CHAPTER II
REVIEW OF LITERATURE
Curiosity about nature and natural products and interaction among them brings information day by day, which helps in improvement of life style and health. Research on natural products and their action in living system is an old day practice and appears that some ingredients of diet have strong pharmacological action with toxicological manifestation in biological system. Primitive men were very conscious of the dangers of the lurked in the food that was available to him and carefully adhered to pattern of experience passed on to him by previous generations. Many of the powerful drugs now in use come originally from herbs on first recognized to be poisonous and later cautiously utilized for their medicinal value.

In recent decades, there has been a growing concern over the presence of pharmacologically active agents and natural toxicants in food. Research in the area of pharmacological and food toxicants have primarily been directed towards caffeine. Caffeine is an active chemical under the group methylxanthine, which is found in tea and other beverages and also found in medicine.

Earlier reports indicated the use of caffeine containing compound by uncivilized men as beverages to stimulate mental activities and to increase capacity to
work. Decoction of tea leaf is used as an antidote against poisoning by alkaloids and heavy metal in early-uncivilized men.

Scientific analysis and experimental study of pharmacologically important ingredient of plants for the treatment of disease probably started taking shape following the introduction of experimental procedures in animals by Franzous Magendie (1783–1855) and Claude Bernard (1813-1878), after that chemical were tested in animals and subsequently used for human after standardization (Satoskar et al 1999).

Different workers reported pharmacological effect of caffeine and other related compounds, although, there were no written record about the first report of pharmacological properties of caffeine but Lewins in 1904 reported that abortion was the first toxic sign described for caffeine. Uncivilized people could observe lethality of caffeine also. Salent and Reiger (1912) first determined the lethal dose of caffeine and reported that the maximum fetal dose of caffeine administered peritoneally to rabbit, ginea pig, and cat varied from 150–250 mg/kg. Later on Kisskalt (1915) reported that the LD₅₀ of caffeine to be approximately 260 mg/kg for white rat. Poe and Johnson (1953) demonstrated that the lethal dose of caffeine or theophylline was approximately 200 mg/kg when given intraperitoneally to young rat and that caffeine was slightly more toxic in adult rats (LD₅₀ 167 mg/kg). The contribution of Boyd and associates in 1960, 1965 represent the most comprehensive evaluation of the toxicity of caffeine. LD₅₀ of caffeine administered orally to Guinea pig was found to be 220 mg/kg and
with the common cause of death had been attributed to the respiratory failure following convulsion. Peters and Boyd (1965), reported that the sensitivity of rats to the lethal effect of caffeine increased with age. Peters (1967) again reported the more toxic nature of caffeine in male rats than in females. He also stated the high daily doses (185mg/kg) of caffeine that killed susceptible rats in 2 to 3 days leaving only those that were resistant to caffeine and other methylxanthines.

Death due to excessive caffeine ingestion was not very common, and only a few cases had been reported in literature. In case of lethal poisoning of a 15 months old hospitalized child, severe cerebral edema was reported. The child who received by intake 20% caffeine solution (about 18 grams) started to vomit slowly afterwards, had tetanic spasms and died 5 hours later. Death had been documented in human after intravenous administration of 3.2 grams caffeine (Jokela and Vartiwiner 1959). Two suicidal death by caffeine had been reported where the estimated amount of ingested caffeine were between 6.5 to 12.0 grams (Alstott et al 1973). Death after ingestion of an unknown quantity of caffeine by a 34 years old female was preceded by episodes of weakness, vomiting and convulsions and her post mortem caffeine concentration were 1060 micro grams per ml and 1080 micro grams per grams in blood and brain respectively (Turner and Cravery 1977). A fetal case of caffeine intoxication in a 19 years old female was associated with ingestion of an over the counter appetite suppressant (Mc Gee 1980), blood obtained from the heart at autopsy had a caffeine level of 181 micrograms per ml and a large amount of caffeine found in gastric content. Several cases in literature indicated that nearly every case of xanthine toxicity
was goitrogenic (White 1956 and Banner 1980). The estimated amount of those commodities required to provide 5 to 10 gram caffeine following consumption of approximately 75 cups of coffee, 125 cups of tea or 200 cola beverages at one time. (Curotolo and Robertson 1983).

