Microbial and chemical analysis of illicit drugs samples confiscated from different areas of Pakistan

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Abstract: The microbial and chemical analysis of illicit drug samples from different areas of Pakistan i.e. Quetta, Karachi, Lahore and Islamabad was conducted in a cross-sectional study at National Institute of Health, Islamabad. The drug samples were confiscated by Anti Narcotics Force (ANF), Pakistan. Microbial analysis was done by estimating bio-burden which revealed the presence of gram negative and positive bacteria's, fungus, *Streptococcus, Staphylococcus* species. Trypton soya agar was used for total aerobic count, MacConkey agar for gram-negative bacteria, Sabouraud dextrose agar for fungus and Vogel-Johnson agar for *Streptococcus* and *Staphylococcus* species. Colour tests were applied to identify the drug samples. Qualitative and quantitative analysis of suspected samples of Heroin, morphine, cocaine and acetic anhydride was made by employing different chromatographic techniques i.e. Thin-layer chromatography (TLC) and High-performance liquid chromatography (HPLC). The samples were found to be adulterated with paracetamol, diazepam and Dextromethorphen. Acetic anhydride was adulterated with hydrochloric acid (HCl). There is lack of information providing structured advice on responses to the consequences of illicit drug adulteration. Robust and rehearsed interventions and communication strategies would provide a basis for response for a wide variety of organisations. Research into the usefulness of media warnings about adulteration of illicit drugs is required.

Keywords: Illicit drugs, heroin, paracetamol, diazepam and dextromethorphen, acetic Anhydride. TLC, HPLC, Pakistan

INTRODUCTION

In modern era, there has been widespread examination that 'Illicit' drugs which are structurally varied set of chemical agents possess tremendously elevated ability for biological effects in humans and also in non target organisms. They contain other substances in addition to the purported active ingredient that can have serious adverse health consequences or even cause premature death (Daughton, 2011). The word ''illicit'' is given to these drugs because of their way of production, circulation, their mode of getting and also their use. These drugs are utilised for non-medical reasons. On the basis of their origin and biological effects, these illicit drugs are illustrated in a variety of ways i.e. these may be naturally producing, semi artificial (chemical managements, such as analogs, of materials obtained from natural resources), or artificial (produced completely by laboratory production and management). The key groups are opiates, other central nervous system (CNS) depressants (sedative-hypnotics), CNS stimulants, hallucinogens, and Cannabinoids (Daughton, 2011). Number of drug abusers that becomes sick or those bringing to the hospital having unusual drug problems, illicit drug adulteration brought the interest of health or drug services (Cole *et al.,* 2010). The identification of adulterants and information of their unfavorable effects, an early warning system helps in enhancement of understanding of, public health reactions to illicit drug adulteration (Cole *et al.,* 2010). Benign substances i.e. sugars are the substances that will boost or imitate the effects of these drugs i.e. procaine in cocaine, or also are the substances that will assist in taking of illicit drugs such as caffeine in heroin, are normally used as adulterants in illicit drugs but these adulterants are characteristically altered with time. The reasons of these alterations are the accessibility of other materials, in addition to other substances that act as enhancers and also because of customer liking for a specific mixture of active component and adulterants. Because of the unclean or unsterile process of making and production of these drugs, their unsatisfactory wrapping and unsuitable storage, by-products of the process, bacteria or other biological agents can also be added as adulterants in these drugs (Cole *et al.,* 2010).

Illicit drugs are seldom employed and sold in market in their pure form (Lindholst *et al.,* 2008). In order to give an additional convenient amount these drugs are often combined with other substances (King, 1997). With the aim of making the drug to appear as that there is a large

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quantity of drug than is really present and also to increase the dealer's profits, the drugs e.g. diacetylmorphine (Heroin), amphetamine and cocaine are frequently combined or cut with a different types of materials, adulterants and/or diluents (Kaa and Kaempe, 1986;Kaa, 1994; Fucci and Giovanni, 1998). The increasing problem of drug abuse is a point of attention to government authorities and also to society (López-Artíguez *et al.,* 1995).

Detection of these hazardous adulterants is significant because they appear more poisonous than the drug itself i.e. cocaine contaminated with atropine or phenytoins (Katz *et al.,* 1993) are examples of precarious combinations sold on the European drug marketplace. Comprehensive information of these drugs provides information about their supply ways (Slooten *et al.,* 1975). Dissolution of these drugs in commercially obtainable acids, such as citric and tartaric acid increases the risk of tissue breakdown at the inoculation place and in this way they fasten bacterial expansion (Dancer *et al.,* 2002). The presence of adulterants in recreational heroin, amphetamine, and cocaine specimens has reduced considerably but this indicates the occurrence, diversity and amount of adulterants and diluents that have altered over time (Andreasen *et al.,* 2009).

Bacterial diseases are a general threat related with illegal drug employment and amongst inoculating drug abusers; these can produce diseases related to bacteria, fungus and virus specifically (Brazier *et al.,* 2002; McL auchlin *et al.,* 2002; Brett *et al.,* 2005; Cole *et al.,* 2010). Among the drugs addict's, intradermal insertion is most general (Graham *et al.,* 1999) that produces unfavourable health consequences due to microbial residence (Dancer *et al.,* 2002). Illicit drugs insertion produced several microbial diseases (Horsburgh *et al.,* 1989; Brettle, 1992; Peat *et al.,* 2000). Amongst illicit drug users (IDUs) through 2000, in the duration of the researches, *B. cereus, C. botulinum* and *C. Perfringens* were isolated from both heroin or drug inoculation tools (Dancer *et al.,* 2002).

Heroin chemical composition differs with diacetylmorphine ratio, which is in the form of salt or free base and also differs with the quantities and uniqueness of diluents and adulterants that have been added. Different studies suggest that brown heroin is made up of about 35-45% of diacetylmorphine hydrochloride content (Kaa and Bent, 1986; Fuente *et al.*, 1996) while white heroin is more pure having about 85-95% of diacetylmorphine hydrochloride ratio (Huizer, 1992; Fuente *et al.*, 1996). Pure heroin accessibility is increasing so it is adulterated with more drugs (e.g. paracetamol, quinine, strychnine or other poisons) or other essences such as sugars (glucose, lactose, sucrose and mannitol) and yet starch or powdered milk (Kaa and Bent, 1986; Fuente *et al.*, 1996; Risser *et al.*, 2000). The main aim of our study was to analyse qualitatively and quantitatively various illicit drugs using various chromatographic techniques and to analyse them for the presence of certain microbes collected from various regions.

