Wastewater is the source of containing several toxin chemicals which are liberating in environment in an uncontrolled manner. In ideal conditions, domestic wastewater should not have any toxic elements but wastewater releasing from hospitals, research laboratories, maternity centers and industries without treatment may contain a variety of hazardous chemicals which pose a long-term risk to health human or environment. Releasing these effluents directly into the environment not only contaminate the surface water but also sometimes mix with potable water. Using treated wastewater for irrigation, cleaning purpose may be positive think to discharge urban sewage water in which health centers effluent should not mix in any circumstances. The urban sewage water contains of plenty of nutrients which are useful for irrigation. Wastewater contains tons of microorganisms which may be source of cancer causing. Due to these unhealthy microorganism present in untreated wastewater can be a cause of epidemic diseases for the farmers who are directly using it. Therefore, the World Health Organization (WHO, 1999, 2000) highly recommended for the treatment of wastewater before releasing into the environment and using in fields for agricultural purpose.

Exposure of such new invisible hazardous pollutants and chemicals are not life threatening to human health but their effects are carcinogenic, mutagenic and tetratogenic, neural toxic probably which are studied by regulatory framework and standard estimation.

Concern become more intense if the wastewater releasing from health care establishments like hospitals, diagnostic research centers, nursing care, dispensaries as health care industries wastewater are heavily loaded with pathogenic bacteria, metabolized drugs, analgesic medicine, anesthetic gases, hormones, radio nucleosides, and other hazardous substances such as antineoplasm, antiseptic and halogenated compounds and their subsidiary by-products (Emmanuel et al., 2002). Vulnerable population consists of patients, haulers, health care workers, waste handlers and other inhabitants who are residing near the chemical industries and urban sewage system having regular exposure with these toxins (SAICM, 2011 and Government of India,1998)
Per capita productions of health center waste water are estimated to be 1000 litres/person/day has been reported by Tsakona et al. (2006). From the last decades, environmentalists and toxicologists have research that wastewater liberating from health centers are endowed with disastrous characteristics of bearing specific genotoxic and carcinogenic agents which are capable of causing fatal effects on global environment and aquatic ecosystem (Sprehe et al., 1999). Therefore, for the conservation of global environmental probity and to maintain sustainability for the next generations, the concern on increasing environmental impacts from health centers wastewater and the viable diminution measures has become an area of concern for environmental protection and management. Moreover, increase in demands for healthcare facilities, advancements in medical technology and rapid development in the drugs, the number and complexity of health centers activities have increased and this has led to lethal effects through the increase in wastewater production by health centers.

Wastewater generating from healthcare facilities contain high range of hazardous pathogens, toxin chemicals, bactericide, antibiotics and more harmful materials like antidole, antiseptics and radio nucleosides than domestic sewage (Ruth Stringer et al., 2011). While in some countries (e.g., Japan, China, Greece, US) (Pauwels and Verstraete, 2006; Kosma et al., 2010; Liu et al., 2010) wastewater from big hospitals is pretreated on-site, in many other countries, including India, it is connected directly to a municipal sewerage system and treated at municipal wastewater treatment plants (WWTP) along with domestic and industries sewage (Gupta et al., 2009). Many of the chemical compounds present in health centers wastewater resist treatment in normal wastewater treatment plant (WWTP), as their conventional activation process are ineffective in removing them (Kummerer, 2001, 2004, 2009; Kolpin et al., 2002; Petrovic et al., 2003; Snyder et al., 2003; Carballa et al., 2004, Glotermann et al., 1978). Further, theses effluents percolate with underground water table and contaminate the potable water.

In general, the effect of a complex mixture of several chemical substances in health centers sewage can be i) additive, ii) synergistic, iii) antagonistic (Calamari and Alabaster, 1980). When theses toxin substances, react with each other they show
more catastrophic effects and potency with other substances than as alone. Physico-chemical characterization and by-products of such chemical through biological interactions can sometimes become more calamitous than those initial one (Hermens et al., 1984). In the case of health centers wastewater, the problem become denser when several hazardous substances get mixed and disposed of directly into the environment water bodies, which is capable of magnifying the toxin impacts and cause communicable disease.