Another case of death of a 5 years child due to overdose of caffeine containing nonprescription stimulant of appetite suppressant was reported later. Their postmortem caffeine concentration in blood ranged from 129.9 to 343.9 micrograms per ml (Garriot et al 1985). However, lethal toxicity due to caffeine from coffee, tea and soft drinks was very unlikely.

Chronic toxicity of caffeine appeared to be related with bio-disposition and plasma half-life. Bio-disposition of caffeine was investigated to attenuate the pharmacological nature of caffeine or to assess the toxicity. Metabolism of caffeine was extensively studied in both human and other animal system. In 1956, Cornish and Christman, studied the metabolism of methylxanthine in human and found that after ingestion 1 gram theobromine, the majority of urinary excretory products were 7 methyl xanthines, 3 methyl xanthine and unchanged theobromine. A small amount of 7 methyl uric was also excreted suggesting the demethylation occurred more rapidly at 3 than 7 position. There might be formation 7- methyl uric acid being formed by the oxidation of 7 methyl xanthine or by the demethylation of the 3 –7 dimethyl uric acid formed by direct oxidation of theobromine. Thus majority of theobromine was excreted as monomethylxanthine. In contrast, methyluric acid were the predominant
excretory products of the thephylline and approximately equal amount of methylated xanthine and methyluric acid were present in urine after caffeine administration Elkins et al (1981) reported that caffeine was eliminated rapidly in young children than the adults. Neims (1981) emphasized the distribution of caffeine and reported that 99 percent of the filtered caffeine was reabsorbed in the kidneys cerebrospinal fluid. Caffeine clearance rate and its metabolism were intensively studied in rat. The use of radiotracers had led to identification of mono and dimethylxanthine and also uric acids. Technique of radiotracers also led to the discovery of the formation of trimethylallantoin and several uracil derivatives. Bioavailability of caffeine in mammalian system was well documented. Distribution of radioactivity by whole animal body autoradiography had been reported in C57 BL/65 mice after a tail vain injection of [I-Me 14 C] or [14C] caffeine at dose of 0.7 or 11 mg /kg. This study showed that bile and gastrointestinal content was the major site of deposition of radioactive caffeine (Lachance et al 1983). Caraco et al (1990) put forward the hypothesis that caffeine could induce its own metabolism and therefore heavy coffee drinkers were relatively resistant to the effect of caffeine. Very recent investigation also revealed pronounced action of caffeine on cytochrome enzyme system (Bruce 2001).

Many pharmacologically active compounds had a considerable effect on physiology and biochemistry as studied by different workers. Most of the physiological and biochemical alterations were associated with change of enzyme activities. Much of the early works on caffeine were associated with the efficacy of
enzyme system but the findings were left unsubstantiated. Mc Neil et al (1973) reported that caffeine and theophylline inhibited catechol-o-methyltransferase, which inactivated the contractile response of a number of adrenergic amines. Burge (1980) found that the caffeine produced an increased activities of the enzyme catalase with a resulting increase in oxidation by stimulating the synthesis of this enzyme in liver.

Bose et al (1960) reported that in-vitro AMP, theophylline and theobromine showed maximum inhibitory action of the cholinesterase, while caffeine, alloxan and ATP were intermediate and both xanthine and hypoxanthine were least inhibitory. Nachmansolhi and Schneemar (1945) evaluated the effect of caffeine and theobromine as colenesterase inhibitor. Berkowitz and Spector (1971) found that single or multiple dose of caffeine increased the concentration of 5-hydroxyindole acetic acid in rat indicating that caffeine did not prevent serotonin deamination. Caffeine either prevented the release of brain serotonin or was able to increase serotonin synthesis.

