

EFFECTS OF MICROCYSTINS TOXINS CONTAMINATED DRINKING WATER ON HEPATIC PROBLEMS IN ANIMALS (COWS AND BUFFALOS) AND TOXINS REMOVAL CHEMICAL METHOD

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ABSTRACT

In present study, it is investigated the toxins in drinking water samples due to microbe's activities, very harmful health effect on humans and animals and especially their liver functions can disturb badly. Liver and kidneys problems in cows and buffalos have a major economic impact on the beef and milk processing business for the reason that liver disease problem can shrink body size and animal performance. For this purpose draw the blood samples of both cows and buffaloes for LFTs (liver function tests) and RFTs (renal function tests) medical laboratory test. This study had the documentary proof the bad health of large animals (cows and buffaloes) that was due to microcystins toxins effects, these findings in case of cows and buffaloes with hepatic problems, collected the 116 samples of buffaloes and 116 of cows, but 82.2% and 87.93% seen as positive in range for microcystins, respectively. In details, animal samples was 116 collected including 50 (43.1% of total sample) cows and 66 (56.8% of total sample) buffaloes but find 47 out of 50 (94%) of cows and 63 out of 66 (95.45%) buffaloes suffered from liver diseases were investigated. Liver swellings were confirmed by performing the LFTs biochemical tests profile

of liver and disease sign and symptoms.

Water sources treatment is only solution of these problems; in this case we use the coagulation process with ferric chloride solution of different doses. In this study, it has proved that microcystins are removed using the concentration of ferric chloride dose is 16 mg/l.

Liver function tests were also played very important role to know the actual working positions of liver function, so values of Rfts indicated the abnormalities of liver in cows and buffaloes due to continues taking the microcystin toxins from water and food sources.

Keywords: liver function, proteins, blood testing, serum albumin

INTRODUCTION

The cyanobacteria are named as blue-green algae and its name due to the existence of photosynthetic pigments inside cells of cyanobacteria. A cyanobacterium is a main group of bacteria that take place all over the world. Cyanobacteria of freshwater may accumulate on the surface of water supplies in form of blooms,

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it may high concentrate on the surface in form of blue-green is known as scums. Cyanobacteria some species produce toxins, these toxins are categorised according to their type of action with other such as neurotoxins (e.g. anatoxins), hepatotoxins (e.g. microcystins), skin toxin produce irritants as feeling on skins and many other types of toxins producing from various other sources. Hepatotoxins and neurotoxins both are produced by cyanobacteria as normally and it is found on surface of water and they are related to water supplies as drinking source (Abenavoli *et al.*, 2011).

The toxin of Microcystins is the most commonly known as hepatotoxin, and it is the common dose variant with an LD₅₀, have value 50.0 µg/kg in mice observed by method of intra peritoneal injection. Microcystins is 200 times more toxic and poison than cyanide metal. This toxin has structural variants include amino acid substitutions and alterations such as methylation and demethylation. Drinking water supplies contaminated with Cyanobacteria toxins is a main cause of a health hazard for human beings, domestic animals both large and small, and wildlife animals (Badar *et al.*, 2016). Cyanobacteria can be produced both toxins microcystins and nodularin which are known as the hepatotoxins, and they are powerful tumour promoters and can bind to serine and threonine protein phosphatase enzymes and slow the protein activity by reaction mechanism. The results of this hepatotoxicity from the entering ability of microcystins and nodularin to hepatocytes process where they make strong cause hyperphosphorylation of liver proteins and destruction of liver cells (Ayers *et al.*, 2008).

Basically, Liver is important one with the largest part of animal's body and works as

a central title role in the process of metabolic chemical reactions which essential for the life and survival. It controls several important functions in the body such as proteins, carbohydrates and fat absorption in animals's body and also excrete the substances formed in the duration of body growth (Bakoyiannis *et al.*, 2013).

Liver function medical tests are very important for the identification of hepatic illnesses in both animals and animals such as, estimation of serum albumin, globulin, ALT, bilirubin, AST and GGT levels along with several specific enzymes as well. But, there is lack of information in literature regarding work on these tests for hepatic insufficiency in buffaloes and cattle. 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 (Crump *et al.*, 2003).

