CHAPTER V
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Findings of the present investigation covering different physiological parameter has clearly revealed certain degree of toxic injury induced after caffeine consumption in the experimental mammal and a partial inhibition of these toxic damages following simultaneous application of dietary ascorbic acid. Further, the findings also depicted recognizable toxic effects following ingestion of caffeine in physiological, biochemical and histological level. Some of the significant physiological alteration as appeared in present experimental results is also in conformity with earlier findings, although some contradictory results has also been recorded. As scientific conception changed gradually and new information added in scientific research, but action of caffeine on some selected parameters are still contradictory and need further detailed investigation in this line.

Caffeine action on food and water intake also studied previously and reported that caffeine showed anorectic effect on rat. This anorectic effect of caffeine was biphasic being significant at 0.5 and 1 hour after the caffeine administration, than vanishing for three hours and significant again 6 hours after the caffeine administration (Racotta et al 1994). A group of scientist studied the relation between food intake and caffeine consumption and reported that consumption of food caused greatly increased water and caffeine consumption (Newland et al 1992). Current
observation was also inconsistent with earlier findings as the animals refused to take food after caffeine administration. Impact of caffeine on respiration was already studied and reported that caffeine directly effect the respiratory center of medulla. (Chopra et al 1984).

Caffeine increases the locomotor's activity (Holtzman et al 1991). It was established fact that caffeine effects hypothalamus pituitary adrenal axis and thus exerted a behavioral change (Marzouk et al 1991). Rats also showed spasmodic movement of limb after caffeine consumption. Loss of weight due to caffeine consumption was also analyzed and reported that caffeine in acute dose can reduced the rate of body weight.

Biological system is a complex one, different physiological processes are linked in a chain like fashion, therefore, shift in one system can interfere with others and caffeine can cause a significant change in this manner in different metabolic pathways. Liver represents the principle center of metabolism and main detoxificating organ of the body. Biosynthesis and conversion of different blood constituents and detoxification of undesired metabolites or foodstuffs are among primary functions of the liver, which is being regulated by the enzymatic activity. Caffeine is metabolized in the liver. However, the magnitude and severity of toxic effect depend on concentration of toxicants in body fluids half-life and the rate of metabolic conversion.
Metabolism of caffeine was studied in isolated rat hepatocytes and detected different metabolic intermediates mainly 1,7 dimethylxanthine, 3,7 dimethylxanthine, 1,3 dimethylxanthine and 1,3,7 tri-methyl uric acid. It was also reported that two pathways of caffeine metabolism namely N demethylation and C-8 oxidation appeared. They also reported that capsaicin stimulated both the pathways of caffeine metabolism while dehydrocapsaicin seems to be inhibit N-demethylation pathways without effecting C-8 oxidation route (Bouraoui et al 1995).

Metabolism and elimination studies of caffeine demonstrated that caffeine activates CYP3A2 activity. Studies conducted with antibodies directed against cytochrome P450 reductase and cytochrome b5 indicated that the later protein was involved in microsomal cytochrome 450 activation by caffeine. Anticytochrome b5 (antibody) caused a 50 percent decrease in the extent of caffeine mediated cytochrome P450 activation (Caroline et al 1997). Metabolism of caffeine was studied in Wistar rat in different strains and reported that in some strain C-8 hydroxylation of caffeine had larger V max than Wistar rats (Morita et al 1998). It was also reported, hepatic caffeine metabolism reduced in hypophysectomized rat treated by SC, ACTH and T4. (Bienvenu et al 1990)

Desmond (1980) reported the effect of cirrhosis on the deposition and elimination of caffeine. Elimination half-life was prolong in cirrhotic than normal. The plasma clearance was higher in control as compared with cirrhotics. The plasma binding of caffeine was also lower in cirrhotic. The plasma clearance of unbound
caffeine therefore was reduced in cirrhotic demonstrating impaired elimination of caffeine in cirrhotic. As the caffeine level was found significantly higher in caffeine fed rat as compared to the control in the present study which could be explained on the earlier findings that following caffeine ingestion caffeine might be bound to the blood protein thereby causing an elevation of caffeine in blood. Vitamin C supplemented group showed a declined in caffeine level as compared to only caffeine treated rat which might be due to the detoxifying effect of vitamin C. Fluctuation of caffeine level in blood with the progress of treatment as significantly (P<0.01) higher level of caffeine was observed again on day 30 of caffeine administration which might be due to accumulation in blood and surpassing the tolerance level. However elevation of caffeine level in blood even after dietary vitamin C supplementation in the present study needs further investigation in this line.