Most of the caffeine research were concentrated in neurophysiology and neurobiochemistry. Relation between cerebral blood flow and caffeine consumption was investigated using position emission tomography and reported that a mean dose of 250 mg caffeine produced approximately a 30 percent decrease in whole brain cerebral flow (Cameron et al 1990). The effect of caffeine in cerebral blood flow was also measured and confirmed the earlier observation (Mathew and Wilson 1990).
Research in the area of physiology and biochemistry was directed toward cardiovascular system, endocrine physiology and metabolic processes with carbohydrate metabolism. In 1967, Ardlic et al observed the inhibitory effect of caffeine in platelet aggregation. The inhibition appeared to be in competitive and effect of methylxanthines were not mediated through inhibition of platelet sulfhydryl group, of calcium. Cardiovascular system showed high response after caffeine and theophylline administration. Caffeine, theophylline and the bromine showed vasodilator action on the coronary vessels with caffeine being the weakest and theophylline being the strongest. Champman and Muller (1974) studied on the contraction on the heart and they demonstrated the requirement of double bounded imidazole ring to evoke the contracture. Methylxanthine enhanced the beta adrenergic receptors mediated responses on the coronary artery via blocked of catecholamine uptake (Kalsner et al 1975). Field et al (1945) observed the shortening of time for clotting of blood. Caffeine also gained the attention for its pronounced effect on fibrinogen synthesis.

Caffeine also associated with elevation or decline of blood glucose level. Much of the work on caffeine research indicated that caffeine appeared as a causative agent of hypoglycemia although contradictory results were also available. Soeren and Graham (1988) also reported that blood glucose level did not change after caffeine consumption. Ferrauti et al (1997) studied effect of caffeine on glucose homeostasis and reported that caffeine improved glucose homeostasis. Investigation on the correlation of insulin and glucose with caffeine concentration in blood suggested that.
level of insulin and glucose did not change from base line after caffeine consumption. (Arciero 2000). Recently a group of scientist studied the influence of hypoglycemia on visual sensation in presence of caffeine. (Owen 2001)

It was well established that caffeine had protective capacity on β cell from inhibitory action of alloxan (Lacy et al 1975). Increase of insulin following caffeine administration was established in third international caffeine workshop in 1980 and the report was published by Ferastron (1981) Macchchsettus Institute of Technology, Cambridge. Earlier investigation report suggested that caffeine did not enhance the biosynthesis of insulin but subsequent findings on caffeine indicated a completely opposite action and proved that caffeine caused the increase blood glucose level nonesterified fatty acids and insulin secretion. When the rats were given high doses of caffeine profound neuroendocrine changes with blood hormonal level occurred that were similar with stress (Sachs and Forster 1984). McCarty (2001) studied the insulin resistance and visceral obesity in relation to exercise and found that pre-administration of caffeine had beneficial effect of exercise on visceral obesity.

Blood protein level also changed after caffeine consumption as available in the literature. Field et al (1945) reported that methylxanthine like caffeine caused increased plasma binding fibrinogen. Nagata et al (1988) reported that caffeine were commonly correlated with increasing sex hormone binding globulin.
Formation of urea from ammonia in rat liver was investigated and reported that caffeine inhibited in vitro formation of urea Bernheim and Bernheim (1945). Generation of cyclic CAMP inhibit urea transport and this findings suggested that inhibition of urea transport could be brought about by oxidation of one or more component in its metabolic pathways (Franki et al 1975). All the methylxanthines including caffeine got the clinical attention for their pronounced effect on nitrogen metabolism and formation of urea and uric acids. They found that excretion of uric acid in mammal increased when caffeine was ingested. Caffeine did undergo biotransformation in human body to form methylated derivatives of uric acid Ramalakhmi (1999).