Toxins accumulating within the blood were responsible for other end-organs being damaged. Some of these factors may be due to changes in the cellular component of blood, but many of these deleterious effects are due to changes in the humoral component of circulating blood. These toxins may arise either as a consequence of a failure of normal hepatic functions, or elsewhere in the body as an importance of simple liver disease (da Hora VP *et al.*, 2011).

These toxic factors in the blood affect the function of many organ systems, such as the systemic and portal vasculature and the brain, as well as the liver itself. The exact nature of these toxins is unknown and may be different and multiple for each organ system damaged. Ammonia, aromatic amino-acids, tryptophan, indoles, mercaptans and endogenous benzodiazepines are implicated in the

development of hepatic encephalopathy (de la cruz *et al.*, 2011).

Whereas, prostanoids, inflammatory cytokines, nitric oxide and oxidative stress, are all considered to be important factors in the development of the haemodynamic and renal changes seen in liver failure. It is, however, the substances that are directly hepatotoxic that are particularly important in terms of recovery, as they may perpetuate liver injury invoking a downward spiral with further reduction in functional liver mass and increased toxin load. It is notable that many of the suggested toxins are insoluble in water and exist in the circulation bound to albumin (Eckburg *et al.*, 2005).

The most commonly used chemicals in this process include aluminium or ferric chloride. More recently some synthetic organic polymers gained some approval. It is very effective for removing the cyanobacteria cells and it is possible to removal soluble microcystins by strong chemical coagulants such as polyaluminium chloride, alum and ferric sulphate. The effectiveness depends on coagulation doses but high dose can produce fungus or algae (Tehrani *et al.*, 2012).

In present study, we are investigating contaminated drinking water toxic effect that microcystins toxins on 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,

1. Ground water

2. Canal water
3. Upper water storage tanks

And the temperature of the day when collect the samples was 27°C. All sample collect in sterilized PVC bottles as the container and water sample container were filled 100% of the volume capacity. Water sampling is followed the standards methods of water sampling.

Collection of blood samples

Blood sampling has been taking day time at 11:00 am after breakfast at their normal body temperature and blood pressure. In animals blood samples collection after surveys and interviews we selected animals (60 cows and 40 Buffaloes) from houses of diseases infected persons. Selected animals blood samples are for toxins analysis and to estimate the live functions strength due to bioaccumulation of toxins. All the samples shifted to Microbiological Division of Chemical Biotech Lab for Biochemical and Microbiological analysis and detail of temperature and humidity given in Figure 1.

Animal blood sampling

Collect the random animal blood sampling from different places (animals use for meat and milk) was with the frequency of samples (n=116). All samples were collected by syringe in sterilized blood vessel used as container and blood sample 5 ml collected by volume and actual capacity of container was 5 ml. The temperature of the day when collect the samples was 16°C.

Microcystins toxin testing method

ELISA is the most reliable method for rapid screening of samples for detection of microcystins because its sensitivity and specificity

very clear with ease operation in devices. ELISA assay provide the information about the total toxin concentration in the sample. If the water samples very clear or filtered, then the testing were started with following assay protocol with better sensitivity, where the analysis of the three microcystin-LR calibrators are 0.16, 1.5 and 2.5 ppb performed, were diluted as 1:3 by adding 100 μ L of each to 200 μ L of kit water and then give the concentrations of calibrators as 0.05, 0.2 and 0.83 ppb respectively (Ethelberg *et al.*, 2004).

Liver function tests

Biochemical Testing methods are available for liver function Tests of animals (Buffaloes and Cows) to know how liver damage due to excess inhaling the toxins (Falconer *et al.*, 2005).

Blood samples is used for complete count with regard to the liver enzymes, was investigated in a whole blood by help of clinical method (Scott, 2013).

Serums of samples were collected after mechanical centrifugation of the samples blood, and start the analysis of clinical chemistries of blood samples. It was used the Chemical reagent (in form kits) to determine concentrations of following parameters in the serum as,

1. ProteinAspartate 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)

In the experiments, it was used the coagulant Ferric chloride as chemical formula ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$). Different composition of solutions

was prepared using calculated amount of Ferric chloride salt dissolved in deionised water. Solution of different concentration used in this study as given (3, 6, 9, 11, 16).