Ascorbic acid appeared as an important reducing agent involved in activation of detoxifying enzymes. Impaired uptake of ascorbic acid by cell due to presence of some chemicals, which interfered with membrane transport system caused an elevation of blood ascorbic acid with concomitant depletion of tissue ascorbic acid. Dietary supplementation of ascorbic acid helped in uptake of ascorbic acid by cells and therefore level of serum ascorbic acid manifested in high level. Result of present experiment indicated that after caffeine administration serum ascorbic acid level elevated significantly which was due to impaired vitamin uptake by cells. Caffeine is a causative agent of hyperglycemia an elevated glucose might be a factor that utilized the membrane transport system of vitamin C (Garg et al 2000). There is a report that
in diabetic condition, supplemented ascorbic acid competes with glucose transport and ascorbic acid transported into the cells results in depletion of serum ascorbic acid level. In the present experiment the higher serum level of ascorbic acid almost remained through the experimental period.

Blood glucose level was found to be elevated in caffeine fed and also in caffeine and ascorbic acid supplemented rats in present investigation. Further a decline in blood sugar level following prolong exposure to the caffeine and caffeine plus vitamin C in present study indicate homeostatic adjustment of glucose level due to hormonal interaction. It was also reported that caffeine and other methylxanthine caused modest elevation of arterial blood glucose. Adenosine and its antagonist had been shown to increase hepatic glucose release. Besides adenosine antagonism can reduced glucose uptake by contracting rodent muscle as reported. It was possible that caffeine induced increased in adrenaline that enhanced hepatic glycogenolysis. (Vergauwen et al 1997). Liver phosphorylase activity is stimulated by AMP. It is inhibited by ADP, ATP and glucose. Liver phosphorylase inhibitor neutralizes the stimulatory effect of AMP. Xanthine and other xanthine derivatives strongly inhibited activity and inhibition was independent of other effectors. (Ercan Fang et al 1997). Glucose induced insulin release exhibits a diminished potentiatory responsiveness to acetylcholine but not to caffeine. (Shi 1997).

The glucose level in blood was under control of different factors and studied by different workers. Glucose uptake by adipocyte was controlled by cyclic
AMP, thyroid hormone and insulin each of which can act independently. Maximum glucose uptake was achieved in presence of a combination of low concentration of cyclic AMP, of insulin and in absence of thyroid hormone. (Correze et al 1979). Glycogenolysis, a process of carbohydrate metabolism mostly regulated by glucagon and caffeine, which is also known to inhibits c AMP phosphodiesterase also potentiated the stimulatory effect of glucagons on the glycogenolysis in a dose dependent manner (Ogihara 1993).

In the present investigation, at the onset of the caffeine exposure an increase in the blood insulin level was recorded with simultaneous increase of blood glucose level. However in the subsequent period, the glucose level declined towards control while in blood insulin level was still maintained higher in control to conventional fact that insulin level fluctuates along with the fluctuation of blood glucose level.

Release of insulin after caffeine consumption was already experimentally proved. Increase of insulin secretion, following caffeine administration was established and presented in the third International Caffeine Workshop held in 1980 and report was published by Femstron (1981) in Massachusetts Institute of Technology, Cambridge. Although, regulation of glucose level was primary function of insulin, but other functions also regulated by insulin. Sometimes, especially in stress condition resistance of insulin also developed. (Tripathi 1994). Although, release of insulin following caffeine consumption was confirmed but real process was still to be
remained for investigation. Already established fact was that caffeine caused glucose induced insulin release. It was well established fact that caffeine had a protective capacity on β cells from inhibitory action of alloxin (Lacy et al 1975). It was also proved that caffeine caused increased blood glucose level, non-esterified fatty acids and insulin secretion (Avogaro et al 1973)

It was suggested that thyroxine modify insulin release in response to glucose. There was a relation between alteration of serum insulin level and production of urea. As the insulin level depleted production of urea enhanced due to breakdown of amino acid and protein and initiation of gluconeogenesis. This experimental finding was not inconformity with present result.