Level of serum cholesterol changed following the consumption of caffeine therefore associated with cardiac function and was reported to have and appeared possible deleterious effect on health. It is reported that caffeine with normal diet did not enhance the cholesterol level in rat. (Rakicioglu et al 1998). Scientists studied the effect of a combination of caffeine carnitine and choline on body fat and reported that body fat decreased after consumption of caffeine and serum leptin concentration in rats was attributed to increase fat utilization for energy. (Sachan and Hongu 2000)

Caffeine had a strong stimulatory or inhibitory effect on endocrine system. Studies carried out on human given high dose of caffeine (500mg) revealed stress like stimulation of pituitary adrenal axis (Spindel 1984). They also reported that high doses of caffeine markedly inhibited the thyrotropin and growth hormone
secretion in rat. Willett (1987) reported that no change of T₃ and T₄ and TSH appeared after caffeine therapy. Subsequent studies suggested that caffeine consumption had no significant alteration of catecholamine and thyroid hormones (Pochlman et al 1989). High doses of caffeine inhibited TSH secretion through stress mechanism (Anonymous 1981). Caffeine appeared to be a stimulator of corticosterone secretion. Caffeine did not effect prolactine secretion in either male or female rat nor estrogen had any synthesizing effect. Caffeine enhanced the speed of recovery of the hypothelamo pituitary adrenal axis in rat. (Marzouk 1991).

Alteration of cell morphology following caffeine consumption was also indicated in the literature. Change of blood cell morphology due to caffeine administration was already published. (Barthakur et al 1999).

Caffeine also caused cell wide cytoplasmic oscillations of calcium (Mc Donough et al 2000). Contractility and intracellular calcium concentrations of renal arterial smooth muscle were also changed following caffeine application (Murphy et al 2001). Caffeine showed a stimulatory effect on the cell division which was blocked by external stimuli (Zolzer 2001). Caffeine paused to be an inhibitory factor of tubular reabsorption of sodium and water and appeared as diuretics (Tripathi 1994, Satoskar et al 1999). Besides, caffeine also exerted stimulatory effect on the blood flow whereby contributed in diuretic nature of caffeine. (Satoskar et al 1999)
Caffeine altered level of minerals that associated with change of physiological processes. In neural cell, calcium signaling depends on the efflux of calcium from the intercellular stores in to the cytoplasm via caffeine sensitive ryanodine receptors of the endoplasmic reticulum. Caffeine also caused cell wide cytoplasmic oscillations of calcium (Mc Donough 2000) Contractility and intracellular calcium concentration of renal arterial smooth muscle also changed with caffeine application. (Murphy 2001) Caffeine has a stimulatory effect on the cell division which are blocked by external stimuli (Zolzer 2001) Negretti (2000) reported that some polyunsaturated fatty acids caused an increased in caffeine induced Na⁺, Ca²⁺ exchange current.

Some ingredients of food interfered with the absorption and utilization of essential minerals and vitamins. Phylic acid present in many food grains and pulses interfered with the absorption of mineral like calcium and iron. (Rao 1986) Similarly absorption of mineral from gastrointestinal tract and their excretion through kidney were also effected following caffeine administration.

Reports from Food and Drug Administration critically analyzed the risk of caffeine exposure on health and they proposed to remove caffeine from its list of substances that were generally recognized as save (Hussain 1989).