MATERIAL AND METHODS

Bio burden (detection of microorganisms)

Media and chemicals

Vogel-Johnson agar (Oxoid), Sabouraud dextrose agar (Oxoid), Tryptone soya agar (Oxoid), Mac-Conkey agar (Oxoid), Distilled water, Normal saline

Collection of drug samples

One hundred (100) different illicit drugs samples were collected from various regions of Pakistan and transported to the NIH laboratory through ANF, Pakistan

Bio-burden testing procedure

Preparation of media and isolation of pathogenic microorganisms

Drug samples were subjected to total aerobic viable count (TAVC) by pour plate method and for presence or absence of bacteria and fungi. Mac-Conkey, Sabouraud dextrose, Tryptone soya and Vogel-Johnson agar media were prepared according to the instruction of the manufacturer. One ml of each sample were poured according to the marking of the plates and then poured 10-15ml of the sterilized medium in their respective petri plates. After solidification of the media, these plates were incubated at 35˚C for 72 hrs except Sabouraud dextrose agar. Sabouraud dextrose agar plates were incubated at 20-25˚C for 5-7 days. After the completion of incubation period, total aerobic microorganisms were counted on Tryptone soya agar plate. Growth of *streptococcus* and *staphylococcus* species were observed on Vogel-Johnson agar, gram -ve bacteria growth were observed on Mac-Conkey agar and fungus and moulds on Sabouraud dextrose agar medium. All this procedure was performed in the laminar flow hood.

Qualitative analysis

Qualitative analysis has been done with colour tests and TLC technique by employing Kits provided by United Nations Office on Drugs and Crime (UNODC) Colour test kit.

Thin layer chromatography (TLC)

Preparation of samples and standard solutions

Standard solutions

Standard solutions of heroin, morphine was prepared at a concentration of 1mg/ml in methanol. All standards were combined into a single standard solution. 5µl spot of the standard solution(s) was spotted to the TLC plate.

Sample preparation

Five (05) mg of sample was dissolved for each 1ml of methanol and placed 5µl spot onto the plate.

Commercially available TLC plates, coated with activated silica gel G having thickness of 0.25mm were used. The ‘’spotting line’’ was made 1cm from the bottom of the plate. The samples was spotted about 0.8cm apart from each other. The spot size was ≤ 2mm. The spots were dried by cold or hot air between applications. Clear glass TLC tank and lid was used. The tank was contained with developing solvent. The developing solvent in the TLC tank was 0.3 and 0.5cm in depth. Plates were placed in the tank. For the developing systems, the solvent was renewed after a maximum of 3 runs, or, ideally, after each development. We used ethyl acetate (35ml), methanol (10ml) and ammonia (5ml) as developing solvent. Development line was 14cm long. An analysis was terminated when the solvent migrated to the development line. The plates were removed from the developing tank as soon as the solvent reaches the development line. Plates were dried prior to visualization. Drying was accomplished at room temperature. Ultraviolet (UV) light, usually at 254nm was used. Acidified potassium iodoplatinate spray reagent was used. The data obtained was recorded at each stage, including the colours and retardation factor (*R*f) values of the compounds observed at each stage of the visualization process. By using this approach, it was possible to compared different samples (UNIDCP, 1998).

High-performance liquid chromatography (HPLC) were employed for the investigation of heroin and its metabolites by using in street samples with various analytical methods e.g. HPLC coupled with ultraviolet (UV), fluorescence, or electrochemical detection.

Heroin samples were supplied by anti-narcotics force of Pakistan. Reference standards includes: Pure caffeine, paracetamol, diazepam, dextromethorphen, morphine, cocaine, acetic anhydride and ephedrine were purchased from PDH laboratories (Pvt) limited, Lahore, Pakistan. HPLC-grade methanol was used, distilled water, produced by NIH laboratory. Dioctyl Sulfosuccinate sodium salt and sodium acetate were obtained from PDH laboratories (Pvt) limited, Lahore, Pakistan. All other types of materials and reagents were of analytical grade and provided from commercial sources.

The method development was performed with the Shimadzu HPLC system (Japan) consisting of LC-9A, LC-20AT pump coupled with UV-Vis detector SPD-6AV at 285 nm, with system controller SCL-6B. The data and peak areas were processed with chromatopac C-R4A.The analytical column was octadecylsulphonate (ODS) CTO-6A (stationary phase) with particle size of 5µm maintained at room temperature 35-37˚C. The samples and standards were injected with an HPLC syringe having capacity of 20µl sample loop. All types of the calculations related to quantitative analysis were carried out with external standardization by the computing the peak areas.

Preparation of samples and standard solutions

Heroin samples were taken accurately by weighing 20 mg in volumetric flask and dissolved in 5 ml mobile phase. After sonication for 2 minutes, 1ml from solution was taken and again dissolved in 10ml mobile phase. Dilution of 400µl was prepared. Morphine samples were taken accurately by weighting 10mg in volumetric flask and dissolved in 10ml of mobile phase. After sonication for 2 minutes, 1ml from solution was taken and again dissolved in 10ml mobile phase. Dilution of 400µl was prepared.

Operating conditions

Detector: UV at 285nm, Temperature: 35-37˚C, Run time: 40min, Flow rate: 1ml/min, Injection volume: 20µl. Mobile phase: 0.01M Dioctyl Sulfosuccinate sodium salt in methanol (60%) i.e. 1.33g in 300ml of methanol and 0.005M Sodium acetate in distilled water (40%) i.e. 0.136g in 200ml of distilled water.

Cocaine

Samples were taken accurately by weighting 10mg in volumetric flask and dissolved in 10ml of mobile phase. After sonication for 2 minutes, 1ml from solution was taken and again dissolved in 10ml mobile phase. Dilution of 400 µl was prepared.

Acetic anhydride

2g of acetic anhydride was accurately weighed in 50ml of N/1 sodium hydroxide (NaOH) in a stopper flask and allowed to stand for 1 h. Excess of alkali was titrated with N/1 hydrochloric acid (HCl) using phenolphthalein solution as indicator (BP, 1968).

