provided the information about toxin concentration in samples of blood. As water samples were very clear or filtered and then the testing was started to follow the protocol with the better sensitivity. It was the analysis of three microcystins calibrators as 2.5, 1.5 and 0.16 ppb performed. calibrators were diluted as per 1:3 by adding 100 µL of each to 200 µL of kit water and then give the concentrations of calibrators as 0.05, 0.2 and 0.83 ppb respectively [4, 16].

### Liver Function Tests

The Biochemical Testing methods were applied to available for liver function tests of animals (Cows) to know how liver damage after accumulations of toxins in the body [5]. The Blood samples were used for complete liver testing for the liver enzymes by using advanced clinical methods as determinations tools [6].
The Serums of blood samples were collected by mechanical centrifugation method and started the clinical analysis. Some Chemical reagents in form kits were used to determine the concentrations of the parameters like as Protein, Aspartate amino- transferase (AST), Alanine amino transferase (ALT), G-glutamyl transferase (GGT), Alkaline phosphatase(ALP), Total Bilirubin, Direct bilirubin.

### Preparation of Coagulants Solutions

### Ferric Chloride (FC)

Coagulant Ferric chloride was used in the experiments and it had a chemical formula ($FeCl_3$. $6H_2O$). The Different composition was prepared of solutions as (3, 6, 9, 11, 16) by using calculated amount of Ferric chloride salt as dissolved to the deionised water.

### Coagulation Experiments

Coagulation experiments were performed using two jar test equipment at 27$^o$C room temperature of the system. In the process where it was taken five jars with said concentrations. It was taken 2.5 litters untreated drinking water sample volume of was taken as the actual capacity of each jar was 5 litter. It was calculated the efficiency of coagulant Ferric Chloride in different concentration as mentioned as 3, 6, 9, 11, 16 for removing the bio toxins from drinking water samples [7].

## RESULTS

The drinking water supplies chain was contaminated with Cyanobacterial toxins that were main causes of a health hazardous for human and animals. Cyanobacteria species can be produced both type of bio toxins nodularin and microcystins that mostly known as the hepatotoxins. So, they are promoters of powerful tumour and can be bonded to threonine and serine protein phosphatase enzymes. Toxins can slow down the protein binding activity by strong chemical reaction mechanisms. **F**igure-2 is clearly showing below the cyanbectrial presence in the different drinking water samples.

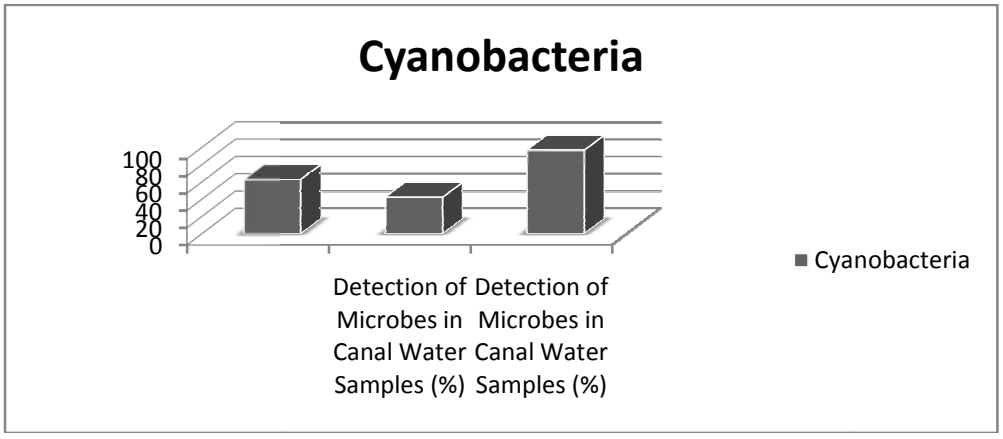


**Fig. 2-**Detection of Cynobectira in Different Water Samples Analysis

**Toxins Analysis in Blood**

It was showed in Table -1 that values of micocystins toxins in blood samples of cows wre not upto standard as seen no good sign for good health. And these effects may be appeared in their meats and milk production. High concentration of toxins may be affected on the food chain that travels from cows and their milk to humans.

**Table 1- (**Mean±S.D) values Blood Samples Analysis of COWS

| Cows Blood Samples | Microcystin (Toxin) (mg/l) | |
|---|---|---|
| | Mean±S.D | Range |
| | 5.7±0.5 | 1-7.9 |

But find 47 out of 50 (94%) of cattle and 63 out of 66 (95.45) buffaloes suffered from liver abscesses were investigated. Liver swellings were confirmed by performing the LFTs tests profile of liver and disease sign and symptoms.

**Effect of toxins Accumulation in Body on Liver Functions Performance**

The values of the LFTs were showed the enzymatic activity that was high in range and solid cause to liver damage which was supported to actual observation as bad health of cows seen on sampling time as shown in table-2. Generally, the regenerative power of liver has to help the minimize this problem as cell membrane may injury. The enzymatic activities in liver might be occurred due to sudden increasing in enzyme synthesis to damage liver. These may be attributed to decrease enzyme synthesis that may it is due to changes in absorptivity of hepatic cells.

The Results were cleared about amylase activity in this study that increased in the samples of cows. Basically, Amylase is release by exocrine of pancreas in the mammalians system which is helped by the functions of liver. These activities are occurred due to pancreatitis or due to the damage of the amylase secretary cells. If this is possibile then the greater amounts of amylase has secreted into the intestine. Which can cause of the so enhanced starch digestion and transferred itself to the degradation products into portal blood. And then into liver and hepatic cells through assimilation, which may also be caused for hyperglycemic response in animals.