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Soeren and Graham (1988) studied the insulin response in rat with exercise and reported that insulin concentration declined with exercise and there were no significant difference in any of the trial after ingestion of caffeine. In an early report it was found that during exercise, insulin level declined and simultaneously applied glucose polymer maintain the insulin and glucose level, while the application of caffeine did not able to maintain insulin level.
Metabolic rate and substrate utilization in normal weight man after caffeine consumption was studied considering insulin, glucose and free fatty acid as a parameter and found that plasma glucose, insulin and carbohydrate oxidation did not change significantly. (Acheson et al 1980)

In an another observation on the effect of caffeine on insulin and glucose and reported that after caffeine ingestion there was an initial hyperinsulinemia followed by progressive fall of plasma insulin level to subnormal level. This fall in plasma insulin coincide with depletion of pancreatic insulin store and thus enhanced sensitivity of insulin secretory process without a change in sensitivity of biosynthetic process. (Dunlop et al 1982). Intravenous administration of caffeine caused an increased in serum concentration of glucose, insulin, urea and free fatty acid. (Sachs and Forster 1984) After study of medical sport report, in the area of intravenous caffeine on liver glycogenolysis during prolong exercise and found no change in level of glucose and insulin. (Winder 1986).

Caffeine caused no change in insulin level although in some cases glucose level changed due to fall of plasma norepinephrine and increase of. Calcium agonist enhanced the insulin secretory responses (Taljedal et al 1994). Caffeine also contributed release of calcium which had impact on secretion of epinephrine and nor epinephrine both of them regulate glucose level in blood. Molecular and biochemical studies revealed that caffeine caused depletion of sarcoplasmic reticulum calcium stores. (Janiac et al 2001)
In the present experiment in case of serum protein level no significant difference was observed between treated and nontreated rats upto 60 days of exposure. Therefore from the result it appeared that caffeine was unable to alter the protein metabolism upto 60 days of exposure. However the present study revealed significantly lower level of blood protein following 90 days of treatment groups which may be explained on the basis of cytotoxic effect of liver and kidney.

Caffeine caused the hypothyroidism, which also effects the elevation of the urea in blood. Further, in hypothyroid condition increase of gluconeogenesis caused on amino acid breakdown and also amino acid deamination both of which contribute urea elevation. Cerebral blood flow also effected after caffeine administration in normoglycemic and hypoglycemic rat (Harinaka et al 1997). Further, amino acid breakdown enhanced in hypothyroid condition. Literature clearly indicated that caffeine consumption related with hypothyroid and decline of protein level might be associated with hypothyrisism after caffeine consumption.

It was already established that caffeine had an impact on cholesterol level in blood. Caffeine directly antagonized adenosine A1 receptors on adipocytes and caused increased plasma free fatty acid concentration. Again, it was also reported that caffeine had no impact on serum cholesterol level when rats were given cholesterol free diet, but elevated cholesterol after cholesterol rich diet (Rakicioglu et al 1998). In the present experiment, it was found that cholesterol level declined after caffeine consumption and this finding was although supported by most of earlier workers but
contradictory information also available regarding caffeine consumption and elevated blood cholesterol level. Recently, it was reported that enzyme cytochrome P 450 was associated with the lower level of cholesterol in blood. Cholesterol metabolism was regulated by liver microsomal enzyme, cytochrome P 450 (7α hydroxylase) and brokendown the cholesterol into bile acid. Lower level of cholesterol after caffeine consumption was probably associated with cholesterol metabolism. Since caffeine activates cytochrome P450 7α hydroxylase, therefore, caffeine enhanced cholesterol metabolism resulted in declined in blood cholesterol level. (Srabani 1997).

Endocrine glands are regarded as vital center and controlling factor of the body functions which are responsible for regulation of enormous activities and in regulating many physiological activities following interaction with the exogenous chemicals. The toxicity of caffeine was observed in some selected endocrine glands.