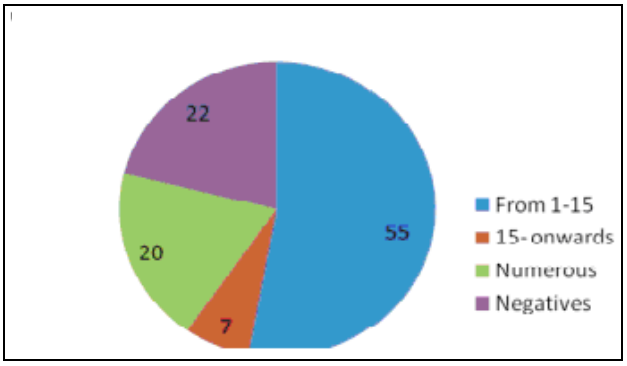

Fig. 1(a): Figure showing total aerobic count of microbes

Microbial analysis

Bio-burden

The first part of our research was the microbial analysis of heroin samples. This was further divided into two parts i.e. estimation of total aerobic count on Trypton soya agar and estimation of microbial growth on different media. In the first case the samples having total aerobic count was done. Those samples having range of 1-15 colonies of microbes were 55 in numbers, those having more than 15 colonies were 7 in numbers. In this analysis some samples have numerous growth i.e. uncountable growth were 20 in

numbers and those having no growth i.e. negatives were 22 in numbers as shown in fig. 1(a).

Microbial growth

The second portion of the first part was the estimation of microbial growth on their respective media. Gram-negatives bacteria show their growth on MacConkey agar, fungus species grow on Sabouraud dextrose agar while *Streptococcus* and *Staphylococcus* species showed their growth on Vogel-Johnson agar. These results indicate that heroin samples contain several microbes that arrive during the drug preparations in laboratories. Unclean and unhygienic handling of drug also assists in their growth. The samples of gram-negative bacteria having single colony were 17 in numbers, those having double colonies were 14 in numbers, numerous growth were 7 in numbers while negative growths were 60 in numbers. This is shown in fig. 1(b). For fungus species the samples having single colony were 12, those with double colonies were 10, numerous growth were 18 and negative growth were 57 in numbers. This is clear in fig. 1(c). For *Streptococcus* and *Staphylococcus* species samples with single colony were 12, with double colonies were 5, with numerous growth were 35 and with negative growth were 48 in numbers as indicated in fig. 1(d).

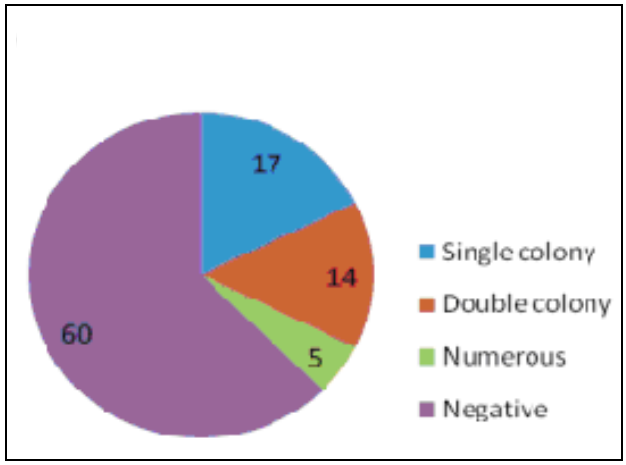

Fig. 1(b): Fig. showing gram -ve bacteria colonies

Chemical analysis

Colour test

The second part of the research was the chemical analysis of drug samples. The first portion of this part was the colour test. Different colour tests were performed for different drugs, which were confiscated by the anti narcotic force of Pakistan. The colour tests were performed by using UNODC drug kit, containing specific reagents for each drug samples.

Thin layer chromatography (TLC)

The second portion of chemical analysis was the thin-layer chromatography. TLC for heroin and morphine samples was done. In three different solvent systems each gives its respective Rf values. The data is shown in table 1.

High-performance liquid chromatography (HPLC)

The last portion of chemical analysis was the quantitative analysis of heroin, morphine, cocaine and acetic anhydride samples. HPLC gives quantitative values of drugs along with their adulterants.

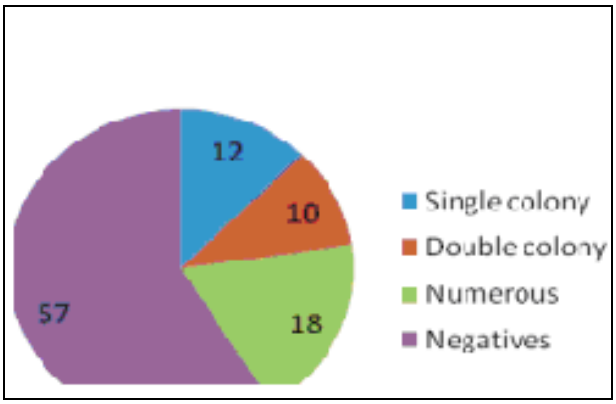

Fig. 1(c): Fig. Showing fungus species colonies

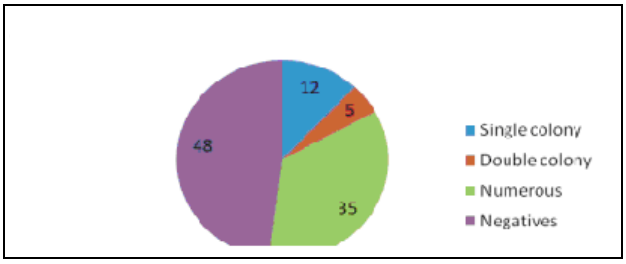

Fig. 1(d): Figure showing ratio of *Staphylococcus* and *Streptococcus* species

Percentage of heroine samples

According to our results heroin samples which were adulterated with paracetamol was 20, with diazepam were 10, with dextromethorphen were 35 and those which cannot be quantified were 34 samples as shown in figs. 2a, 2b and 2c.

