CHAPTER I
INTRODUCTION
Importance of various plant ingredients and their effects on animal system has been recognized since early days of scientific research. Investigative study on various plant alkaloids is considered inevitable from the scientific standpoint for obvious reasons. It is established that many of the plant extracts have rich value as therapeutics and common food, although possess some undesirable effects at higher doses. Therefore the jovial nature of this kind of highly valued plant alkaloids is inviting great deal of scientific attention to exploit their full potentiality for commercial burst and human welfare.

One of such extensively used and naturally occurring chemical is caffeine. This popular compound is belonging to purine or methylxanthine group that is available as an active principle of some tropical plants mainly tea, coffee and mate. Various workers have forwarded explicit reasons for its popularity as beverages among majority of global masses at different times.

Caffeine is used therapeutically to alliviate neonatal aponea, to control asthalmatic manifestation and to releave bronchial spasms (Stavric 1992). Caffeine is used for stimulation of central nervous system, antidepressant, diuretics and also used as a sex stimulant in a combination of strychnine, caffeine and theophylline. (Walter et al 1976)
Use of caffeine and caffeine containing plant extracts were old day practice. Earlier people used the caffeine containing plant extracts as antidote against poisoning of alkaloids and heavy metals (Chopra 1984). Therapeutic importance of caffeine was also established very earlier. Recently caffeine is used in most of the medicines in combination with other pharmacologically important chemicals.

Toxic nature of this important compound was also observed in earlier periods. Disorders of gastric and intestinal functions, paleness and yellowness of the skin, troubles in the nervous system like headache, hypochondria, weakness of memory, disturbances of the sight and also atrophy of the liver were common pathological disorders observed by the people after caffeine and caffeine containing food and drink consumption (Chopra 1984). Although this pathological disorders were observed by the people but concomitant research in therapeutic value of caffeine suppressed the toxic effect of caffeine and the compound became popular among the people. Besides, the refreshing nature of this chemical compound also gained popularity.

Although, study on the beneficial and harmful effects of caffeine on animal system had been started earlier but recent analysis in the same area added new information in pharmacological and toxicological research. Caffeine administered in small doses could increase B M.R. and release catecholamine. Further, recent studies showed that it
inhibited the immunoresponse to some extent by inhibiting the release of histamine and other mediators from mast cells. (Tripathi 1994)

Caffeine intake mainly comes from tea, coffee, cola drinks and prescription and nonprescription drugs such as over the counter analgesics (Beaver 1984, Gilbert et al. 1976). Undoubtedly, caffeine's stimulatory effect on human mental activities is the reasons for its use in the form of beverages by million of people. Nursing baby also get caffeine from the mother, as caffeine is available in mother's milk of habituated mother (Parsons, Pelletier and Neims 1976; Rya 1985, Tyrala and Dodson 1979). Since caffeine is an ingredient of habituated drink and food, so it is difficult to avoid use of caffeine.

Caffeine has remarkable effect on some physiological processes of the body, generally in the amount normally ingested from different sources. Caffeine is expected to produce a variety of biological effect such as diuretics, cardiac muscle stimulation, central nervous stimulation, smooth muscle relaxation, stimulation of gastric acid secretion and elevation of plasma free fatty acids and glucose. (Graham 1978)

Larger doses of caffeine can produce insomnia, headache and jitters, nervousness and even delirium. Non-users are more susceptible than users as reported by Gilbert (1981).
There are several literature available in connection with the mode of action of caffeine in animal body. Action of small doses of caffeine is found to be mainly on that part of the brain, which is connected with the mental functions. It facilitates the perception of sensory stimulus as well as association of ideas. Further, its overdose produces a condition of weakness, drowsiness and fatigue, but in low dose intake such conditions disappears and mental activity is resumed. Larger doses of caffeine give rise to confusion of thought, association with subjective affection of sense organs such as flashes of light before the eye, and ringing in the ears. Parts of the nervous system, which are affected by caffeine, found to be variable in different group of animals. In human beings, caffeine mainly affects the brain, while in other animals spinal cord is mainly effected (Chopra et al 1984). Caffeine stimulates higher brain centres, respiratory tract, vagal, and vasomotor centers in the medulla (Walter et al 1976). Normal renal function also effected by caffeine and acts as diuretics. It enhances the urinary excretion of sodium and water usually by inhibiting reabsorption of sodium in renal tubules and thus enhancing greater loss of body fluids and acts as diuretics. (Walter et al 1976)

