Introduction

The skeletal muscle relaxants are a heterogeneous group of medications that are not chemically related. Because of this, there may be important difference in efficacy safety, functional ability and adverse events assessment. Again, there is very limited published evidence regarding their clinical effectiveness **(Chou *et al*., 2004).**

In this study, the chosen drug is tizanidine hydrochloride (sirdalud or zanaflex) which represent the centrally acting skeletal muscle relaxant (acting mainly at the level of spinal cord) **(Martindal, 1998).** Tizanidine is commonly used in chronic spasticity condition, migraine, tension-type, headache and musculoskeletal pain **(Wagstaff and Bryson, 1997, Lake and Saper, 2002 and Freitag, 2003)**. Tizanidine has a sedation activity as it acts as CNS depress **(Miettinen *et al*., 1996 and Takenaka *et al*., 1996)** also it has a visual hallucinations **(Wallace, 1994)**.

The chemical structure of tizanidine **[**5 choloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiodiazole] **(Kotzung, 2001)** is related to other α2-adrenoceptor agonists such as the antihypertensive drugs clonidine and moxonidine **(Koch *et al*., 1989).**

According to **Lawson (1998)** the mechanism of action of tizanidine is the reduction of :-

1) The release of excitatory amino transmitter activity.
2) Post-synaptic excitatory transmitter activity.
3) Synaptic transmission of nociceptive stimuli in the spinal pathways.

Thus, it is likely that tizanidine corrects more than one pathophysiologic abnormality by reinforcing pre-synaptic inhibition as well as post synaptic (reciprocal and non reciprocal) inhibition. Also, tizanidine seems to have little effect on mono-synaptic activity.

Granfers *et al*. (2003) reported that tizanidine reduces spasticity presumably by increasing presynaptic inhibition of motor neurons, by stimulating presynaptic α_2-receptors tizanidine inhibits the release of catecholamine (norepinephrine, i.e noradrenaline) and leads to decreased activity of the sympathetic nervous system and hence to the hypotension and bradycardia associated with tizanidine use.

Identification of studied drug, tizanidine hydrochloride (sirdalud) by using different techniques and methods are of importance for toxicological analysis. Therefore, one aim of the present study is to demonstrate a quick and reliable methods of analysis for identification and detection of drug.

Colormetric test methods usually include all the analytical techniques in which the analyte either absorbed electromagnetic radiation in the range of 185-800 nm or can be made to undergo a chemical reaction where a reaction product will absorb light in that range. This includes spot tests and the more sophistical ultraviolet or visible spectrophotometry. Spot tests are simple and this simplicity makes them valuable. Since spot tests usually screen for a class or a broad category of compounds, appositive result is usually considered to be presumptive only **(Steinert and Coffman, 1992)**. Many substances give distinct colors when brought into contact with various chemical reagents **(Clarke, 1986).** In the present study several spot tests are used to demonstrate the

advantage of the method for the detection of studied drug at different doses.

Ultraviolet/visible spectrophotometric methods are commonly used for quantifying drug. The utility of spectrophotometry comes from the fact that each substance has an absorption spectrum determined by its own structure. So it can be used for identification and characterization of the studied drug by evaluating its ultraviolet spectral data.

Toxicological laboratories usually employ a variety of chromatographic techniques and instruments for the identification and quantification of drug in biological samples. The most techniques often utilized are thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). TLC method is employed in the present study for the detection of studied drug. The separated sample components are detected by using the appropriate procedures of identification as ultraviolet illumination, spraying with specific color reagents as well as calculating R_f value for studied drug.

