GENERAL INTRODUCTION
It is well known that most of the people of the world consumes tea (containing known methylxanthines (MXs) like caffeine and small amounts of theophylline (Th) and theobromine) that prepare from the leaves of a plant (*Thea sinensis*) widely distributed geographically, a bush native of Southern China and now extensively cultivated in other countries. It is believed that paleolithic man discovered the tropical MX(s) containing plants throughout the world and made beverages from them. The basis for the popularity of all the MX(s) containing beverages is due to its stimulant and antispasmodic actions that elevate mood, reduce fatigue and increase capacity for work (1-3).

The sources and the contents of MX(s) containing beverages as they are usually prepared are described in Table 1.

**Table 1**: The sources and the contents of MX(s) in beverages (4)

<table>
<thead>
<tr>
<th>Source</th>
<th>MX(s)</th>
<th>Content (mg)</th>
<th>ml (approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffea arabica (Coffee seeds)</td>
<td>Caffeine</td>
<td>75</td>
<td>125 (in an average cup of coffee)</td>
</tr>
<tr>
<td>Thea Sinensis (Tea leaves)</td>
<td>Caffeine</td>
<td>50</td>
<td>125 (in an average cup of tea)</td>
</tr>
<tr>
<td></td>
<td>Theophylline</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cola acuminata (Guru nuts)</td>
<td>Caffeine</td>
<td>30</td>
<td>200 (in one bottle of cola drink)</td>
</tr>
<tr>
<td>Theobroma Cocoa (Cocoa, chocolate)</td>
<td>Theobromine</td>
<td>200</td>
<td>125 (in an average cup of cocoa)</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Xanthine is a dioxypurine and is structurally related to uric acid. Methylation of this xanthine compound at different position(s) formed different MX(s). Caffeine is chemically known as 1, 3, 7 - tri-methyl xanthine, Th is called as 1, 3 - dimethylxanthine and 3, 7 - dimethylxanthine is known as theobromine. The structural formulas of xanthine and the three naturally occurring xanthine derivatives are shown in Fig. 1.
Fig. 1: Structures of xanthine and its methyl derivatives

They are alkaloid in nature having both hydrophilic and lipophilic character. They are soluble in water and alcohol (1-3). 1 gm caffeine dissolves in 46 ml water, 66 ml alcohol, 50 ml acetone, 530 ml ether and in 100 ml benzene at room temperature.

At low to moderate concentration of caffeine apparently activates all the mentioned cellular actions but different position of substitution activates separately the one type of cellular action and it is established through the derivatives of the mother compound (1-3, 5).
Fig. 2: The structure activity relationship of MX(s)

In general both adenosine receptor and phosphodiesterase activities are reduced in derivatives that lack substituents at position 1 or contain substituents at position 7, as compared with the corresponding 1, 3 dialkylxanthine (6-8). For example, the order of potency for the naturally occurring MX(s) is \( \text{Th} > \text{caffeine} > \text{theobromine} \). Addition of aromatic, cyclohexyl or cyclopentyl groups at position 8 usually markedly increases the affinity for adenosine receptors but reduces inhibition of cyclic nucleotide phosphodiesterase (9). Although neither caffeine nor Th discriminates between the subtypes of adenosine receptors, certain 8-substituted derivatives of 1, 3 - dipropylxanthine display marked selectivity for \( A_1 \) receptors, while some analogs of caffeine display appreciable selectivity for \( A_2 \) receptors.