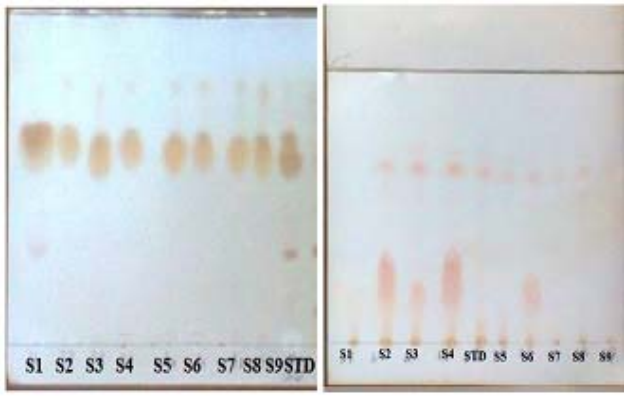

Fig. 2(a): TLC of Heroin and Morphine samples

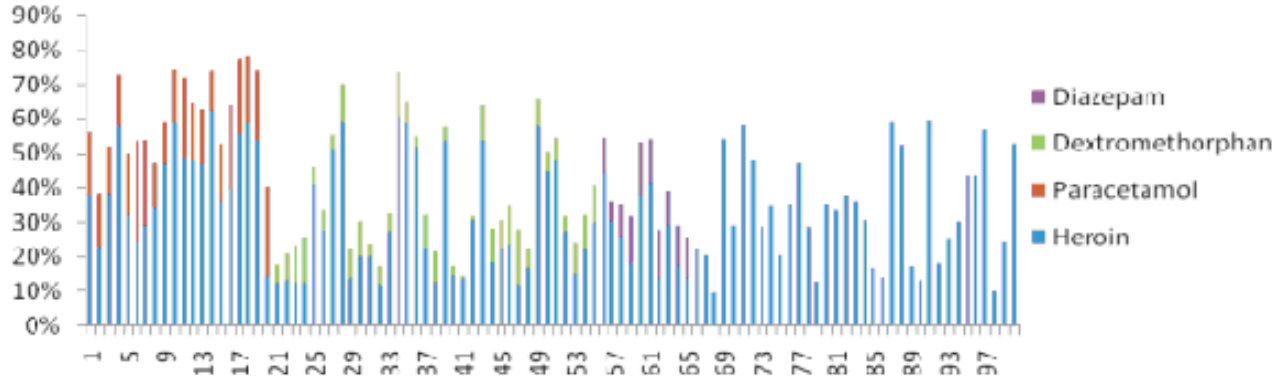

Fig. 2(b): Figure shows adulterated heroin samples

Percentage of morphine samples

Morphine was also subjected to HPLC for quantitative analysis. Among morphine samples those having concentration between 1-5% were 49, from 6-10% were 99 and more than 10% were 39 in numbers as shown in fig. 3a, 3b.

Percentage of cocaine samples

10 cocaine samples were subjected to HPLC for quantitative analysis. Those having concentration up to 10 % were 3samples and those having concentration beyond the 10 % were 7 in numbers as shown in fig. 4a and 4b.

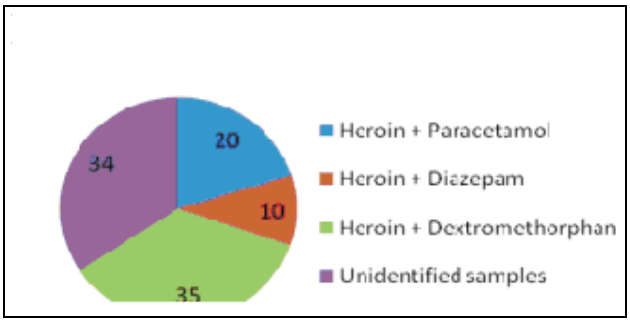

Fig. 2(c): Figure showing ratio of adulterants in heroin and unidentified samples

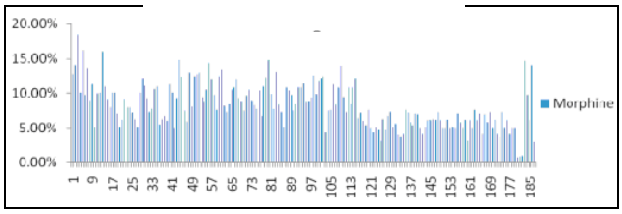

Fig. 3(a): Figure showing concentration of Morphine samples

Identification and quantification of acetic anhydride

20 acetic anhydride samples were subjected to HPLC for quantification. According to fig. 5a and 5b, those samples having concentration up to 10% were 4, up to 20 % were 5, up to 30% were 7, up to 40% were 2 and up to 50% were also 2 in numbers.

Samples of acetic anhydride adulterated with HCl

30 samples of acetic anhydride adulterated with HCl were quantified by HPLC. According to fig. 6a and 6b, there were no sample of acetic anhydride having concentration up to 10%, up to 20% were 11 and up to 30% were 19 in numbers. Acetic anhydride samples adulterated with HCl ranges from 30-35%. From 30-31, there were 10, from 32-33 were 16 and from 34-35 were 4 samples.

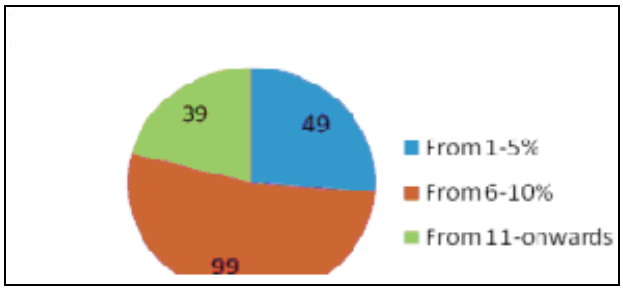

Fig. 3(b): Figure showing concentration of morphine samples

Drug samples from different cities of Pakistan were confiscated by anti-narcotics force of Pakistan. The ratio of these drugs obtained from different cities is given in the fig. 7 and table 2.

MacConkey agar is selective for gram -ve bacteria and is a differential medium that differentiate between lactose fermenters and non-lactose fermenters for gram -ve bacteria. In lactose fermenters it gives yellow colonies (*E. coli*) while in case of lactose non-fermenters it gives pink colonies (*Salmonella typhi*).