Coagulation experiments

At room temperature (27°C), Coagulation experiments were performed using two jar test equipment. In this process, it was taken the untreated water from different three drinking water samples from different sources mentioned previously. The in each jar, sample volume of untreated water was taken 2L, but actual capacity of jar was 5 litter. To calculated the performances efficiency of Ferric Chloride in different concentration (mentioned as 3, 6, 9, 11, 16) as coagulation process for removing the toxins from drinking samples of water (Ho *et al.*, 2011).

RESULTS

Drinking water supplies contaminated with Cyanobacteria toxins is a main cause of a health hazard for animals, domestic animals both large and small. Cyanobacteria can be produced both toxins microcystins and nodularin which are known as the hepatotoxins, and they are powerful tumour promoters and can bind to serine and threonine protein phosphatase enzymes and slow the protein activity by reaction mechanism.

In Figure 2, it is clearly observed the cyanobacteria population in different samples of drinking water as shown, respectively.

Toxins analysis in blood

1 show that different values of micocystins in blood samples of cows and buffaloes which is not good sign for good health and have bad

effects on their milk and meats production. Excess concentration of toxins found in animals' blood samples are meant that food chain including animals drinking water source is the cyanobacterial contaminated. Cows have more immunity of detoxification of toxins as compared to buffaloes and that is why buffaloes' blood samples with more toxins values as observed in Table 1. 116 blood samples were taken from buffaloes and cows, but 82.2% and 87.93% seen as positive in range for microcystins toxins, respectively. In details, animal samples was 116 including 50 (43.1% of total sample) cattle and 66 (56.8% of total sample) buffaloes. But found 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

According to literature it is confirmed that liver function, performance is disturbed due to accumulation of toxins in the body because the work function of the liver is to detoxify the toxins and other harmful substances but if the toxin load increased liver function values changed and disordered.

Especially ALT liver function tests depend on liver health and blood purifications, its value increase showed that blood has high level toxin concentrations. Animals were also infected because their SGPT values in the range of 78 to 82 which is no good sign for milk producing animals, similarly SGOT was high, which was showed, that liver status was not good and these effects may appear in their meat and milk production. Identification of toxins in blood samples showed that toxins present in food chain and this was a serious threat to all

living things.

The results of complete LFTs blood tests revealed that all animals had the liver enzymes, there were mild elevation of ALT (80.50 ± 3.90 ; reference range 5 to 40 units/L) and GGT (24.80 ± 4.30 , reference range 12 to 64 units/L) in buffalo animals given in Table 4.3 and, while AST and ALP were also not within reference range in all examined animals (AST: 84 ± 4.1 , reference range 5 to 42 units/L, ALP: 416 ± 10 , reference range 410 to 420 units/L, all the units values given from kit bio-check company).

Similarly in Table 2, it was clearly showed those cattle's liver enzymes in abnormal form as given values in ascending order because cattle had to use canal water for drinking purposes due to some economic reasons under our surveys. We had already discussed the quality results of canal water as previous given in Figure 1.

Liver damage situation is a very serious health issue across world due to drinking of bad quality water. Liver function tests are usually represent and recognized as the reliable indicator of liver performance of detoxification function. Inside the liver, enzymatic activity have been raised up, this is may be due to synthesis of enzymes, their low levels indicate that the enzymatic inhibition due to liver injury without specific regeneration. Among liver enzymes, amylase GOT, GPT and ALT were elevated in the samples of animals blood, it was showing acute liver damage (hepatitis), while in samples of animal's blood, all these enzymes were inhibited showing hypocondition or dysenzymia.

The raised values of LFTs showed the enzymatic activity in the blood which attributed to liver damage, while their low values of LFTs showed in Table 2, the regenerative power of liver to minimize and cell membrane may injury.

The increased enzymatic activities in the

Table 1. (Mean±S.D.) values blood samples analysis of animals.

Type of animal blood samples	Microcystin (Toxin)	
	Mean±S.D.	Range
Cows (mg/l)	5.7±0.5	1-7.9
Buffalos (mg/l)	9.7±1.4	7-11

Table 2. Live function clinical test of animals (buffaloes and cows).