Caffeine as well as other MXs (Th and theobromine) are absorbed readily after oral, rectal or parenteral administration (1-3). Caffeine administered with liquid or uncoated tablets is rapidly and completely absorbed from the digestive tract. More than
99% of an orally administered dose of caffeine is absorbed, with peak plasma levels occurring within 15 to 45 minutes (10, 11) following its administration. A 250 mg dose yield peak plasma concentrations between 5 and 25 μg / ml with 15% protein binding (10). The half-life of caffeine in plasma shows considerable variation between persons, with reported value ranging from 3.0 to 7.5 hours (10-15). Caffeine as well as other MXs are distributed into all body compartments, they cross the placenta and pass into the breast milk. The apparent volume of distribution is similar for caffeine and Th and usually in between 0.4 and 0.6 litre / kg (1-3). MX(s) are eliminated primarily by metabolism in the liver (1-3). It has been also known that from 0.5% to 3.5% of an administered dose of caffeine is excreted through urine (13, 16, 17). The major metabolite of caffeine is 1, 7 - dimethylxanthine, although numerous other metabolites exist (11). In addition to metabolic conversion and urinary excretion, caffeine is also excreted into saliva (13, 18, 19), semen (20), and breast milk (21-23), and is present in umbilical cord blood (24-26). The clearance of caffeine is significantly influenced by disease, concurrent drug use, or pregnancy. Patients with alcoholic liver disease dispose of caffeine slowly (27, 28). Caffeine clearance is stimulated by smoking (19, 29). The elimination of caffeine is surprisingly slow in the newborn (26, 30). The slow elimination of caffeine at birth is thought to be due to a deficiency of N-demethylation (31, 32).

Caffeine is well absorbed orally. It is extensively metabolized in liver microsome by oxidative N-demethylation and ring oxidation to methylxanthines and methyluric acids (17, 33, 34). Their metabolism is enhanced by polycyclic aromatic hydrocarbon in animals (35-37) and by cigarette smoking in man (19, 38). These data suggest the involvement of cytochrome P450 (cyt. P450) in the metabolism of caffeine. Additional studies have shown that of all the MXs, only 1MX is a substrate of xanthine oxidase (39-41). There are 14 possible MXs and methyluric acids (MU) which could be formed from caffeine (Fig. 3). The primary degradation of caffeine in man is by oxidative N-demethylation with the help of the enzyme N-demethylase to theobromine, paraxanthine, Th and by direct ring oxidation to 1, 3, 7 - trimethyluric acid. These compounds are further metabolized to dimethyluric acid, monomethyluric acid and monomethylxanthine (Fig. 3) (42). Its 0.5 to 3.5% of the amount intake has been reported to be excreted as unchanged form through urine. Its elimination kinetics varies considerably among individuals. Half life of caffeine in plasma of adult human is 3 -7 h, this increases by about two fold in women during the later stages of pregnancy or with long-
Fig. 3: Possible metabolic pathway of caffeine and its metabolites in man. Demethylation processes (-), ring oxidation processes (---)
term use of oral contraceptive steroids (1-3, 43). The half life in preterm infants at birth is 65.0 to 102.9 h (30, 44); in term infants at birth is 82 h (45, 46); in 3 - to 4½ month old infants is 14.4 h (46), and in 5 to 6 months old infants, the half life is 2.6 h (46). Neonates eliminates caffeine very slowly with average half life of 100 h (2).

Caffeine, Th and theobromine share in common several pharmacological actions of therapeutic interest. They affect the different physiological systems. They act on the kidney to produce diuresis, stimulate cardiac muscle and relax smooth muscle specially bronchial muscle. They also act on respiratory, gastrointestinal system and on central nervous system (CNS) (1-3). The potency of these action of xanthines depends markedly on the difference of their structure. One particular xanthine is usually more suitable than another for any specific therapeutic aspect.

Comparative pharmacological actions of these three methylxanthines (4) is presented in Table 2.

Table 2: Pharmacological actions of three methylxanthines

<table>
<thead>
<tr>
<th>Action</th>
<th>Caffeine</th>
<th>Th</th>
<th>Theobromine</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS-stimulation</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Heart-stimulation</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Blood vessel-relaxation</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Bronchi-dilation</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Kidney-diuresis</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>increased contractivity</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Gastric mucosa irritation</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Number of + sign represents the degree of response.