Circulation is effected by caffeine in a complex manner. In the therapeutic doses it increases the absolute strength of the heart. Diastolic relaxation is reduced after toxic doses by central vagus stimulation and quicken after toxic doses by direct stimulation of the musculature on the exitomotor apparatus. These renal vessels are dilated as also the central and coronary vessels (Chopra et al 1984). Further studies on
blood pressure of regular coffee drinkers and decaffeinated coffee drinkers confirmed the influence of caffeine on elevation of blood pressure (Dusseldrop et al 1989)

Transient rise of blood pressure after a few hours of caffeine consumption and level falling and returning to baseline thereafter was found (Ammon et al 1983). A decrease in the cerebral blood flow after caffeine administration was also reported (Labow 1983, Mathew et al 1983), and this also reduced the placental blood supply while the maternal serum epinephrine increased (Kirkinen et al 1983) Caffeine influences on secretion of catecholamine and there was marked increased of catecholamine from the isolated perfused rat adrenal gland (Lim et al 1991) Motor nerve conduction velocity showed a significant decrease in caffeine treated animals. But no change was recorded in the amplitude of extra cellular muscle action potentials (Raya et al 1994).

Caffeine essentially a planner, is an inducers of CYPIA2 in rat liver (Ayalague et al 1995)

Caffeine entered the brain rapidly and seems to reach equilibrium within 5 minutes. The average brain plasma ratio was 0.64 for old and 0.95 for young rats.

Biochemical components of the animal body also recorded alteration in their concentration in blood level due to consumption of caffeine. A correlation between caffeine consumption and rise of blood cholesterol level has been established (Tverdal et al 1990). Epidemiological studies also revealed the association between
coffee consumption and the serum level of cholesterol (Pietinen et al 1990) Blood glucose also changes following caffeine consumption (Fernstrom et al 1981)

Caffeine causes the rise of total cholesterol in serum when added with normal diet. Despite these increases, the ratio of HDL-cholesterol to total cholesterol and LDL-cholesterol remained unchanged (Rakicioglu et al 1998)

Caffeine and theobromine derivatives caused a primary increased in the rate of glycolysis of glycogen to lactate. (Gemmill 1974)

Caffeine enhanced the rate of liver glycogen synthetase activity as reported by Gilboe and Nuttall (1984).

In vitro formation of urea from ammonia in rat liver is inhibited by methylxanthine and this inhibition is overcome by addition of ornithine or glutathione. (Bernheim and Bernheim 1945)

Excretion of uric acid is found to be increased after caffeine consumption as reported by Wardell et al (1925) but elevation of uric acid is still controversial.

Alteration of neurotransmitter metabolism also occurs due to caffeine administration. Acute effect of caffeine on rat brain especially on tryptophan, serotinine and 5 hydroxyindol acetic acid increased (Fernstrom et al 1981)
Caffeine and theophylline inhibit catechol-o-methyl transferase which inactivates the contractile response of a number of adrenergic amines. (Mc. Neil. et al 1973)

One of the interesting properties of caffeine is that it causes the lowering of blood cholesterol level (Sieger et al 1972) On the other hand smoking has been shown to enhance metabolism of caffeine. (Parsons and Neims 1978)

Carcinogen activating and detoxifying enzymes in liver of mice is also inhibited by caffeine (Gandhi et al 1992). Caffeine induced stimulation of motor activity in rat against by dopamine has been reported (Garrett et al 1994). Plasma renin activity and nor-epinephrine concentration rise significantly following a single dose of caffeine. (Robertson et al 1978)

Caffeine also affects secretion of different hormones. Caffeine has an inhibitory effect on T.S.H. secretion and act as a goitrogenic agent (Wolff 1969). Caffeine and theophylline can facilitates nor epinephrine release from sympathetic nerve endings (Wennmalm and Wennmalm 1989). Growth hormone secretion and thyrotropin release in rat also inhibited by caffeine (Spindel 1984). Caffeine has an impact on the action of corticotropic hormone releasing hormone as reported by (Racotta et al 1994).