Goitrogenic nature of caffeine was already furnished in the literature and also in conformity with this experimental result, which indicated toxic impact of the drug on thyroid gland in experimental mammal (Wolff and Varrone 1969).

Almost all goitrogens appeared as hypercholesterolemic (Rao 1986) but caffeine appeared as an exception as also found in the experiment but further study was required for clear sinereo. Caffeine impaired the serum thyroxine level either by breakdown of thyroxine in to bile or synthesis and secretion of thyroid hormone. There was information that stress inhibited thyroxin secretion
(Chatterjea and Shinde 1995). Stress induced vesoconstriction in the thyroid probably decrease the thyroid hormone release. On the other hand glucocorticoids liberated by stress increase urinary excretion of iodine (Chatterjea and Shinde 1995). Under stress enhancement of gluconeogenesis and hyperglycemia is a common feature. There is a perfect regulatory mechanism, which immediately recovers hyperglycemia and brought the glucose towards normal by increased insulin release.

Caffeine in acute and chronic doses caused in the change of growth hormone, thyroxin and thyrotropin. A high dose of caffeine has a biphasic action on T4 with an increase at 4 hours and a decrease in 24 hours as has been reported. (Clozel et al 1983). In our present experiment, it was found that depletion of thyroxine level appeared throughout the experimental period.

Caffeine caused the hypothyroidism, which also effects the elevation of the urea in blood. Further, in hypothyroid condition increase of gluconeogenesis caused on amino acid breakdown and also amino acid deamination both of which contribute urea elevation. Information was available regarding level of serum uric acid and coffee consumption and reported that there was a clear inverse relationship with coffee consumption and level of serum uric acid (Kiyahara et al 1999). There was information that lactic acid competes with uric acid secretion. Uric acid excretion was enhanced by glucocorticoids. In the early period of experiment under severe stress more glucocorticoids perhaps secreted that enhanced excretion of uric acid. In the latter part of the experiment homeostasis mainly stress to a lesser extent and thus
reduced glucocorticoids and than uric acid level was high than middle. Caffeine caused increased uric acid level in urine and might helped in lowering of blood uric acid level. (Sumbaev and Rozanov 1997) Cerebral blood flow also effected after caffeine administration in normoglycemic and hypoglycemic rat (Harinaka et al 1997)

In case of erythrocyte membrane dynamics as revealed by SEM studies also exhibited similar findings as indicated by alteration in the membrane structure by toxic impact of caffeine showing formation of tear drop cell, echynocytes, membrane internalization and ring shaped cell which has been found to be revived to normal discocyte structure following supplementation with dietary ascorbic acid. This findings also supported by earlier findings and publications (Barthakur et al 1999)

Scanning Electron Microscopic examination of organs reveled similar changes as observed in histopathological studies which further suggested that loss of cellular integrity and alterations in cell function had grossly effected which was again confirmed after various biochemical studies. Histopathological studies on liver also reveal the toxic influence of caffeine on liver as recorded by cytopathic change in hepatocytes alteration of liver archetacture and loss of cellularity after caffeine administration which further confirmed by the changes recorded in biochemical parameters due to impaired metabolic function of the liver, which have been found to be restored partially following dietary supplementation ascorbic acid which found to revive the cellular damage leading to restoration of cell functional status, as recorded in the case of urea, uric acid, cholesterol etc. Earlier investigation on the impact of
caffeine established that caffeine induced fatty liver formation. The investigative study suggested that the result indicated an increased peroxidability in the liver of caffeine treated animal due to an increased in the triglyceroid content. A decrease of vitamin E also occurred that also had an impact on lipid peroxidation. (Dianzani et al 1991)

Recently, a group of scientist reported that depress of antioxidative function of liver and reduction of ascorbic acid level were found following administration of some hepatotoxic material (Gonskii I Ya-I et al 1996).

It is established that intracellular cyclic AMP production has the inhibitory effect on cell growth (Lions et al 1989). Caffeine was appeared as intracellular cyclic AMP producer and might be responsible for inhibition of cell growth that impaired normal functioning of different glands like thyroid and liver with declined in the thyroxin level.