The actions of MX(s) on the circulatory system are complex and sometimes antagonistic, and the resulting effects largely depend on the conditions prevailing at the time of their administration, the dose used, and the history of exposure to
MX(s) (2). Systolic blood pressure increases 10 mm Hg 60 min after ingestion of caffeine (47). Heart rate decreased during the first h, then increases above the baseline value during the next 2 h (47). Plasma epinephrine, norepinephrine, and plasma renin activity significantly increases for 1 and 3 h after caffeine ingestion (47). However, complete tolerance has been found to be developed to all these effects of caffeine following 1 to 4 consecutive days of its administration (48). In patients with heart failure, however, the venous pressure is initially rather high; consequently, the cardiac stimulation and decreased venous pressure produced by MX(s) leads to a marked increase in cardiac output that persists for 30 min or more (49). At higher concentration, MX(s) produces tachycardia, although the sensitive individuals also experience other arrhythmias, such as premature ventricular contractions (50). It dilates systemic blood vessels (including coronaries) by direct action; however, cranial vessels are constricted by caffeine and that may be considered as the basis of its use in migraine (4, 51-54).

The most important action of MX(s) in this respect is their ability to relax the smooth muscles of the bronchi, especially if the bronchi have been constricted either experimentally by a spasmogen or clinically in asthma (1-3, 55). Th has been found to be the most effective among the xanthines. It may be mentioned that the concentrations of Th greater than 50 μM are required to relax bronchiolar segments of human lung previously contracted by carbachol (56); this corresponds to the concentration of free drug attained at the top of the therapeutic range (20 μg/ml) (57).

It has long been known that caffeine increases the capacity for muscular work in human beings (58). For example, the injection of caffeine (6 mg/kg) improves the racing performance of cross-country skiers, particularly at higher altitudes (59). At therapeutic concentrations, caffeine can improve diaphragmatic contractility and reduce diaphragmatic fatigue in normal human subjects (60). MX(s) increases the release of Ca²⁺ from sarcoplasmic reticulum by their direct action (4). At low doses, twitch response to nerve stimulation is augmented, while at toxic doses, contracture is produced (4). In addition, it facilitates neuromuscular transmission by increased acetylcholine (ACh) release (61). Its central action relieves
fatigue and increases muscular work (62-64). Thus, athletic performance may be improved for short periods.

Physiologic doses of caffeine stimulates the respiratory rate with the degree of respiratory stimulation correlating closely with the plasma level of caffeine (65, 66). Caffeine has also been shown to increase minute ventilation in patients with chronic obstructive pulmonary disease (67). The mechanism for caffeine's respirogenic effect appears to be an increased sensitivity of the medullary respiratory center to CO₂ (68-70).

The acute ingestion of caffeine produces mild increases in urine volume and urinary sodium excretion in humans (47, 71, 72). Tolerance to these effects develops after habitual consumption of the drug (71, 73). It has been suggest that the diuretic action of MX(s) results primary from a decrease in tubular reabsorption of sodium and water (74-76).

The administration of caffeine (4 to 8 mg / kg) to normal or obese human subjects elevates the concentration of free fatty acids in plasma and increases the basal metabolic rate (49). Caffeine increases blood glucose in subjects who are obese (77), in poor health (78), or who have maturity-onset diabetes (79). The acute ingestion of caffeine has many significant effects on metabolism, it increases metabolic rate and elevates plasma free fatty acid levels and, perhaps, cortisol level (80).

Man is relatively sensitive to the effects of MX(s) on gastric secretion. It is also observed that moderate oral or parenteral doses of caffeine cause secretion of both acid and pepsin (81, 82). Caffeine acts synergistically with histamine (83), but not with pentagastrin (84) in stimulating acid secretion; atropine diminishes (85), and cimetidine completely abolishes (86) the acid secretory response to the drug. The mechanism of caffeine-induced secretion of gastric acid has been hypothesized to be inhibition of adenosine 3' - 5' monophosphate phosphodiesterase activity in gastric mucosal cells (87). Caffeine has been shown
to stimulate water and sodium secretion by the small intestine in experimental animals (88) and humans (89, 90) without altering intestinal transit times (90).