On the otherhand, caffeine has protective capacity on B-cell from inhibitory action of alloxine and act as an antihypoinsulinemic agent (Lacy et al 1975)
Caffeine consumption of about 1 gram daily may lead to caffeinism. The symptoms of which are fever, agitation, depression, irritability, insomnia, tremulousness, tachypnoea, tachycardia, cardiac palpitation, diuresis, nausea, and anorexia (Greden 1974). Psychosis resulting from caffeine toxicity has been described (Ulde et al 1984).

Caffeine also has an impact on water and food consumption. Caffeine consumption causes the requirement of more food and water as has been reported (Newland and Brown 1992). Caffeine also acts as recovery agent, suppressed hypothalamo-pituitary-adrenal axis (Marzouk et al 1991).

Caffeine may also cause some behavioral alteration. A group of scientist studied the effect of nicotine, caffeine and their combinations on locomotor activity of rat and reported that in nicotine tolerant rat, caffeine and nicotine in combination significantly increased locomotor activity. Acute exposure to nicotine depressed locomotor activity in nicotine-naïve rat, which can be antagonized by simultaneous injection of caffeine (Cohen et al 1991).

Caffeine administration causes the delayed mineralization in the fetal skeleton which is related to inhibition of endochondrial bone formation at the early stages of proliferation of undifferentiated mesenchymal cells to cartilage specific cells as well as later stages of bone formation. (Barone et al 1993)
Some organs of the body are greatly affected by caffeine administration. Liver is the main target organ, which is greatly affected by the toxicity of caffeine and becomes hypertrophied. Besides, lung heart and kidneys were also affected by caffeine (Poe et al 1953). Rats given daily doses above the maximum LD 50 exhibited a stressor reaction in the form of hypertrophy of adrenal cortex and atrophy of thymus. (Poe et al 1953)

The caffeine is known to be a causative agent of birth defect in offspring when given pregnant rat (Hussain 1989). High doses of caffeine had been associated with an increase in cleft palate and malformation of extremities in experimental animals. (Hussain 1989)

The thyroiditis, occasional dermatitis, some degree of nephritis and loss of red pulp of spleen are also seen after caffeine exposure (Kisskalt 1915). An youth had become accustomed to consumption of large quantities of caffeine and had suffered from symptoms of anemia, suffocation and hallucination. All methylxanthine group of compound qualitatively have similar action but there are marked quantitative and pharmacokinetik differences. (Tripathi, 1994). Caffeine was ranked as exhibiting the greatest effect on the brain and skeletal muscles, theophylline as most potent on the heart, bronchi and kidney, while theobromine was ranked as weak or moderately strong in comparison. (Czok 1974).

Caffeine is a lipophilic molecule, which rapidly penetrates biological membrane, and hence it is rapidly and completely absorbed through intestine and
reportedly distributes freely into the total body fluids (Axelrod 1953). In human system caffeine is rapidly and completely absorbed from gastrointestinal tract (Axelrod 1953) Caffeine is distributed in various tissues in approximate proportion to there water content; the drug passes rapidly to the central nervous system (Axelrod 1953). Caffeine enters the human body through cigarettes also besides drugs, beverages and food. Caffeine consumption increases in those persons who are professional tea testers, and suffers from hepatic disorder, headache and weakness of memory due to frequent intake of tea (Chopra et al 1984).