Chronic administration of caffeine for long a term caused some changes in kidneys and liver of rats, receiving caffeine at high doses, but no tumor was observed.
Caffeine administered to whister rats in their drinking water for 15 months developed cancer of pituitary, thyroid, mammary glands and uterus. As analysis were made critically between caffeine and carcinogenesis, three major neoplastic entities being observed were the tumor of liver, lung and lymphatic tissue (Stalder 1984). Rajaraman (1984) studied the effect of methylxanthine on cell function and structure on the ovary of the Chinese hamster and suggested that methylxanthine that have a methyl group at the seventh position lacked reverse transforming potential causing an increase in surface fibronectin, cell suspension adhesion strength and anchorage dependence on growth. It was established that caffeine induced fatty liver formation. This sequel seemed to be attributed an increased peroxidebility in the liver of caffeine treated animal due to an increased in the triglyceroid content. A decrease vitamin E which also occurred might contributed to the lipid peroxidation (Dianzani et al 1991). Caffeine potentiated the effect of the nonsteroidal anti inflammatory drugs mfenemic acid on the rat medulla resulting in the quantitative increase in the interstitial tissue between adjacent afferent and efferent vasa recta (Hewilson et al 1991). Nutritional supplements, which could able to reduce the toxicity in the mammalian system got great attention to researchers and some nutritional supplements such as vitamins, were recognized. Most of the antioxidant had the property and they absorbed the free radicals. The positive role of ascorbic acid against toxic injury was also described. Vitamins as antagonistic factor for toxic effect on certain pathological conditions was recommended since early days of experimental research (Rao 1986). Vitamin C is a potent antitoxic factor with the property of neutralizing chemical stress in
physiological level and acts as a potent antioxidant in cellular level to prevent specific cell injury. (Satoskar et al 1999)

Different workers studied hypoglycemic nature of vitamin C. As observed in hypovitaminosis condition, hyperglycemia, reduced liver glycogen and reduced sugar tolerance was observed (Harkart and Mathur 1976). Hypoglycemic nature of ascorbic acid was also observed by other workers (Zahair 1998). Ascorbic acid involved in the carbohydrate metabolism and in oxidation of phenylalanine and tyrosine. Scorbustic animal exhibited hyperglycemia, lowered glucose tolerance and resistance to insulin. (Satoskar et al 1999)

Ascorbic acid caused the release of ferrous iron from ferritin and thus caused the diuresis and induced vasoconstrictor effect. Ferrous iron was used by the body for the synthesis of hemoglobin (Rao 1986).

Ascorbic acid also exhibited a hepatoprotective nature. In induced liver toxicity it caused the reducing effect on SGPT, SGOT level bringing towards normal condition (Ghosh et al 1996). Vitamin C also prevented the lipid peroxidation in the lens of diabetic rat (Naziroglu et al 1999).

Ascorbic acid could modulate growth of early preneoplastic aciner lesions induced in rats' pancreas (Woutersen et al 1999). Ascorbic acid also appeared as a protective agent against induced diabetic rats (Garg et al 2000).
Ascorbic acid in mega doses given for long periods could cause ‘rebound scurvy’ or stoppage probably due to enhancement of its own metabolism or tissue acclimatization. The risk of urinary oxalate stones might also increase. Tripathi (1994). Ascorbic acid in high doses might able to clog up arteries as presented in American Heart Association Meeting in San Diego (Basu 2000).

It is well-established fact that daily prophylactic administration of vitamin exerted a significant good effect on common cold. (Satosker 1999)

There was evidence that elevated dietary intake of ascorbic acid protected the body against toxic substances (Schlegel 1970, Kamm et al 1973). Vitamin C maintained reduced state of epinephrine (Kutsky 1973) while vitamin C depletion caused decreased synthesis of progesterone in adrenal cortex or ovary (Kutsky 1973).

It was well established that the low tissue concentration of ascorbic acid reduced the detoxification capacity of animals by causing an impairment of microsomal hydroxylation in the liver (Rao 1986). In addition, ascorbic acid was known to stimulate ion transport through inhibition of 3′5′ cyclic Adenosine monophosphate phosphodiesterase .

Simultaneous administration of caffeine and ascorbic acid yielded a beneficial effects on blood picture of albino rat as reported previously. (Barthakur et al 1999)
Recently it was reported that some toxicants like copper gluconate, a nutrient supplement, caused toxicity in the mouse liver and kidney also elevated blood ascorbic acid level (Hojo et al 2000). In rat, administration of ascorbic acid for a short period also caused an elevation of urea, although prolong administration declined the urea level toward normal (Maturova et al 1978)

Extensive review of literature indicated that controversy still remained in respect of toxicity of caffeine in the mammalian system and thus invited its critical analysis and also called upon the study on the antitoxic substances, which might bear meaningful application in human life