A recent study found that caffeine consumption during pregnancy decreases the birth weight of the newborn (91), in a dose-related manner. Previously, it has been found that consumption of caffeinated beverages decreased fertility in women followed for a year after discontinuing birth control (92). There is no clear explanation for the association between caffeine and low birth weight or reduced fertility. Significant concentrations of caffeine are present in the umbilical cord blood of newborns (25), and a neonatal withdrawl syndrome, consisting of irritability, jitteriness, and vomiting has been reported in infants born to mothers who consumed large amounts of caffeine during pregnancy (93).

Caffeine is a CNS stimulant that can elevate mood, reduce fatigue and increase capacity to work (3, 94, 95). Persons ingesting caffeine or caffeine containing beverages usually experience less drowsiness, less fatigue, and a more rapid and clearer flow of thought (3). The ingestion of 85 to 250 mg of caffeine, the amount contained in 1 to 3 cups of coffee, produces an increased intellectual effort and decreases reaction time. However, tasks involving delicate muscular coordination and accurate timing or arithmetic skills may be adversely affected (1-3). Ingestion of caffeine 30 to 60 min before going to bed increases sleep latency (72, 96-98), decreases total sleep time (99-101), and significantly worsens subjective estimation of sleep quality (72, 97-101). Caffeine increases vigilance and decreases motor reaction time in response to both visual (102-104) and auditory (105) stimuli. Caffeine has significant effects on the electroencephalogram (EEG) spectrum. Higher doses of caffeine produces nervousness or anxiety, restlessness, insomnia, tremors, and hyperesthesia. At still higher doses, focal and generalized convulsions are produced (3).

Many therapeutic uses have been proposed for caffeine, including use in artificial insemination of hypokinetic sperm, minimal brain dysfunction, Parkinson's syndrome, atopic dermatitis, and neonatal apnea (106-108). 6 mmol of caffeine increased the motility and forward progressiveness of freshly ejaculated hypokinetic
human spermatozoa for up to 5 h (109). Caffeine has also been shown to increase glucose and fructose use, increase lactate production, and decrease oxygen consumption in normokinetic and hypokinetic sperm, and increase sperm penetration of mid-cycle cervical mucus (110, 111). Recent evidence suggests that caffeine disrupts the normal architecture and alters the elemental composition of spermatozoal heads (112, 113).

Insomnia, restlessness, and excitement are the early symptoms of caffeine toxicity, which may progress to mild delirium, emesis and convulsions. The muscles become tense and tremulous. Tachycardia and extrasystoles are frequent, and respiration is quickened. The short-term lethal dose of caffeine in adults appears to be about 5 to 10 gm, untoward reactions may be observed following the ingestion of 1 gm (15 mg / kg; plasma concentrations above 30 µg / ml) (3). The acute lethal dose of caffeine in adults appears to be roughly 5 to 10 gm either intravenously or orally (114, 115). This quantity of caffeine would be approximately that contained in 75 cups of coffee, 125 cups of tea or 200 cola beverages. Doses in this range lead to agitation, tachycardia (116), convulsions (114, 115), coma (117), and death due to violent shock, pulmonary edema and atelectasis (118), ventricular fibrillation (119), and cardiopulmonary arrest (118). Roughly equivalent doses (approximately 100 mg / kg body weight) of caffeine lead to similar symptoms in infants and children (120, 121), though no deaths have been reported.