One major worry for the flow of health centers wastewater into the aquatic environment is its genotoxicity to the aquatic inhabitants. If uncontrolled, such toxicities are capable of causing acute, chronic or even lethal impacts on the survival of the aquatic organisms and their future generations (Shaner, 1997; Roberts et al., 2006). Concerns regarding the exposure of humans are mostly associated with exposure through drinking water produced from contaminated surface and underground water (Tyagi et al., 2007)

There are several disadvantages associated with animal and plant bioassays such a problem with their standardization, time consuming and expensive cost of experimentation etc. On the other hand, short term genetic bioassays affect the DNA of organisms, which is a well known cellular component (Roopadevi and Somashekar, 2012). Thus the present obtained from these bioassays are admissible to human health.

It is very difficult to correlates the results of each test as screening of toxicity bioassays develop their own reactivity and standards. Hence, a battery of microbial bioassays comprising bacterial (prokaryotic) and chromosome aberration in Swiss albino mice (eukaryotic) bioassays can meet the feasible challenges of screening wastewater.

The present study is aimed at studying toxic and genotoxic potential of the final discharged from health centers. Three hospitals situated at different prime locations in Jaipur (Rajasthan) were selected for this study, namely one state run biggest hospital GH (Government Hospital) with bed strength of 2000 per ward and
two biggest private hospitals of Jaipur city i.e. PH-I and II (private hospital-PH) with the bed strength of 430 and 350 respectively. Two diagnostic laboratories DL-I and DL-II were also taken for study. The wastewater samples were taken directly from main sewer of GH, DL-I and DL-II as they don’t have wastewater treatment facilities.

WTP established in PH-I and PH-II are effectual functioning in removing genotoxins to treat their wastewater before releasing it into the public sewer system or in environment. Volume of waste water being treated in the ETP of PH-I and PH-II is about 50,000 liters and 78,000 liters per day respectively. Final treated effluent (after chlorination and filtration) from ETP is used for gardening purposes in hospital arena. Therefore, samples were collected both before and after treatment. Untreated effluent, samples were procured from the main sewage, where the entire wastewater of health centers collected. Filtrate and treated wastewater were collected from different stages of treatment plant.

The finding study correlates with other microbial genetic bioassays like between treated and untreated effluent samples. Use of genotoxicity bioassays employing both prokaryotic and eukaryotic assays was also studied and compared for their capability to determine toxicity level.

For this purpose, three genotoxicity bioassays namely Ames Assay, SOS Chromotest and Chromosome aberration were executed with samples collected during the years 2012 and 2013. Sampling was done during summer, rainy and winter to account for seasonal variation. The wastewater samples from all the health centers were collected in the months of May, August and November 2012 for physico-chemical analysis, Ames Assay, and again in the months of January and March of 2013 for SOS Chromotest and Chromosome aberration. Sampling was done twice in every month.

Wastewater samples from different health centers were collected in sterilized autoclaved sampler containers and were stored at 4°C for further studies. Samples were collected by grab sampling. Samples were collected from health centers
between timing i.e 8a.m to 6p.m as maximum health care activities occur during this course of time. They were transported in ice cold box to prevent from transfer heat effect. The samples were also tested in their (raw) crude form for their mutagenic and genotoxic potential.

In most cases this hazardous effluent is discharged directly into urban sewer network without treatment or in some cases with partial treatment. In present study, the genotoxicity and mutagenicity of (i) Untreated Liquid effluents generated by the important hospital and diagnostic laboratories and (ii) Filtrate and treated effluent at different stages was studied.

Three short – term microbial bioassays were performed to evaluate toxicity and genotoxicity of different health centers waste water in which two of them were employing prokaryotic organisms (bacterial system) and one employed eukaryotic organisms (Swiss albino mice). These assays included Salmonella Mutagenicity Ames assay, SOS - Chromotest and Chromosomal Aberration. All the raw, filtrate and treated samples of different health centers were subjected to each of these bioassays one by one for analysis of their genotoxic potential.

The data obtained from physico - chemical quality analysis as well as genotoxicity estimation of health centers waste water samples in the present study reported that untreated wastewater from GH, DL-I and DL-II laboratories are releasing (without any treatment plant) into the city sewerage system are hazardous enough. The treated influents collected from PH-I and PH-II are less toxic as compared to filtrate and untreated effluents.

The Salmonella Ames assay being the standardized and most common method of genotoxicity evaluation was performed with collected samples from all five health centers. In this assay, Salmonella typhimurium viz strains TA 98, TA 100 and TA 102 were used to determining frame - shift mutagens and base - pair substitution mutagens, respectively in health centers wastewater. Exposure of such mutagens present in samples give rise to induced revertants on a minimal media (absence of histidine) which were observed for the presence of a reverse mutation
event taking place in previously mutated strains (that were not able to grow in a media without histidine).