Metabolism of caffeine was studied in mouse liver by (Szczawinska et al 1980) and later studies reported that liver microsomes from AKR/ J mice metabolized caffeine into paraxanthine, theophylline, theobromine, tri-methyl-uric acid and 6-amino-5 [N-formylmethylamine]-1-3dimethyluracil. Caffeine metabolism in rat is accelerated by administration of enzyme inducing agents. (Welch et al 1977)

Caffeine is known to be a good substrate for cytochrome P448 and delayed maturation of caffeine metabolism in human neonates may be related to particularly slow rate of development of this pathways relative to other functions of the hepatic mono-oxyg enase system (Aranda et al 1981). Although a considerable amount of caffeine accumulates in the body of moderately heavy coffee drinkers during the day, but there is no day to day accumulation. (Axelrod 1953)

It has been reported that smokers tend to excrete caffeine from their system more rapidly than nonsmokers, and that the use of caffeine increased requirement for
anesthetic drugs during surgery. In a study, carried out at University of Utah, School of medicinal research, compared the use of anesthetics in a group of patients not using caffeine with a group that used it. Result clearly indicates that use of caffeine increased the requirement of anesthetics and muscle relaxants and also prolonged the length of recovery time for anesthesia. The use of anesthetic drug necessary to induce unconsciousness was nearly 50 percent higher in the user of caffeine than nonuser and nearly double to maintain unconsciousness during operation. (Hussain 1989).

Clearance of caffeine from the body of the organism also depends on some other factors. A decrease in caffeine elimination was also found in non-pregnant woman taking oral contraceptive steroid. (Patwardhan et al 1980).

It was also observed that there was a delay in caffeine elimination in breast-fed infants as compared to formula fed infants during caffeine maintenance therapy (Le Guennec et al 1985). Later, it was suggested that delay was due to some component of human milk, namely free fatty acids and lipase activity, which affected caffeine metabolism by inhibiting liver cytochrome 450 (Le Guennec and Billon 1987).

An organism response to any toxic substance is very complicated mechanism as it interfere with the physicochemical condition of protoplasm, colloidal chemical state and enzymatic systems and effect the biochemical processes at molecular level. Even some amount of toxicants may be lethal for one animal may not be lethal for others. The lethal dose of caffeine was slightly more toxic in old rats than young rats. (Poe et al 1953). Sensitivity of rat to the lethal dose of caffeine increases
with age, Peters and Boyd 1965). Caffeine was also much more toxic in male than female rats (Peters 1967, Lethal dose of caffeine in human being was recorded as 10 gms. (Goodman and Gilman 1975), but there was a report of taking 24 gms of caffeine orally and the patient survived (Benowitz et al 1982). Death due to caffeine ingestion is not very common and only a few cases have been reported in literature. Indeed, risk assessment for a prolong and high dose of caffeine exposure and searching of chemicals with recovery effect following reduction of the toxicity of caffeine is very important. Quick metabolism of caffeine also reduced the toxic injury. Vitamin C is not involve in the enzymatic demethylation of caffeine, but all the studies investigating the relationship between Vitamin C and drug metabolism in guinea pig have indicated that vitamin C depletion near scorbutic level is required before any alteration in drug metabolism is observed (John et al 1982).

Isocaffeine, a derivatives of caffeine, was only about 3% as potent as caffeine. (Snyder et al 1982). Diet and metabolic condition also markedly influence magnitude of toxicity. Acute starvation has been reported to augment the susceptibility of the albino rat to the lethal effect of caffeine (Peter 1967). Poor nutritional conditions or deficiency of dietary factors suppress the enzymatic detoxification process and unable to reduce toxicity but subsequent supplementation of dietary factor found to have enhanced the detoxifying power.

Detoxification system of the body although involves in the metabolism of toxic substances in detoxification but their complete elimination and complete recovery was not possible in the normal physiological process and hence a search for
antitoxic and regenerative factor to normalize the cytotoxic stress produced by the
different chemicals was initiated from the very onset of toxicological research and
some chemicals were found to reduce toxic injury. Glucose is an antagonizing agent,
which can reduce the toxic injury of caffeine in liver cells (Chauchard et al 1945).