Blood Parameters Unit	Buffaloes blood samples			Cows blood samples		
	Mean±SD	Range	Reference range	Mean±SD	Range	Reference range
SGPT (ALT) U/L	80±3.9	78-82	5.0-40	50±2.9	47-53	5.0-40
SGOT (AST) U/L	84±4.1	80-87	5.0-42	72±3.1	68-74	5.0-42
Alkaline Phosphatase (ALP) U/L	416±10	410-420	98-279	299±7.8	290-310	98-279
GGT U/L	16.7±1.6	15-18	6.0-8.5	4.2±1	15-18	6.0-8.5
Total proteins g/dl	20±2.2	18-22	12-64	7±1.2	12-64	3.5-5.0
Globulin g/dl	11±0.9	10-12	1.2-3.2	7±0.7	6-12	1.2-3.2
Bilirubin total mg/dl	10±0.7	8-12	0.2-1.2	3±0.4	1-5	0.2-1.2

Table 3. Renal function clinical test of animals.

Parameters	Units	Buffaloes		Cows		Reference range
		Mean±SD	Range	Mean±SD	Range	
Urea	mg/dl	4.1±1.1	4-5	3.2±0.6	2.5-4	0.9-1.1
Creatinine	mg/dl	4.7±0.5	4-5.3	3.7±0.4	3-4.3	1.2-1.9
Ammonia	µg/dl	127±10	120-140	116±10.4	110-127	13.0-108.0
Glucose	mg/dl	47±3	45-52	60.7±4	55-63	75-115
Calcium	mg/dl	9.3±0.9	7-11	7±0.9	6-10	1.3-4.6
Potassium	mg/dl	9±3	10-11	7.2±0.5	6-9	1.5-5.4
Sodium	mg/dl	1.4±0.3	0.9-2	0.97±0.3	0.7-1	0.9-3

Table 4. Different coagulant doses (Ferric Chloride) for removing toxins from canal drinking 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

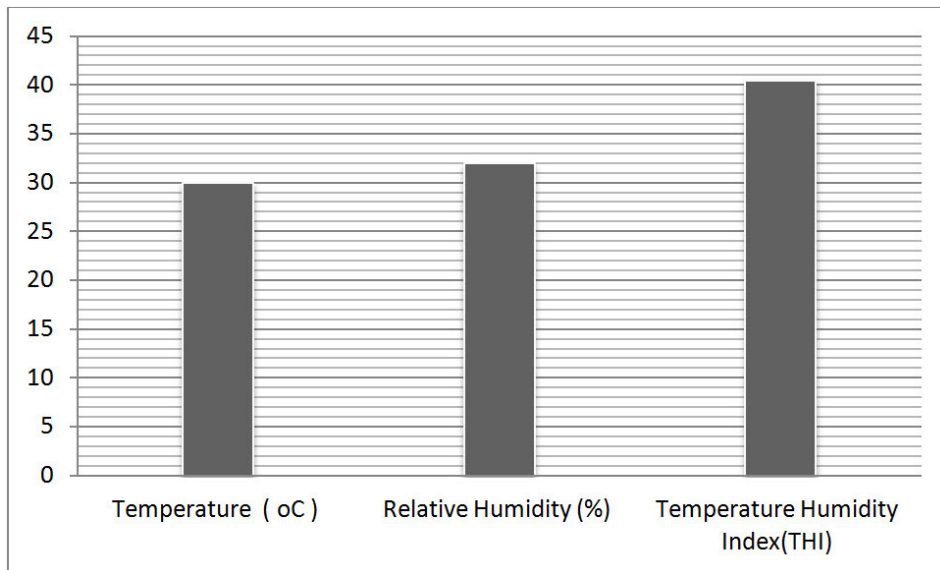


Figure 1. Condition of temperature and humidity during samples testing in laboratory.