It is noted that long-term ingestion of caffeine can produce tolerance, a widely occurring adaptive biological phenomenon (122, 123). Tolerance is a requirement of higher dose of a drug to produce a given response. When caffeine is consumed on a regular schedule (124-128), complete tolerance develops (counts as locomotor activity) within a few days. In fact, tolerance to the effects of caffeine on CNS have been observed in relation to locomotor activity (LA) (123, 125, 126, 129, 130), operant conditioning (124), cerebral electrical activity (131, 132) and caffeine-induced seizures (133, 134). In animals, tolerance is associated with an increased number of adenosine receptors (A₁) in the brain, a shift of brain A₁ receptors to a high affinity state, and an increase in functional sensitivity to adenosine (135-137).
Several hypothesis have been formulated concerning possible mechanisms of action of MX / (caffeine) at the cellular level. Three main mechanisms of action have been described, which are in chronological order of their discovery (137) : (a) intracellular mobilization of calcium, (b) inhibition of phosphodiesterases and (c) antagonism at the level of adenosine receptors (138-143). In addition, a hypothesis has been formulated suggesting as a fourth mechanism of action of MX on the CNS, binding of caffeine to benzodiazepine receptors (137). There are several other types of actions that have received relatively little attention to date, but that might prove to be very important in certain effects of the MX(s). These include their (MXs) potentiation or inhibition of prostaglandin synthesis (144) and reduction in uptake and / or metabolism of catecholamines in nonneuronal tissues (145, 146).

The effect of MX(s) on the mobilization of intracellular Ca\(^{2+}\) has been first demonstrated in skeletal muscle (147). Caffeine, at a concentration of 1-2 mM lowers the excitability threshold and prolongs duration of the active period of muscle contraction by promoting translocation of extra cellular calcium through plasma membrane and sarcoplasmic reticulum (147-149). Similar observations have been made subsequently on normal cardiac muscle (150). The effect of caffeine also depends on intra or extra cellular concentration of Ca\(^{2+}\) (151). Recently, it has been shown that caffeine sensitizes the muscular contractile apparatus to the concentration of intracellular Ca\(^{2+}\) (152-154), the direct interaction of MX with calcium channels has been observed in the sarcoplasmic reticulum (155).

The inhibitory properties of MX(s) on cyclic nucleotide phosphodiesterases activity were discovered by Sutherland's group (156, 157), who used caffeine and Th in their research on regulation of glycogen metabolism and on peripheral lipolysis. After identifying the major role of cyclic adenosine 3', 5' - monophosphate (cAMP) in the regulation of these processes, the authors observed that MX prevents enzymatic breakdown of cAMP by inhibiting cyclic nucleotide phosphodiesterase (156, 157). This discovery represents a possible mechanism of action of MX(s), i.e., accumulation of cAMP and potentialization
of its effects in order to stimulate the action of substances, such as catecholamines (139, 158).

The possibility that central stimulant effects of MX(s) result from competitive antagonism of the depressant effect of endogenous adenosine is appealing for many reasons: (i) structural similarity between adenosine and caffeine and (ii) most pharmacological effects of adenosine in nerve tissue can be suppressed by relatively low concentration of circulating MX(s) e.g., less than 100 μM, which is attained after drinking 1-3 cups of soft drinks (3). But this concentration apparently has neither direct effect on cAMP metabolism, nor on calcium shifts (141, 159). Moreover, administration of adenosine and its derivatives usually produce effects opposite to those of caffeine (160). These effects include depression of spontaneous electrical activity of the neurons (161, 162), inhibition of synaptic transmission (163, 164) and release of neurotransmitters (165, 166). Adenosine and its derivatives also influence on the behavioral activity (167-171). It causes a dose-dependent decrease in LA that can be eliminated by small dose of caffeine or Th (139, 141, 172). The relative efficacy of various xanthine compounds in stimulating LA is related to the relative affinity of these substances to adenosine receptors (141).