The high performance liquid chromatography (HPLC) has been developed for the analysis of many drugs in postmortem samples and biological fluids. **Chan and Chan (1984)** presented a sensitive and specific procedure for screening acidic and neutral drugs in postmortem blood. The procedure employs high performance liquid chromatography with a reversed phase column and an ultraviolet detector. **Koves and Wells (1992)** used photodiode array/HPLC system for the detection and quantitation of basic drugs in postmortem blood, reported that the chromatographic conditions developed are suitable for the screening of several basic, amphotric and neutral drugs. Again, **Koves (1999)** demonstrated that HPLC method is reliable and convenient for general

drug screening and quantition. **Klys *et al*. (1997)** stated that the analytic method (FPIA, HPLC and GC-MS) have been used for qualitative and quantitative evolution in wide range of xenobiotic concentration in biological matrix. **Elliott and Hale (1999)** described a method for detection and quantifying etrophine using HPLC with UV diode array defection and demonstrates the advantage of the technique for the detection of this drug at low doses. **Giorgi *et al*. (2000)** reported that due to the good sensitivity and specificity of both HPLC and GC-MS methods recommend their use in toxicological analysis in both human and veterinary medicine. Recently, **(Qi *et al*., 2003; Mahadik *et al*., 2003, Kaul *et al*., 2005, Puranik *et al*., 2006 and Sivasubramanian and Devarajan, 2009)** they indicated that HPLC method developed for tizanidine is highly senisitive, accurate, precise, specific, rapid and more stable method.

Hilberg *et al*. (1999) reported that in special cases where the diagnosis of overdose is to be used as judicial evidence, a single sample of blood may prove insufficient. They add that analysis of several samples of blood and tissue will increase the possibility of reaching a correct conclusion. In the present study drug concentration in different tissues (liver, kidney, spleen, femoral muscle, brain and hair) are estimated.

Granfors *et al*. (2003) reported that tizanidine is widely distributed throughout the body.

Apple (1989) examine some cases in which death caused by tricyclic antidepressant overdose, concluded that liver TCA concentrations should be quantitated to specific manner of death. **Casarett and Daull (1996), De Graaf *et al*. (1996), Singh *et al*. (2002)**

and Chou *et al*. (2004) indicated that accumulation of chemicals in liver tissue induce liver failure associated with death in most cases.

According to **Heazlewood *et al*. (1983), Tse *et al*. (1987), Koch *et al*. (1989), Mathias *et al*. (1989) and Emre *et al*. (1994)** the bioavailability of tizanidine has been estimated to average 20% to 40%, as a result of extensive first pass metabolism in the stomach when it is administrated orally and its half-life is 4.16 h. the major excretory route of tizanidine is being via the kidney. **Casarett and Doull, (1996)** reported that the specific structure of the kidney favors the accumulation of chemicals in its tissue.

Garriott, (1991) reported that for most common basic drugs, the drug concentration in muscle and blood were often near unity and therefore muscle is proposed as a useful alternative specimen to postmortem blood.

Casarett and Doull, (1996) mentioned that the blood-brain barrier prevents the access of hydrophilic chemicals to the brain except for those that can be actively transport. Since sirdalud is water soluble drug, therefore its transport to brain cells is restricted.

Henderson, (1993) suggested that drugs may be incorporated into hair:-

1. From the blood during formation.
2. From sweat and sebum after formation.
3. From the external environmental after formation and after the hair has emerged from the skin.

Several authors reported that hair pigmentation has some influence on the drug concentration in hair samples, since the measured concentrations drugs are higher in pigmented hair than in non pigmented one **(Bathory *et al*., 1990, Uematoso *et al*., 1990, Uematsu *et al*., 1992 and Wilson *et al*., 1995).** Hair is quite on attractive indicator in forensic since it is easy to obtain, store and resist to autolysis and putrefaction **Traqui *et al*. (1995).**

Although, several investigations have been reported in which the effect of different drugs on some blood metabolites and enzymes is described, there have been little information about the effect of studied drug as such parameters. Therefore, the present study attempted such experiment via the determination of concentrations of some metabolites (glucose, protein, lipid, triglycerides and cholesterol) and enzymes (AST and ALT). besides, the determination of liver protein, glycogen and enzymes (AST and ALT) in rats administrate different doses of the drug.