Thin-layer chromatography

Table 1: Thin-layer chromatography of heroin and morphine samples

Rf values			
Solvent system	A	B	C
Heroin	57	49	47
Morphine	19	20	37

DISCUSSION

The current study aimed to examine the different drug samples that were used by the youngsters in the whole world and also in Pakistan. The strength of this study was the microbial analysis and chemical analysis of drug samples. These samples were obtained from different districts of the country. They were from Quetta, Karachi, Lahore and capital of the country. These drugs were confiscated by the anti-narcotics force of Pakistan. Different number of drug samples obtained from these cities indicated drug prevalence in particular city. According to our samples Quetta was leading in heroin and morphine samples, which were then followed by Karachi.

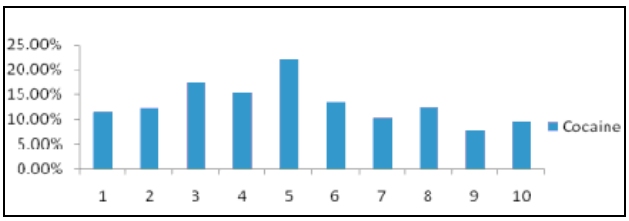

Fig. 4(a): Figure showing cocaine concentration

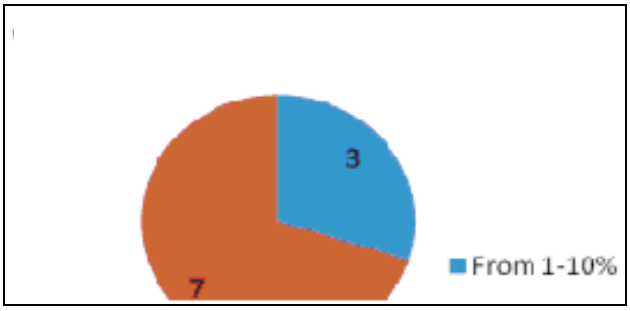

Fig. 4(b): Figure showing cocaine concentration

The main findings of our research were divided into two parts, one was the estimation of microbial analysis or bio-burden of heroin samples and the other part was related to the chemical analysis. For microbial analysis we took 1g of heroin samples and allow them on petri plates having different culture media to estimate the microbial growth. For total aerobic count we used Trypton soya agar. For the detection of gram -ve bacteria we used MacConkey agar, for fungus species Sabouraud dextrose agar and for *Streptococcus* and *Staphylococcus* species Vogel-Johnson agar was employed. The heroin samples used for microbial analysis were randomly chosen.

The second part was the chemical analysis of drug samples. For this part we have done different colour tests, TLC (Thin layer chromatography) and HPLC (high-performance liquid chromatography) for quantification. The colour tests were performed by using the UNODC drug kit. This kit contain reagent specific for each drug. It gives particular colour change, specific for each drug, indicated the particular drug. For example purple colour indicate heroin while light purple to grey colour was the indication of the morphine. TLC technique was performed for heroin and morphine samples. We used three solvent systems, each drug give its own Rf value in each solvent system. The last task was the quantification of drug samples using HPLC. HPLC quantified different drugs along with their adulterants and gives their respective values.

According to results of our study it is suggested that as heroin availability is increasing day by day, it has been adulterated with different substances. These substances produce certain sever effects on the body along with the psychological effects produced by heroin itself. In our study heroin samples obtained from different cities of a country were subjected to chemical analysis. Mostly these samples were adulterated with diazepam, dextromethorphan and paracetamol while in rest of the samples other adulterants were present but cannot be identified (shown in the table 4.4). These heroin samples were also quantified along with its adulterants. Similarly samples of morphine and cocaine were also subjected for quantitative analysis. Acetic anhydride samples were adulterated with HCl and were also analysed quantitatively.

Heroin, cannabis, cocaine and the amphetamines are commonly employed illicit drugs. Among illicit drugs commonly employed adulterants are lidocaine, procaine, caffeine and piracetam and normally employed diluents are carbohydrates such as glucose, lactose, sucrose, starch and polyalcohol's such as mannitol (López-Artíguez *et al.,* 1995). Despite significant declines in cultivation trends, the area known as the golden triangles remains a source of opiates. Opiate use has generally stabilized in the region of late. However, since 2009, there has been an indicator to suggest that heroin use is re-emerging as a threat in the region (UNODC, 2011).

Large amount of heroin intake produces hypoxia, which consecutively produces placental vasoconstriction; hence in this way it causes damage to the foetus. Intravenous inoculations of heroin and other drugs produces several problems which includes infected emboli, external body embolization, endocarditic, endocarditic-related septic pulmonary emboli, valvular deficiency skin and soft tissue diseases e.g. abscesses, cellulites, supportive thrombophlebitis, necrotizing fasciitis, wound botulism, sepsis, osteomyelitis, subdural abscess, cerebrovascular accident, mycotic aneurysm, AIDS, hepatitis, fungal infections and tuberculosis (Purushottam *et al.,* 2013).

In comparison to these countries in Pakistan opiate utilisation is superior than the universal approximate while the confiscation of opium and morphine sustained to be mainly concentrated. In Pakistan information of single heroin abduction incidences propose that of the heroin delivers with a known target other than Pakistan. Their proportion planned for the Asia-Pacific region

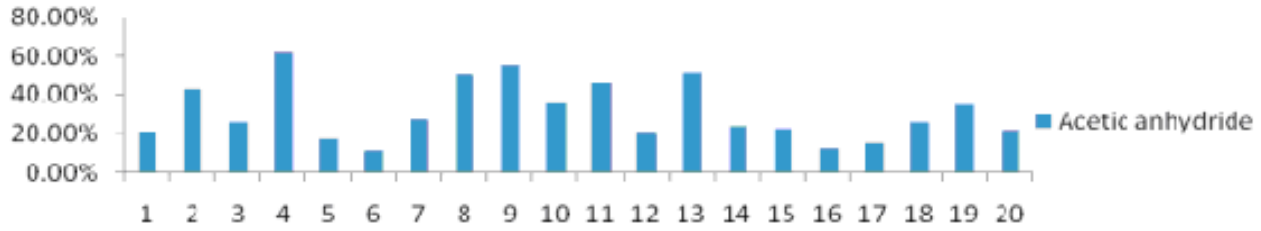

Fig. 5(a): Figure showing acetic anhydride concentration

Table 2: City wise distribution of drugs

Number of samples of drugs obtained				
Cities	Heroin	Morphine	Cocaine	Acetic anhydride
Quetta	40	87	5	19
Karachi	30	50	3	01
Islamabad	10	20	0	30
Lahore	20	30	2	0

lowered from 42% in 2009 to 34% in 2010. In Pakistan, from 2008 confiscations of cannabis resin amplified sharply, with 212 tons being seized in 2010, almost twice the 2007 level (UNODC, 2012).