Attenuation of toxic injury with the help of dietary factor is a major
callenge before the of present day pharmacological research. It is well established
that vitamin C plays an important role in endowing the organism with increased power
of resistance. Role of ascorbic acid an antagonistic factor to toxic effect of certain
pathological conditions was recorded since early days of experimental research.
Ascorbic acid can protect the body against toxic substances (Schleł et al 1970) and
has a direct impact on adrenal cortex and able to reduce metabolic stress (Kutsky
1973).

The role of ascorbic acid in maintaining the normal cell structure and
membrane component was reported by Rouller (1964). Deficiency of vitamin C leads
to hyperglycemia, induce liver glycogen and sugar tolerance indicating the
involvement of ascorbic in carbohydrate metabolism. Decreasing effect of
hyperglycemia by ascorbic acid has been reported recently (Clarke et al 1996). The
depletion of adrenal ascorbic acid level, after administration of toxicants, indicates the
metabolic requirements of ascorbic acid to maintain normal physiology or reduce toxic
injury.
Therefore, another facet of the experiment has been designed to investigate possible protective effect of dietary vitamin C against toxic impact induced by oral administration of caffeine covering histological, biochemical, and hormonal level. Ascorbic acid also has a protective effect against caffeine induced abnormality in rat erythrocyte. (Barthakur et al 1999)

Earlier observation suggests that serum lipid increased up to 8 weeks following caffeine administration but urinary ascorbic acid remained as same during experimental period but was significantly higher than the respective controls (Quazi et al 1985). Recently, a group of scientists has reported decrease of antioxidative function of liver and reduction of ascorbic acid level following administration of some hepatotoxic material (Gonskii Ya-I 1996).

Ascorbic acid also has a hepatoprotective effect and lowering of SGPT, SGOT, acidphosphatase and alkalinephosphatase following ascorbic acid supplementation has been reported (Ghosh et al 1996) Abolition of cytotoxic nature of interferon alfa on pancreatic islets by ascorbic acid is recently demonstrated (AL-Zahair and Hoda 1998) and has been shown as antihypercholesterolemic agent. It is well established that vitamin C involved in maintenance of peroxidase system of detoxifying mechanism. (Kutsky1973)

An enormous findings of the action of caffeine on biological system has led to the development of curiosity among the scientists. Now the general tendency is that to bring forth all the possible and inconclusive functional interrelationship
between different systems and organ of the body in caffeine fed animal. As known that, several biochemical constituents reflect the functional status of glandular function of different systems of the body. Effort to make known the hidden facts through such studies is unquestionable. As a matter of fact, the present study is directed to probe into the possible influence of caffeine on certain biochemical constituents like glucose, insulin, cholesterol urea, uric acid in addition to the caffeine and vitamin C level on blood.

Further, thyroid hormones have recognized for their importance in regulating general metabolism, development and tissue differentiation. Hence, study of goitrogenic effect if any may be considered essential in relation to different important functions of the body. Especially, it is considered essential in cases of exogenous substances having goitrogenic effect belong to the common food or drinks. A clue towards goitrogenic effect of caffeine in large doses has already been furnished in literature. However, the operating modes of its action on thyroid gland have remained to be elucidated till now. Hence, the change of the thyroid hormone profile in blood following short and long term caffeine administration also require meticulous study from the scientific point of view.

Available literature also suggest that many of the unwarranted loss of body weight following consumption of caffeine containing herbs such as ma huang (Ephedra sinica) was combined with increased heart rate and blood pressure, and thus avoided by people with the apprehension of developing ailing heart condition, hypertension, diabetes or thyroid diseases, (Tyler 1994).
Keeping in views the above mention facts and also as a scientific pursuit to uphold the health status of the much population, the present study was undertaken to see the toxic effect of much consumed compound caffeine following its high doses and long term exposure by taking rat as a model animal. It is expected that findings of the present study would help substantially in understanding this cytotoxic effect of dietary caffeine and possibly the ameliorating effect of vitamin C on the toxicity caused by caffeine.