Liver is the major organ for drug metabolism, but drug metabolizing enzymes are also present at other sites, such as the intestinal wall, kidney, lung and skin **(Krishna and Klotz, 1994) and (Meyer, 1996). Granfors *et al*. (2003)** add that tizanidine is mainly metabolized by CYP_1A_2 in vitro.

The previous authors, indicate that the metabolism of drug usually takes place in two phases, termed functionalisation (phase I) and conjugation (phase II) reactions, respectively. the phase I reaction are mostly oxidative, incorporating a functional molecule such as a hydroxyl (OH^-) group into the drug molecule and other types of phase I reactions are reduction and hydrolysis. CYP enzymes play a major role in phase I (human drug metabolism). Phase II reactions usually conjugate the drug

with endogenous molecules, such as a glucuronide, glutathione and a sulfate group. Phase II enzymes that catalyse conjugation reactions include broad specificity transferases such as glucuronosyltransferases, glutathione-s-transferases and sulfotransferases.

Vearanjaneyulu *et al*. (1995) concluded that the clonidine (imidazoline) analogs produced an increase in blood glucose level in a dose dependent manner and they suggested that hyperglycemia is mediated through stimulation of alpha-adreno-receptor possibly located on pancreatic cells, resulting in possible inhibition of insulin release. However **Satia *et al*. (1995)** stated that centrally acting drugs don't alter blood glucose and urea levels but it decrease significantly triglycerides level in controlled hypertensive and diabetic patients. On the other hand, cholesterol level is elevated significantly in the same conditions. **Cameron *et al*. (1995)** who studied the action of a neuromuscular blocking agent (atracurium) on blood, concluded that in hyperlipidemic patients there is increase in triglycerides concentration. Also, they stated that higher triglycerides concentrations in obese patients are observed when compared with young subjects. Meanwhile, a significant decrease in total cholesterol and low density lipoprotein cholesterol concentrations are noticed.

Serum and liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels are investigated in the present study. **Harper *et al*. (1977), Sykes *et al*. (1980) and Cain *et al*. (1983)** reported that serum level of AST and ALT enzymes are sensitive indicators of liver damage. **Malanga *et al*. (2002), Momo *et al*. (2008) and Henney *et al*. (2008)** indicated that S-AST and S-ALT levels showed insignificantly change subsequent to tizanidine administration. On the other hand **Nance *et al*. (1994), Smith *et al*. (1994), Lukin *et al*. (1995)**

and De-Graaf *et al*. (1996) they found a significant increase in S-AST and S-ALT levels and they attributed this finding to the relationship between muscle relaxant administration and liver dysfunction. However, **Lawson, (1998)** indicated that extensive first-pass hepatic metabolism by microsomal enzymes converts tizanidine to inactive compounds and the physiological role of this action in unknown. **Holtebeck, (2000)** indicated that there was a trend toward increase in liver AST and ALT activity but these changes was not statistically different and resolved an discontinuation of tizanidine medication. Meanwhile, **Toosy and Thompsou, (2000)** reported that tizanidine may cause reversible increase in hepatic transaminases.

In the present study liver protein and glycogen contents are determined. **Goodman, (20003)** interpreted that mobilization of protein for energy necessarily produces some functional deficits. The same author mentioned that liver glycogen is the immediate source of blood glucose under most circumstances, hepatic gluconeogenesis may contributed to blood glucose directly but is more important for replenishing glycogen stores.

In summary, the present work is an attempt to:

1- Employ the different toxicological analytical procedures to reveal which of these methods is more suitable for the identification and characterization of the studies muscle relaxant (sirdalud).

2- Estimate the postmortem drug concentration in some tissues of rats injected intraperitonealy different doses of the drug.

3- Study the effects of the drug administration on some metabolites and enzymes activity in serum and liver of rats.