There are two major subclasses of adenosine receptor (Fig 4), high affinity A₁ receptor and low affinity A₂ receptor (173, 174). Adenosine exerts its inhibitory effects on adenylate cyclase by A₁ receptor (172) and stimulatory effects on adenylate cyclase by A₂ receptor (175). MX(s) has been found to inhibit binding of adenosine receptor ligand and has been found to exert its antagonistic effect on both A₁ and A₂ receptors (176, 177). There is another adenosine recognition site, termed the P site (Fig. 4). The P site is located on the catalytic subunit of adenylate cyclase. It shows a preference for purines that have intact purine rings, such as 2’-5’ dideoxyadenosine (DDA) and is activated by relatively high concentrations of adenosine (7).

Caffeine also binds to benzodiazepine receptor sites although the affinity is rather weak (178). This binding has been suggested as a possible mechanism of action of MX(s) because caffeine antagonizes or modifies the effects of benzodiazepines on animal (179, 180) and on human behaviour (181-183). However, caffeine are
Fig. 4: Interactions of adenosine with adenylate cyclase
much more potent antagonists of adenosine receptors than of benzodiazepine sites (184). Moreover, the interaction between caffeine and benzodiazepines might not be due to competition of the two substances at the level of benzodiazepine receptors, but could imply action on adenosine receptors (185, 186). Nehlig et al. have shown that the toxic effects at high doses of MX(s) would be due to interaction with benzodiazepine receptors (137).

Adenosine appears to act as an inhibitory neurotransmitter as like as γ-aminobutyric acid (GABA) (187-189). Caffeine competitively antagonizes both the adenosine receptors and retard the effect of adenosine. A primary role of adenosine in the CNS appears to inhibit the release of various neurotransmitter and possibly glutamate in particular through presynaptic receptors. Therefore, adenosine antagonists, such as MX(s) can be expected to increase the release of neurotransmitter mainly endogenous catecholamines (190-194). The administration of MX(s) increases the amount of glutamine in the whole brain of mice, while the amount of GABA and glycine are decreased, particularly in the different areas of the posterior part of the brain (195). Modifications in the concentration of these two inhibitory neurotransmitters amino acids could be the source of an increased excitability of CNS (195). MX(s) also competitively inhibits [3H]-diazepam binding, an effect which may be related to its convulsant effect when administered acutely at high doses. The affinity of MX(s) for the receptors of benzodiazepine is, however, lower than that for adenosine (194, 196-199). Very recently, it has been found that MX(s)-induced release of Ca²⁺ from intracellular stores in mammalian neurons inhibits the GABAergic activity through the desensitization of GABA_A receptor (200).

MX(s) / caffeine increases serotonin (5-hydroxytryptamine or 5-HT) concentration in the brain stem, especially in raphe nuclei, and in cerebral cortex and cerebellar regions of the brain from the 1st day of exposure (201, 202). MX(s) / caffeine reduces 5-HT availability at postsynaptic receptor sites (203), effects on motor function and other physiological change regulated by 5-HT as mood and behaviour. MX(s) increase(s) the out flow of Ach from the cerebral cortex (204) and produce(s) either activation or inhibition on Ach release in brain slices; depending on the caffeine concentration and / or frequency of electrical stimulation of the slices (179, 205) under specified experimental condition.
Caffeine can modulate the effects of carcinogenic agents in animals (206, 207). Thus, caffeine potentiates the carcinogenic effects of a number of chemical substances or physical inducers (208-214). Caffeine can increase the incidence of mammary gland carcinogenesis induced by administration of 7, 12-dimethylbenz[a]anthracene (DMBA) in rats. However, this increase is significant only when caffeine treatment begins 3 days after administration of the carcinogen, i.e. during the promotion phase of the tumor (215-218).