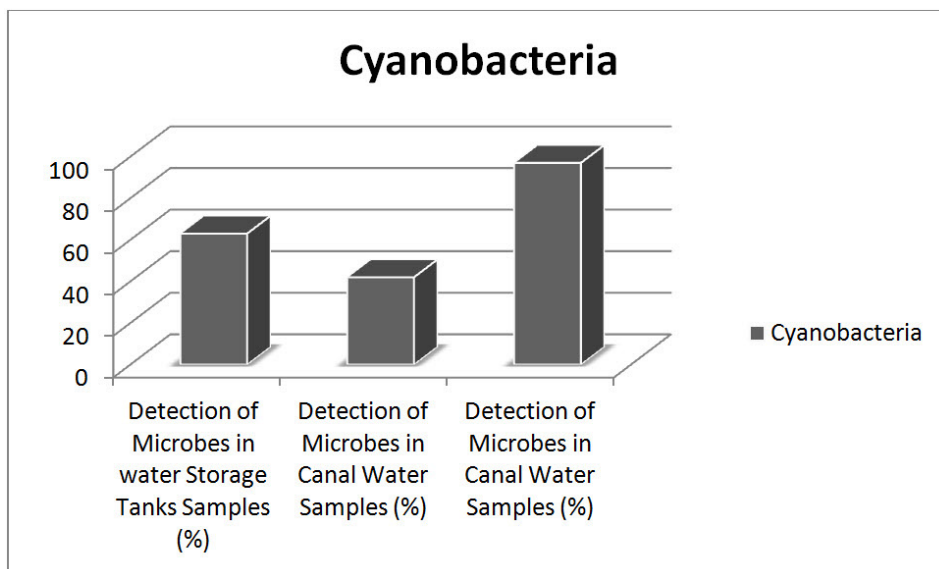


Figure 2. Detection c in different water samples analysis.

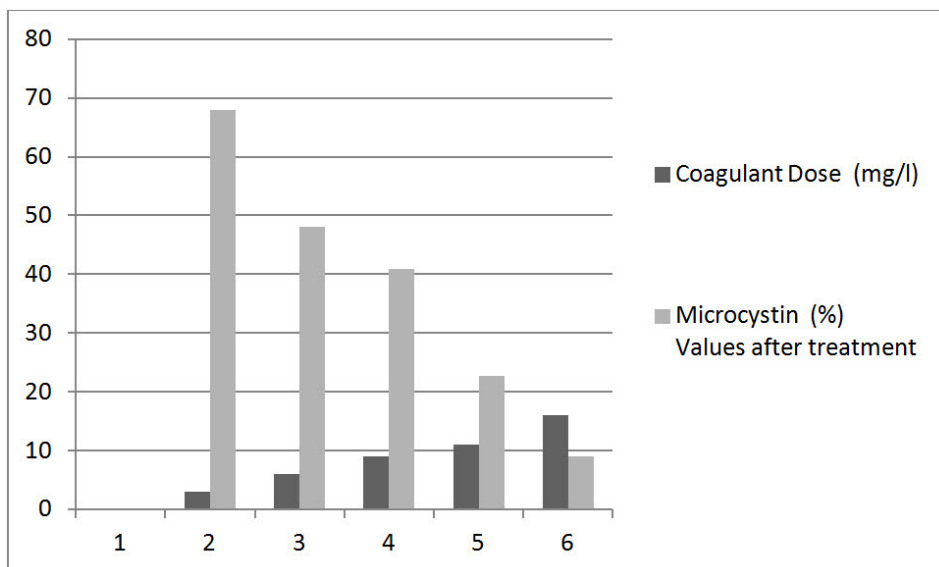


Figure 3. Different doses of ferric chloride used for toxins removing from canal drinking water source samples used for animals.

liver might be due to increased enzyme synthesis to the damage the liver in the collected samples of humans and animals. The decreased activities of enzymes in liver may be attributed to decreased enzyme synthesis and it may also be due to changes in absorptivity of hepatic cells.

Results clear about amylase activity in the present investigation increased in both of samples of cows. Amylase is secreted by the exocrine region of pancreas in mammalians system by help of liver function. The increased activity may be due to pancreatitis or due to the damage of the amylase secretary cells inside body. There is also the possibility that greater amounts of amylase were secreted into the intestine, which causes the consequently 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.