In present study, qualitative and quantitative analysis of heroin samples is done in order to establish percentage of Diacetyl Morphine (Heroin) and to assess the various types of microbes present in the drug. It will help ensure timely medical treatment of the addicts and will also provide platform for enhance public health and awareness of the risks because of its association with the potential health effects that may arise from adulteration.

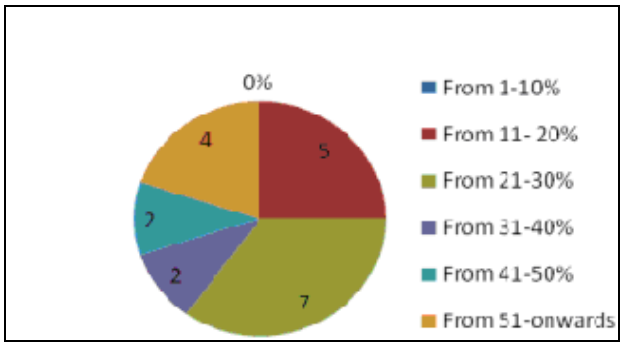

Fig. 5(b): Figure showing acetic anhydride concentration

Our results show that the microbial flora has also been present in the samples. The 100 different samples were selected randomly for microbial analysis. Mostly they contain gram -ve, *Staphylococcus*, *Streptococcus* and fungal species. These were the result of unclean and unhealthy process of manufacturing in the laboratories. Using TSA the total aerobic count of microbes having range of 1-15 colonies of microbes were 55 in numbers, those having more than 15 colonies were 7 in numbers, numerous growth i.e. uncountable growth were 20 in numbers and those having no growth i.e. negatives were 22 in numbers as shown in fig. 1a. Samples numbers used for the estimation of microbial growth on different agar media were 100 that were selected randomly. Those samples having single colony of the microbes were 17, with double colony were 14, and with numerous growth were 5 and those having no growth were 60 in numbers. These results can also be compared with the study conducted by McLauchin in 2002 on micro flora of heroin. According to him different microbes i.e. *Bacillus* sp, *Clostridium* sp, *Streptococcus* sp, *Staphylococcus* spetc were present in heroin samples (McLauchin *et al.*, 2002). *Bacillus* and *Clostridium* species are the main frequently recognized bacterial contaminants (Cole *et al.*, 2011). Disorder of "toxic, spongiform leukoencephalopathy" (SLE) is uncommon but tremendously serious and mostly lethal neurological problem of heroin smoking (Büttner *et al.*, 2000; Brenneisen and Hasler, 2002). Its first incidence was accounted in 1982 from the Netherlands having about 25% of death rate. In fact, street heroin, its adulterant and by-products all robustly enhances the pyrolysis rate (Zelkowicz *et al.*, 2005).

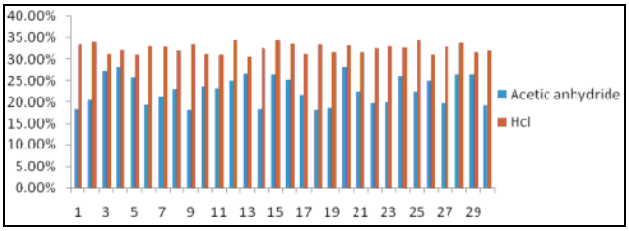

Fig. 6(a): Figure showing acetic anhydride and HCl concentration.

In our results heroin samples adulterated with paracetamol were 20, with diazepam were 10, with Dextromethorphen were 35 and those remain unidentified

were 34 samples (shown in heroin pie chart). Similarly morphine samples those having concentration between 1-5% were 49, from 6-10% were 99 and more than 10% were 39 in numbers (shown in morphine pie chart). In the same way among 10 cocaine samples those having concentration up to 10% were 3 samples and those having concentration beyond the 10% were 7 in numbers (shown in cocaine pie chart). In acetic anhydride samples those samples having concentration up to 10% were 4, up to 20 % were 5, up to 30% were 7, up to 40% were 2 and up to 50% were also 2 in numbers (shown in acetic anhydride pie chart). Acetic anhydride samples adulterated with HCl there were 10 samples having concentration among 30-31, from 32-33 were 16 and from 34-35 were 4 samples. Adulterants are chief materials that are likely accessible and among them most frequently are being caffeine, procaine, paracetamol, sugars (Cole *et al.,* 2011), sucrose, lactose, dextrose, mannitol, caffeine etc (Cole *et al.,* 2010). At minimum dose, they possess least influence on abusers' physical condition. In abusers, that inoculate the drugs, other adulterants possess strong health problems but the amount accounted like strychnine in heroin, are not critical (Cole *et al.,* 2011). The causes for adulteration are complicated and are not easy enough to understand because of following reasons like enhancement of drug effects, alterations in customer styles, novel drug making methods or local/ geographical restrictions (Cunningham *et al.,* 2010). Addition of pharmacologically active compounds alters the drug impact in an unexpectable way (Schneider and Franc, 2011).