**Possible carcinogenic effect of caffeine**

Epidemiological studies have shown that consumption of caffeine (250 - 500 mg/l) is associated with development of cancer of pancreas, kidney, lower urinary tract and ovary (3, 219, 220). Caffeine at doses of 0.05% in drinking water stimulates spontaneous mammary tumorigenesis in mice (221) and is able to accelerate pancreatic carcinogenesis in hamsters when administered during the post initiation phase of the tumor (222). Pituitary adenomas were observed in female rats after 12 months of caffeine administration at doses of 2 g/l in drinking water (223).

At a very high doses (250-500 mg/l), caffeine appears to have some teratogenic activity in mammals (3) because of its structural similarity with purine bases of deoxyribonucleic acid (DNA) (224-226). But these deleterious effect of caffeine are observed only with the concentrations that are much in excess of those that follow the ingestion of beverages and medicine (3). Welsch et al. (218) have shown that caffeine can modify the tumorigenic process depending on the time-span of caffeine treatment, the dose of caffeine used and the animal model examined.

Caffeine has been reported by several authors to antagonize the carcinogenic effect of chemicals *in vitro* and *in vivo*. Caffeine suppressed the carcinogenic effect of cigarette smoke condensate on mouse skin (227), and antagonized 4-nitroquinoline-1-oxide (228) and urethan-induced (229) lung tumorigenesis in mice. It inhibits or delays development of breast cancer in rats (230, 231) or mice (232). When caffeine is associated with a diet high in unsaturated fat, duration of tumor development is reduced considerably as compared to exposure to one of the two agents taken alone (218). The effect of caffeine with other anticarcinogenic agents has also been studied in cultured cells of human osteosarcoma (232) and in patients with osteosarcoma (233), human bladder cancer cells *in vitro* (234) and in animal tumor models (235, 236). Caffeine stimulates the tumor inhibiting effect of several substances, such as cyclophosphamide, mitomycin C, adriamycin,
cisplatin, bleomycin, pleomycin, thiothera and nitrosurea derivatives etc. (233-237). Therefore, these studies suggest that caffeine could be used to enhance the antineoplastic properties of medications in the treatment of cancer (233-237). However, when administered in conjunction with chlorpromazine and nitrosurea, caffeine failed to improve treatment outcomes in humans with metastatic malignant melanoma (238). Likewise, caffeine has no synergistic effect with two other antitumor agents, vincristine and methotrexate (233, 234).

Recent studies have suggested that caffeine or pentoxifylline may reduce or partially prevent late radiation injury in animals and humans (239-242). In the recent study, an increased caffeine intake at the time of radiotherapy is related to decreased incidence of severe late radiation injury in cervical cancer patients (242). Again, it has been shown that caffeine inhibits cancer formation by delaying induction of chemical carcinogenesis of lung (228) and skin tumors (227) in mice. This effects vary in intensity as a function of the time at which the cells are exposed to caffeine as compared to the acting time of the mutagenic agent (243). Inhibitory action is maximal when caffeine is administered 2 h before the genotoxic agent, but it is less marked when the two substances are applied simultaneously. It is nil when caffeine is administered 2-4 h after the genotoxic agent (207).