In the present histopathological study of kidney reveled the nephrotoxic effect as indicated tubular and glomerular degeneration. Further vitamin C supplemented set the cellular configuration of kidney maintain almost similar to control rat (Curran 1985) indicating protective role of vitamin C at cellular level. In thyroid similar pathological alteration was noted showing cyto and histopathological alteration as compared to control (Norman 1989) following exposure to caffeine. Histological structure of kidney also affected by caffeine as reported in the literature. Caffeine also potentiates
the effect of the non-steroid anti inflammatory drugs mefenemic acid on the rat medulla resulting in the quantitative increase in the interstitial tissue between adjacent afferent and efferent vasa recta (Hewitson et al 1991) In puomycin-aminucleoside (PAN) treated rat caffeine caused more severe tubulointerstitial damage like tubular atrophy, presence of proteinaceous material, tubular dilatation, interstitial inflammation, interstitial fibrosis etc and tend to increase glomerulosclerosis. Literature suggested that caffeine affected the kidney by increased activity of the renin angiotensin system. (Tofovic et al 2000)

Caffeine has an impact on cell growth and division and CDC2 kinase is activated. (Minemato et al 2001)

In case of thyroid, histological structure revealed a clear inhibitory effect on thyroid follicle due to caffeine treatment in terms of cellular configuration and colloid biosynthesis. In ascorbic acid supplemented set the histopaternal of thyroid exhibited similar picture to control rat in respect of cellular configuration and colloidal status.

Ascorbic acid also has hepatoprotective effect, lowering of SGPT, SGOT, acid phosphatase and alkaline phosphatase following ascorbic acid supplementation has been reported (Ghosh et al 1996)

Caffeine ingestion increased the urinary excretion of sulphate, effect that might be related with the diuretic nature of caffeine. (Benincosa et al 1995)
Caffeine also has an impact on contractility of renal arterial smooth muscles and might be appeared due to alteration in intercellular calcium concentration (Murphy et al. 2001).

Ascorbic acid, a glucose derivative, had strong protective power against toxic injury in the body (Schlegel et al. 1970). Some physiological processes were stimulated in presence of ascorbic acid. Ascorbic acid was synthesized in the adrenal cortex and liver (Rao 1986). It was also reported that vitamin C was essential for maintenance of adrenal cortex (Kutsky 1973). Adrenal cortex was greatly effected in presence of caffeine and caused adrenal hypertrophy. A close relationship between carbohydrate metabolism and ascorbic acid was established and reported that reduced liver glycogen, hyperglycemia and reduced sugar tolerance occured in hypovitaminosis C conditions. Earlier observation suggested that serum lipid increased upto 8 weeks following by caffeine administration but urinary ascorbic acid remained same during the experimental period and remained significantly higher with respective control. (Quazi et al. 1985).

Adrenal ascorbic acid was rapidly depleted when the gland was stimulated by adenocorticotropic hormone and some toxicants (Rao 1986). Further study also confirmed the earlier observation and reported that when the adrenal gland was stimulated depletion of adrenal cholesterol and adrenal ascorbic acid were found. (Udupa and Udupa 1991). Ascorbic acid was also essential for formation of adrenaline. (Rao 1986)
Further, dietary ascorbic acid found to exert certain protective role resulting restoration histopathological status of the liver and reduce oxidative stress by quenching free radicals (Garg and Bansal 2000)

Toxic injury in cellular level is generally induced by production of free radicals. Vitamin C as an antioxidant interferes with the process of production of free radicals or act as a scavenger for it thus provide effective protection.

Recently, a group of scientist reported that depress of antioxidative function of liver and reduction of ascorbic acid level were found following administration of some hepatotoxic material (Gonski I Ya-I et al 1996).

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Positive response of ascorbic acid also depends upon the level and metabolism of ascorbic acid, which is governed by catabolism of ascorbic acid in the body. Researchers also observed the ascorbic acid catabolism in male and female organisms (Muddeshwar and Nath 1992). Besides other factors also caused the alteration of ascorbic acid catabolism as reported after different experimental observation.
Abolition of cytotoxic nature of interferon alpha on pancreatic islet by ascorbic acid was demonstrated (Zahair and Mohamed 1998).