The colonies were counted after proper incubation and the results were defined in the form of revertants. Hand counting was required due to high range of spontaneous mutant colonies, which are more than 360 revertants per/plate. Genotoxicity evaluation by *Salmonella* test needs the incubation of 48 hours for evaluating the properties of the final growth of mutants on the GM agar plate in spite of determining significant survival. These features are: less number of revertants colonies may be accompanied due to thinning of background lawn, lack of growth and existing of pin point non–revertants colonies (normally when background lawn is absent) is also indication of tremendous death of bacterial cells due to toxicity of test material (Mortelmans and Zeiger, 2000). Sight toxicity or precipitates does not invalidate the experiment, or the concentrations at which they were found, which may affects the inference of results and the way/method of performing experiment (Mortelmans and Zeiger, 2000). The observations were expressed as concentration response curves and MR is considered to be statistically significant if it is 2.0 or more than 2.0. For comparison study of different samples highest concentration was 100µL have been used.

A comparison between untreated and treated effluent from PH-I and PH-II hospitals were representing statistically significant difference (p<0.05) at each concentrations for three strains with and without of S9 mix which further indicates that there was a significant difference existing in terms of number of revertants colonies produced with the samples before and after treatment. Also, a comparison among the final discharge of health centers by applying independent t-test between GH and PH-I untreated samples, between GH and PH-I treated samples; between DL-I and DL-II untreated samples; between PH-I and PH-II untreated samples between PH-I and GH filtrate samples indicating significant level of mutagenicity potential of effluent discharged from different health centers. Thus, on studying the genotoxic potential of different health centers wastewaters GH has proven to be the strongest in terms of mutagenicity while treated effluent of PH-I, PH-II and DL-I and II effluent were found least and weakly mutagenic in comparison to raw effluent of health centers.
Secondly the bioassay, named SOS-chromotest performed by using SOS chromotest kit procured from EBPI, Ontario, Canada with the health center wastewater samples, employing a genetically engineered *E. coli* PQ37 bacterium strain, without metabolic activation which was able to detect genotoxic material that causes damage to DNA of the cell. Based on the Induction Factor obtained, untreated sample of GH, PH-I and II value >1.5 having significant of hospitals were showing SOS inducing activity showing high genotoxicity in untreated wastewater while the Induction factor obtained from filtrate, treated effluents and diagnostic laboratories samples exhibited no significant effect on *E.coli*. The activities of both the enzymes (Alkaline phosphatase and β-galactosidase) and Induction Factor values were less than 1.5 showing weak genotoxic response. It was observed in the month of November 2012, samples of GH hospitals produced IF’s much higher than 1.5 decreasing gradually with the decreasing concentration. The maximum IF of untreated effluents of GH was 11.8 to 2.7 and PH-I and II was 8.5 to 4.6. Turning towards lower concentrations, these samples produced lesser induction factors depending on the dilution of the sample. Thus, according to this assay, the untreated wastewater samples of GH, PH-I and II hospitals were significantly and strong genotoxic at higher concentration.

Lastly the bioassay named, Chromosome aberration was used to evaluate the extent of DNA damage in peripheral blood lymphocytes of Swiss albino mice by giving oral dosing of different health centers waste water in duration of 24 hours, 48 hours and 72 hours for the preparation of chromosomes. Samples were collected in the month of November 2012, January, 2013 and March, 2013 with this assay to determine their cytogenetic potential. When the samples were analyzed with this assay, response observed were more or less similar for all samples in terms of their dose response profiles during all sampling periods representing no seasonal variations.

Hundred well spread cells were scored per Swiss albino mice for the study of chromosomal aberration and approximately 50 cells/concentration of metaphase were analysed. Types of aberration observed are chromosome breaks, gaps and fragmentations. To study the chromosomal assay, the animals were divided in three
groups, (6 animals / groups). The waste water was doses for the period of three continuous duration: 24, 48, 72 hours. The animals of negative control group were given distilled water. The animals of positive group were treated with Cyclophosphamide, which is a well-known clastogene and mutagen (Anderson et al., 1995). Only GH waste water shows one single chromosome aberration at 24 hours. A statistically significant (p<0.05) was observed with all the dose levels tested.

In light of findings of present study it is suggested that all the Health centers; hospitals and diagnostics laboratories should released their wastewater effluents after proper treatment, so the contamination of environment may be avoided.