**Table 2-Live Function Clinical Test of Animals (**Cows**)**

| Blood Parameters Unit | | Cows blood samples | | |
|---|---|---|---|---|
| | | Mean±SD | Range | Reference Range |
| SGPT (ALT) | U/L | 50±2.9 | 47-53 | 5.0-40 |
| SGOT (AST) | U/L | 72±3.1 | 68-74 | 5.0-42 |
| Alkaline Phosphatase (ALP) | U/L | 299±7.8 | 290-310 | 98-279 |
| GGT | U/L | 4.2±1 | 15-18 | 6.0-8.5 |
| Total Proteins | g/dl | 7±1.2 | 12-64 | 3.5-5.0 |
| Globulin | g/dl | 7±0.7 | 6-12 | 1.2-3.2 |
| Bilirubin Total | mg/dl | 3±0.4 | 1-5 | 0.2-1.2 |

**Effect of Coagulant Ferric Chloride on Removing of Toxins**

$FeCl_3$ is an ionic molecule that has a much charge density as to attachment of metals of ferrous which known a heavy metal. The $FeCl_3$ is hydrolysed; it can decompose into negative and positive ions. Fe metal

is electropositive as very soon attached to the organic molecule which is a waste product in waste water as cause of toxicity, seriously. Reaction mechanism of $FeCl_3$ coagulation is explained in literature with good manners as can applicable on this research.
If we are doing the study about compared regarding as toxins removing values after and before $FeCl_3$ coagulation treatment, it is clearly saying that working efficiency of $FeCl_3$ many times better than alum as shown in table -3.

**Table 3-**Different Coagulant Doses (Ferric Chloride) for Removing Toxins from Canal Water Source Samples

| Coagulant Dose (mg/l) Ferric Chloride | Microcystin(mg/l)( Actual value) | | Microcystin(mg/l) (Value after treatment) | | Microcystin (%) Values after treatment | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| 3 | 25 | 17-27 | 17 | 16-19 | 68 | 63-74 |
| 6 | 25 | 16-23 | 12 | 10-14 | 48 | 45-53 |
| 9 | 22 | 16-23 | 9 | 7-10 | 40.90 | 38-43 |
| 11 | 22 | 16-23 | 5 | 4-7 | 22.72 | 20-27 |
| 16 | 22 | 16-23 | 2 | 1.5-3 | 9.09 | 7-11 |

**DISCUSSION**

It was recorded during study that weight loss and dull demeanor, altered appetite was observed in some animals examinations. It was measured 15 to 50 breaths/ minute as respiratory rate and 55 to 100 beats/minute as heart rate occurred.
Liver is very liable for vascular, biliary and parenchymal system injuries. Viral, chemical, Bacterial, toxic or immune-mediated abuses may cause of diffuse or focal hepatic abnormalities. These are not specific signs as considered a general signs for the diseases. The results are similar to those described previously in cattle as report. It was stated that cattle with liver abscesses exhibit many clinical signs [8].
In established countries treatment of drinking water has helped to finish the diseases problem such as cholera and typhoid. However, these diseases as a related other water issues are still remaining a serious problem in under developing states. Present water treatment processes can control the spreading of water related disease as remove the numerous contaminants such as heavy metals and organic chemicals. The presence of disinfection by-products, pharmaceutical residues and disease causing agents such as Cryptosporidium that are unaffected by a conventional water purification methods and processes, so need here to develop the new technologies [9]. The consumer issue as affecting the potable water in developing states is off-flavour and Off-flavour is occurred due to the compounds in water which are notorious and known for their odour characteristics and undesirable taste. A survey was conducted on more than 900 water supply usages in Canada and America that were found 17 % complains about the serious odour and taste problems, but spending on 4.5 % approximately of their total budget for controlling the smell and taste [10].
Nitrogen having biological compounds inside the samples (canal water) can be separated by aluminium sulphate in setting process as if the material is based on organic compound with a minor quantity.
Pietsch *et al.* (2001) initiate that the removal of nitrogenous matter is problematic to attain with simple coagulation in some cases and the nitrogen based compound are separate by microbial degradation [9, 10]. however, Vilge-Ritter *et al*. (2000) was reported that the bio-organic based compounds look like in the water with minor percentage their removal was much poor due to ferric and Aluminium salts that not able to makr coagulation. Removing the algae and cyanobacteria in clarification and coagulation process was dependent on optimization of chemical doses for coagulation by Aluminium coagulants [11, 12]. Specific dose of Coagulant is essential to removal cyanobacteria and algal cell which is relative to the cell number of logarithm. Minimizing turbidity in jar test is not sufficient to remove algae and cyanobacteria toxin. Cyanobacteria will not be removed on insufficient coagulant dose of aluminium sulphate [13, 14, 15].
Aluminium sulphate dose as 20 mg/l can remove about 80 % without polymer addition, as proved in the present research. The toxicity due to neurotoxic bloom of microcystins, the Coagulation has an ability to remove the toxins from water as proved in several chemical studies. These studies based on tested the coagulant as Aluminium sulphate using with different concentrations amount. Clarification and Coagulation studies have mixed results on the cell lysis as the subsequent of cyanobacterial algal toxins released [16, 17].

## CONCLUSION

Data has been showing the very hazards effects of microsyctins on liver functions performances which is caused by polluted drinking water. And it is also needed to treat the drinking water. The treatment of a large volume of drinking water for animals drinking purposes is not as easily practice. But it is made a possible by using the process of coagulation by addition of calculating and specific amount of ferric chloride (Chemical). Coagulation process is removed the more than 97.7% microcystin and it can achieve desire WHO drinking water standards.

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