Although the molecular mechanism by which caffeine post-treatment decreases cell survival is not known, it is generally believed to be caused by caffeine - induced inhibition of post replication repair (244-246), i.e. (a) caffeine inhibit post replication repair synthesis of DNA (247), a phenomenon which itself is poorly understood in mammalian cells or (b) inhibition of ploy (ADP-ribose) polymerase (248). Other studies suggest modifications in S phase (DNA synthesis phase) events of DNA replication such as (c) increased number of sites for DNA synthesis in damaged replication units or replicons (249, 250) or (d) antagonism of the DNA-synthesis inhibition that is induced by DNA damage (251). An alternative mechanism is that caffeine act in G₂ (the interval between the end of DNA synthesis and the begining of mitosis) to induce cells to undergo mitosis before the completion of DNA repair (252, 253). The G₂ phase of the cell cycle has been generally observed to lengthen in many normal or malignant cells exposed to radiation, alkylating agents, or other antineoplastic drugs (254-256). According to this model, caffeine do not necessarily inhibit biochemical DNA repair processes per se ; instead, they act by reducing time available for repair, possibly through a protein which is altered directly or indirectly by DNA damage and which controls the transit from G₂ to mitosis (253, 254). In BHK cell line, caffeine allowed G₂-delayed cells to reach mitosis without
finishing the repair process and consequently caused shattered chromosomes, nuclear fragmentation, and cells death (252). This model is consistant with studies using other cell lines, which demonstrated: (a) prevention by caffeine of $G_2$ delay after damage caused by radiation or alkylating agents (257); (b) enhanced radiation-induced cytotoxicity following caffeine treatment in $G_2$ (258, 259); (c) selective increases in alkylator-induced chromosomal aberrations by caffeine in $G_2$ (260); and (d) minimal or no change in DNA repair when caffeine were added for short periods after DNA damage (261). Caffeine can inhibit or delay mitosis in many kinds of cells, probably due to variations in cAMP concentration that influence DNA synthesis and mitosis (225, 262).

In conclusions, it appears that caffeine have generally no mutagenic risk in mammals and humans, because of the rapid metabolism of caffeine in mammals (263). Caffeine is relatively nontoxic, and even at millimolar concentrations it does not immediately affect cell viability (264). Caffeine and its less toxic analogue, pentoxifylline (265), have a propensity to form complexes with other aromatic molecules and reduces the concentration of the free form of the drug in solution. Thus, caffeine when included into solutions containing active aromatic compounds (e.g. intercalating agents, and / or topoisomerase inhibitors), it can be used to reduce the caffeine concentration of the aromatic molecules in free form, and therefore to modulate their activity (266).

In spite of this informations about the actions of caffeine, a single molecular or cellular mechanisms has been difficult to identified, since caffeine exhibit a variety of modifying effects in mammalian cells as described above. Further, Hilf et al. (267) during his studies on the metabolic changes of the normal tissues of the tumor bearing animal using the endocrine organs of the host have considered elevation of corticosterone status as one of the major index for measuring the stress-induced response. Begg (268) reported that during development of tumor growth, adrenal shows its hypofunctional state by decreasing adrenal osmophilia, reduction in ascorbic acid and cholesterol content for the formation of corticosterone. Again, it is known that development of implanted inducible tumors is a result of stress (269, 270). Stress has been also found to modulate the central GABA binding to its receptor complex (271). Further, it is known that stress induces hypothalamic pituitary adrenocortical (HPA) axis and elevates adrenocorticotropic hormone (ACTH) secretion as well as its level (272) and this stress-induced induction of HPA has been found to be suppressed by GABA (273). Caffeine has been found to affect central GABA. In caffeine nontolerant condition, GABAergic activity is
reduce to cause an increase in LA (129). But in the caffeine tolerant condition, GABAergic activity is pushed up to the control values to restore caffeine-induced LA to that of control values (123). It is well known that induction of superoxide radical and related oxygen species cause cell damage, which have been found to be involved in the formation of malignancy (274-276). It is also known that caffeine acts as an inhibitor of lipid peroxidation (LP) (277) and antioxidant due to its ability to scavenge potentially damaging 'OH and electrons (278).

Considering all those informations, it may be stated that no systemic studies have been done on the effect of caffeine at a dose generally consumed by the people per day in relation to the development of carcinoma induced by chemically or by other means. Therefore, in the present investigation, the object is to find out the effect of caffeine during the development of Ehrlich ascites carcinoma (EAC) cells in female mice.

In order to achieve the above mentioned aspect of caffeine actions, the present investigation has been carried out on the effect of long-term consumption of caffeine at the level of the

(a) development of EAC cells in female mice

(b) activities of hepatic antioxidant enzymes of Ehrlich ascites tumor-bearing mice

(c) whole brain GABAergic activity during the development of EAC cells.