Effects of toxins on renal function of kidneys

Liver and kidneys are worked together because if one of them does not give properly routine function its means one of organ in process of damaging. According to the literature, it is sure abnormal values of liver enzyme due to microcystins toxicity may cause of kidneys problems such as metabolic protein convert into ammonia and then urea by liver break down reaction but only kidneys cannot excrete without breaking down. If kidneys work as it is, they must damage because such big molecule can damage the sensitive filtering tissues of kidneys.

Previous studies had shown that liver problems affect the kidneys functions that may lead different bundle of diseases as similar observed in this research. Both human and animals liver and kidneys functions are distributed as shown

in Tables 2 and 3 for liver and renal or kidneys functions tests. A change in the amount of urine produced or blood in the urine may indicate kidney toxicity. Sodium, potassium and calcium are the minerals that show the kidneys performance.

Effect of coagulant ferric chloride on removing of toxins dissolved in drinking water

FeCl_3 is an ionic molecules that have a great charge density due to attachment of ferrous metals known as heavy metal. When FeCl_3 is hydrolysed in water it decomposes into positive and negative ions. Fe metal is electropositive and very soon attached with organic molecules as waste product in waste water and cause of toxicity. Reaction mechanism of FeCl_3 coagulation is explained in literature with good manners as can applicable on this research.

If we are compared toxins removing values after FeCl_3 coagulation treatment it is clearly proved working efficiency of FeCl_3 much times better than alum coagulation as shown in Table 4.

Toxins basically have weak electrical change when it comes with high polar atom then it attracts automatically and this reason is enough for removing the toxins from drinking water samples. One is most important use FeCl_3 coagulation process, its chemical reaction is going to very sudden because FeCl_3 molecule has maximum change density and able to donate the electrons in this chemical reactions. Low concentration of FeCl_3 is used as compare to Aluminium salt in this research, this situation is very favourable toward where residues of FeCl_3 is less in quantity, this is stopped for further microbes growths as shown in Figure 3.

DISCUSSION

Dull demeanor, altered appetite and weight loss were recorded in some examined animals. It was measured 55 to 100 beats/minute as heart rate and 15 to 50 breaths/ minute as respiratory rate occurred. Grunting, arched back and other tucked up abdomen were observed in both cows and buffaloes. Thirty Two cows and fifty two buffaloes had scant hard feces, while one cow and three buffaloes had diarrhea.

The results of complete blood count revealed that all animals had the liver enzymes, there were mild elevation of ALT (60.50 ± 11.90 , reference range 11 to 40 units/L) and GGT (24.80 ± 4.30 , reference range 6 to 40 units/L) in four animals (3 cattle, one buffalo), while AST and ALP were within reference range in all examined animals (AST: 80.64 ± 20.20 , reference range 78 to 132 units/L, ALP: 140 ± 5.50 , reference range 0 to 500 units/L).

Liver is highly susceptible for parenchymal, vascular and biliary system lesions. Bacterial, chemical, viral, toxic or immune-mediated insults may cause focal or diffuse hepatic abnormalities or lesions. These signs are not specific signs and considered as general signs for many diseases. These results are partially similar to those described previously in cattle. Other report stated that cattle with liver abscesses seldom exhibit any clinical signs and abscesses can be detected only at the time of slaughter (Hoeger *et al.*, 2002).

Moreover, Hypoalbuminaemia with hyperglobulinaemia were reported in all diseased animals under investigation. These results are in accordance with those obtained previously in cattle and in camel. With regard to the liver enzymes (AST, ALT, ALP and GGT) and renal function parameters, blood urea and creatinine

were within normal range in our study, only mild elevation in ALT and GGT were detected in cows and buffaloes. Generally, hematology and liver function tests are reliable indicators of liver abscesses. The usefulness of the technique for diagnosis of various liver abnormalities including abscesses in animals has been well documented (Khan *et al.*, 2001) (Bakoyiannis *et al.*, 2013).

In USA, the death rate due to typhoid illness are twenty or more people per hundred thousand of population was measured common, the ratio was 58.7 in Minneapolis, in London the rate was only 3.3 per hundred thousand (AWWA, 1996). However, despite advances in water treatment many people have no reach to adequately treated potable water. The World Health Organisation assessments on 1 billion people internationally no access to safe drinking water sources and 2.6 billion people suffering from poor sanitation. Diseases causes due to unsafe water drinking, poor sanitation and hygiene are as results of expected 1.7 million deaths annually (Lahti *et al.*, 2001).