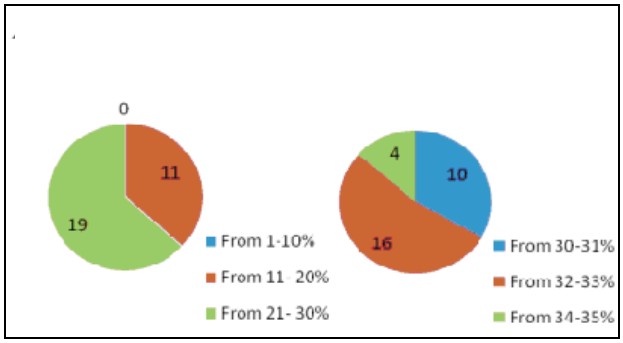

Fig. 6(b): Figures showing acetic anhydride and HCl concentration

The same type of study was also conducted by Zelkowicz and co-workers in 2005 as analysis of simulated heroin by HPLC and also by Khajeamiri *et al* in 2011 as determination of impurities in illicit methamphetamine samples seized in Iran (Zelkowicz *et al.,* 2005; Khajeamiri *et al* in 2011). According to them different adulterants were present in heroin samples like paracetamol, papaverine, acetyl-codeine, caffeine, narcotineetc (Zelkowicz *et al.,* 2005). In order to confirm the presence of morphine, codeine and 6-MAM in the samples, Dordević and Kilibarda use the multicolumn HPLC-UV method (Dordević and Kilibarda, 2007). Column-switching high-performance liquid chromatographic technique was employed by Zhang and his co-workers for the recognition of morphine and O6-monoacetylmorphine (Zhang *et al.,* 2002). Milovanović and his co-workers carry out the HPLC/MS process for usual examination and checking of heroin misuse in order to detect morphine, codeine and 6-mam (Milovanović *et al.,* 2012). In 1998 for efficient detection of 10 drugs of misuse like morphine, codeine, 6-monoacetylmorphine, heroin, levorphanol, pethidine, ethylmorphine, anadol, pentazocine and ethamivan, high performance liquid chromatography method was illustrated by the Ma *et al* (Ma *et al* in 1998). In this technique codeine was employed as interal standard (Ma *et al.,* 1998). For the accurate separation and quantification of caffeine, heroin, acetyl codeine, 6-acetylmorphine, codeine and morphine from one other, Baker and Gough employed high performance liquid chromatography method (Baker and Gough, 1981). The limitation of our study is that the samples were collected at various interval of time, which may gives varied results. A study demonstrated that the heroin have been 'cut to 6 to 7 times before reaching to the dealer' (Richter and Rosenberg, 1968) along with its adulterants. UK, USA, Canada and Australia have done the similar kind of less injurious contamination actions like sugars or caffeine (Coomber, 1997c, 1997d; Coomber and Maher, 2006). From adulterations the drug trader produces the main beneficial trade (Strang and King, 1996; Coomber, 1997c; Coomber and Maher, 2006) and these adulterants have been prepared in the hidden laboratories (Behrman, 2008). Illicit drugs contamination possesses strong substantial effect by the complex connections of deliver, order, and control of these drugs (Cole *et al.,* 2011).

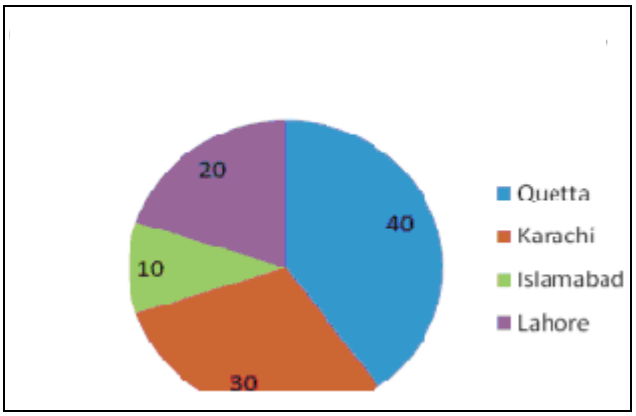

Fig. 7: Figure showing city wise distribution of drug samples.

CONCLUSION

Our findings concluded that the easily assessable illicit drugs are not pure or have been found to be adulterated with several substances i.e. paracetamol, diazepam, dextromethorphen, cocaine. In addition to these adulterants these drugs are also loaded with microbes.

Unclean and unhygienic conditions present in the laboratory during production of the drugs are the reasons for their entrance. So these drugs along with their psychoactive effects also produce severe health consequences due to presence of these microbes. It is further concluded that here is lack of information providing structured advice on responses to the consequences of illicit drug adulteration. Robust and rehearsed interventions and communication strategies would provide a basis for response for a wide variety of organisations. Research into the usefulness of media warnings about adulteration of illicit drugs is required.

REFERENCES

Andreasen MF, Lindholst C and Kaa E (2009). Adulterants and diluents in Heroin, Amphetamine and Cocaine Found on the Illicit Drug Market in Aarhus, Denmark. *The Open Forensic Science Journal,* **2**: 16-20.

Baker PB and Gough TA (1981). The separation and quantitation of the narcotic components of illicit heroin using reversed-phase high performance liquid chromatography. *J. Chromatogr. Sci.,* **19**(10): 483-489.

Behrman AD (2008). Luck of the draw: Common adulterants found in illicit drugs. *Journal of Emergency Nursing,* **34**(1): 80-82.

Brazier JS, Duerden BI, Hall V, Salmon JE, Hood J, Brett MM, McLauchlin J and George RC (2002). Isolation and identification of Clostridium spp. from infections associated with the injection of drugs: Experiences of a microbiological investigation team. *Journal of Medical Microbiology,* **51**: 985-989.

Brenneisen R and Hasler F (2002). GC/MS Determination of Pyrolysis Products from Diacetylmorphine and Adulterants of Street Heroin Samples. *J. Forensic Sci.,* **47**(4): pp.885-888.

Brett MM, Hood J, Brazier JS, Duerden BI and Hahne SJM (2005). Soft tissue infections caused by spore forming bacteria in injecting drug users in the United Kingdom. *Epidemiology & Infection,* **133**(4): 575-582.

Brettle RP (1992). Infection and injection drug use. *J. Infect,* **25**: 121-131.

British pharmacopoeia (BP) (1968). By general medical council, London. pp.247-513.

Büttner A, Mall G, Penning R and Weis S (2000). The neuropathology of heroin abuse. *Forens. Sci. Int.,* **113**(1-3): 435-442.

Cole C, Jones L, McVeigh J, Kicman A, Syed Q and Bellis M (2010). A guide to adulterants, bulking agents and other contaminants found in illicit drugs. Liverpool: Liverpool John Moores University. Available at www.cph.org.uk/showPublication.aspx?pubid=632

Cole C, Jones L, McVeigh J, Kicman A, Syed Q and Bellis M (2011). Adulterants in illicit drugs: a review of empirical evidence. *Drug Test Anal.,* **3**(2): 89-96.

Coomber R (1997c). The adulteration of drugs: What dealers do to illicit drugs and what they think is done to them. *Addiction Research & Theory,* **5**(4): 297-306.

Coomber R (1997d). Dangerous drug adulteration An international survey of drug dealers using the internet and the World Wide Web (WWW). *International Journal of Drug Policy,* **8**(2): 18-28.

Coomber R and Maher L (2006). Street-level drug market activity in Sydney's primary heroin markets: Organization, adulteration practices, pricing, marketing and violence. *Journal of Drug Issues,* **36**(3): 719-754.

Cunningham J, Maxwell J, Campollo O, Cunningham K, Liu L and Lin H (2010). Proximity to the US-Mexico border: A key to explaining geographic variation in US methamphetamine, cocaine and heroin purity. *Addiction.,* **105**: 1785-1798.

Dancer SJ, McNair D, Finn P and Kolstot AB (2002). *Bacillus cereus* cellulitis from contaminated heroin. *J. Med Microbiol.,* **51**: 278-281.

Daughton CG (2011). Illicit Drugs and the Environment. Occurrence, Analysis and Fate Using Mass Spectrometry. Edited by Sara Castiglioni, Ettore Zuccato and Roberto Fanelli. DOI: 10.1002/9781118000816.ch1.

Dordević S and Kilibarda V (2007). Analytical confirmation of lethal heroin overdose by the use of liquid chromatography methods. *Vojnosanit Pregl.,* **64**(11): 739-743.

Fucci N and Giovanni N (1998). Adulterants encountered in the illicit cocaine market. *Forensic Sci. Int.,* **95**: 247-252.

Fuente De la L, Saavedra P, Barrio G, Royuela L and Vicente J (1996). Temporal and geographic variations in the characteristics of heroin seized in Spain and their relation with the route of administration. Spanish Group for the Study of the Purity of Seized Drugs. *Drug Alcohol Depend.,* **40**: 185-194.

Graham CA, McNaughton GW and Crawford R (1999). 'Popping': A cause of soft tissue sepsis in chronic drug abusers. *Eur. J. Emerg. Med.,* **6**: 259-261.

Horsburgh CR, Anderson JR and Boyko EJ (1989). Increased incidence of infections in intravenous drug users. *Infect Control Hosp. Epidemiol.,* **10**: 211-215.

Huizer H (1992). Samenstelling en kwaliteit van illegale heroine in Nederland, 1970-1990. *T. Alc. Drugs,* **18**: 1-12.

Kaa E and Kempe B (1986). Impurities, adulterants and diluents of illicit heroin in Denmark (Jutland and Funen). *Forensic Sci. Int.,* **31**: 195-210.

Kaa E. (1994). Impurities, adulterants and diluents of illicit heroin. Changes during a 12-year period. *Forensic Sci. Int.,* **64**: 171-179.

Katz AA, Hoffman RS, Silverman RA (1993). Phenytoin toxicity from smoking crack cocaine adulterated with phenytoin. *Ann. Emerg. Med.,* **22**: 1485-1487.

Khajeamiri AR, Faizi M, Sohani F, Baheri T and Kobarfard F (2012). Determination of impurities in

illicit methamphetamine samples seized in Iran. *Forensic Science International,* **217**(1-3): 204-206.

King LA (1997). Drug content of powders and other illicit preparations in the UK. *Forensic Sci. Int.,* **85**: 135-147.

Lindholst C, Andreasen MF and Kaa E (2008). Det illegale stofmarked i Aarhus (The illicit drug market in Aarhus); Aarhus University Press: Aarhus, Denmark., **170**: 54-58.

López-Artíguez M, Cameán A and R epetto M (1995). "Unequivocal identification of several common adulterants and diluents in street samples of cocaine by infrared spectroscopy. *Journal of Forensic Sciences, JFSCA.,* **40**(4): 602-610.

Ma C, Duan H, Zhang H, Xu Y and Zhou T (1998). Studies on analytical method for 10 drugs of abuse in urine using HPLC. *Yao. Xue. Xue. Bao.,* **33**(10): 764-767.

McLauchlin J, Mithani V, Bolton FJ, Nichols GL, Bellis MA, Syed Q, Thomson RPM and Ashton JR (2002). An investigation into the micro flora of heroin. *J. Med. Microbiol.,* **51**: 1001-1008.

Milovanović V, Cirić B, Milenković J, Kilibarda V, Curcić M, Vucinić S and Antonijević B (2012). Determination of morphine, codeine and 6-monoacetylmorphine in saliva of substance-abuse patient's using HPLC/MS methods. *Vojnosanit. Pregl.,* **69**(2): 141-146.

Peat M, Budd J, Burns SM and Robertson R (2000). Audit of blood borne virus infections in injecting drug users in general practice. *Commun. Dis. Public Health,* **3**: 244-246.

Purushottam, Bhaskar, and Vincent M. Figueredo. (2013). "Sudden Cardiac Death and Addictive Chemical Substances." *Electrical Diseases of the Heart*. Springer London, pp. 441-460.

Richter RW and Rosenberg RN (1968). Transverse myelitis associated with heroin addiction. *Journal of the American Medical Association,* **206**(6): 1255-1257.

Risser D, Uhl A, Oberndorfer F, Hçnigschnabl S, Stichenwirth M, Hirz R and Sebald D (2007). Is there a relationship between street heroin purity and drug-related emergencies and/or drug-related deaths? An analysis from Vienna, Austria. *J. Forensic Sci.,* **52**(5): pp1171-1176.

Schneider S and Meys F (2011). Analysis of illicit cocaine and heroin samples seized in Luxembourg from 2005-2010. *Forensic Science International,* **30**: doi:10.1016/j.forsciint.2011.06.027. [Consultado 7 Ago 2011] Disponible en: http://www.sciencedirect.com/science/article/pii/S0379073811003124

Slooten VEP, Helm VHJ (1975). Forensic Sci**.,6:** 83-88.

Strang J and King L (1996). Heroin is more than just diamorphine. *Addiction Research,* **5**(1): 3-7.

United National International Drug Control Programme (UNIDCP)(1998). Recommended methods for testing opium, morphine and heroin manual for use by national drug testing laboratories. UN, New York. Pp 28-42.

United Nations Office on Drugs and Crime (UNODC)(2011). World drug report.

United Nations Office on Drugs and Crime (UNODC). (2010). World Drug Report 2010 conducted by UNODC. 31-233

Zhang YR, Wang R and Zhang CG (2002). Column-switching high Performance Liquid chromatographic method for the determination of morphine and O6-monoacetylmorphine in urine. *Fa. Yi. Xue. Za. Zhi.,* **18**(2): 89-91.