In developed countries water treatment and sanitation has removed the problem of diseases such as typhoid and cholera. These diseases, however, among other water related issues, remain a serious problem in developing countries. Modern water treatment processes control the spread of water related disease; remove numerous contaminants, such as organic chemicals and heavy metals, producing safe water. However the presence of pharmaceutical residues, disinfection by-products, and the possibility of disease causing agents as *Cryptosporidium*, which are unaffected by common water purification processes and so, need of new treatment technologies for this purposes (Frank *et al.*, 2008). The single largest consumer issue affecting potable water under developing states is off-flavour. Off-flavour is caused by compounds in

water that are known for their undesirable taste and odour characteristics. A survey conducted of more than 800 water usages in the America and Canada found the 16% of utilities experience the serious taste and odour problems, spending approximately 4.5% of their total budget for taste and odour control (Lehman *et al.*, 2007).

Nitrogen based biological compound inside samples of canal water can be detached by aluminium sulphate setting if the matter is based on organic compound a minor in 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 detached by microbial degradation and zonation processes (Lequin *et al.*, 2005).

Moreover, Vilge-Ritter *et al.* (1999) reported that bio-organic based compounds resemble to if a minor in percentage in the any kind of samples of water; their elimination is so much poor because Aluminium and ferric salts not able to coagulate them. Removal of cyanobacteria and algae in coagulation and clarification process is dependent on optimization of chemical doses of coagulation with Aluminium coagulants (McKaigeny *et al.*, 2013).

Specific dose of Coagulant is essential to removal cyanobacteria and algal cell which is relative to the cell number of logarithm (Perelle *et al.*, 2005). 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 (Radostits *et al.*, 2007).

Aluminium sulphate dosed at 20 mg/l without polymer addition removed about 80% of the toxicity from neurotoxic bloom of microcystins, Coagulation had an ability to eliminate the toxins in water samples in several studies (Cobbold *et*

al., 2004). These studies tested the coagulant as Aluminium sulphate in different concentrations. Coagulation and clarification studies have had mixed results on cell lysis and the subsequent release of cyanobacterial algal toxins (Schlosser *et al.*, 2001). Results of this study shows that microcystin toxins are produced by cyanobacteria that is commonly called blue-green algae although it is really an algae and algal toxins are released from cyanobacteria as a bloom nears the end of its lifecycle, or the cells are lysed (split apart) and the toxins are released.

As previous studies on treatment of potable water using with ferric chloride had shown the results showed that different optimum dose of coagulant can be effected on disinfect the microbes in different types of samples effectively (Schmidt *et al.*, 2002).

Some studies showed a 5 mg/l was ineffective for destroying algal toxin extracts but it is demonstrated that joint flocculation management procedures which involved chlorination 0.7 mg/l dose also successful for killing of microbes (Scott MC *et al.*, 2002).

CONCLUSION

Microcystins are very toxic compounds and their presence may cause of serious acute or chronic toxicity in any level of cattle. Microcystins toxicity can damage the cattle liver easily that can be observed in kidneys functions tests. Kidneys functions tests are also important tools for observing on the filtration process of animals kidneys. High values of Rfts are indicated that kidneys s filtrations process not be normal along losing of animal's weight and healthy habits.

Large volume of drinking water for animals

is not easily practice and it is made possible by using the coagulation process by adding the calculating amount of ferric chloride. This process is showed that more than 98% microcystin are removed and finally achieve the desire WHO drinking water limits. So, we can say that WHO drinking water standard for microcystin is free from any toxicity causes in human and animals.

In present study, it is investigate the toxins in drinking water samples from microbe's activities, very harmful health effect on humans and animals and especially their liver functions disturb badly, for this purpose draw the blood samples of both humans and animals for LFTs medical lab test. Liver abscesses in cattle and buffalos have a major economic impact on the beef and milk processing business for the reason that of liver problem can shrunk body size and animal performance.

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