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Effect of caffeine and coffee on the metabolic rate were investigated and reported that metabolic rate increased significantly during the three hours after caffeine ingestion, while plasma glucose, insulin and carbohydrate oxidation did not change significantly. Plasma free fatty acids level was accompanied by significant increase in fat oxidation. In obese individuals, plasma free fatty acids level did not change significantly while in normal plasma free fatty acid level changed. They finally suggested that caffeine stimulates metabolic rate in both and normal individuals but fat oxidation appeared to be high in normal individuals than obese. (Acheson et al 1980)

Caffeine inhibited oscillations of calcium induced both by nor-adrenaline and vassopression. They reported that this inhibitory action was not due to inhibition of phosphodiesterase enzymes and elevation of cyclic AMP levels. According to them inhibitory effect of caffeine may result from inhibition of receptor stimulated Ins P3,
formation. Inhibition of agonist of caffeine against stimulated Ca²⁺ influx. Direct inhibition of the Ins P₃ sensitive to calcium release channel. (Combettes et al. 1994).

Vitamin C administration in drinking water 2% w/v was associated with significant decrease in the level of hyperglycemia, hyperlipidemia, and hyperketonaemia. Vitamin C treatment selectively reduced the activity and expression of CYP2E protein (Clarke et al. 1996).

Vitamin C appeared to cause decrease in the rate of lipid peroxidation (Evans 2000). Ascorbic acid requires thyroxine for highest activity (Leboy et al. 1997).

Therefore, from discussion of the above facts and factors obtained from experimental findings it may be definitely suggested that the dietary caffeine induced certain levels of toxic injury in overall physiological system biochemical, histological, and some specific haematological parameters. Caffeine administration for a prolong period in experimental model mammal albino rat found to elevate serum ascorbic acid level due to probable decline in Vitamin C transport into the cell or organ which caused deficiency of intracellular vitamin C level as manifested in the cytopathological changes in micro-anatomical and SEM level.

Cholesterol lowering effect of vitamin C on blood has been established and this finding also in conformity with our present day findings (Baser 1991) besides, report also indicated that daily administration of 0.5 grams ascorbic acid caused a
significant reduction of plasma cholesterol while no significant effect on plasma urea nitrogen uric acid and triglyceroids level (Baser 1991)

Ascorbic acid administration caused an increased in the excretion of uric acid and this findings were established earlier (Sutton et al. 1983) Enhancement of uric acid excretion might be one of the cause of lowering of uric acid level in blood. The present experimental findings are clearly supported by this explanation as simultaneous administration of caffeine and vitamin C showed low uric acid level than control although uric acid was the last metabolic product of caffeine metabolism.

The controlling mechanism of urea level in blood depends on different factors and diet may contribute alteration of some degree blood urea concentration. Ascorbic acid, a dietary supplements expected to has power to declined urea in blood but the findings of the present experiment revealed the information that consumption of ascorbic acid had no impact on elevated urea. The previous reports also supported the present findings. Generally oxidizing agents inhibits urea transport. Urea and water transport appears to takes place through independent vasopressin stimulated pathways and some oxidizing agents inhibit urea transport without inhibiting osmotic water flow. It was reported that cyclic AMP and theophylline inhibit urea transport and this can be brought about by oxidation of one or more components in its transport pathways (Franki et al. 1975)
Certain alteration in biochemical parameter in hormonal level also revealed the protective role of vitamin C against the metabolic alteration induced by caffeine exposure.

The role of ascorbic acid as protective factor for many drugs, pesticides, and heavy metals induced toxicity have been described earlier and the present experiment further confirmed the protective role of ascorbic acid against the caffeine which belongs to methylxanthine group of drugs. In conclusion it may be suggested that the person habituated the caffeine-containing beverage and drugs should be supplemented with dietary vitamin C to prevent the possible toxic impact in some extent.

Therefore a general awareness is required regarding indiscriminate use of caffeine containing beverages and to limit its consumption as much as possible and if absolutely necessary it should be taken along with vitamin C to reduce the toxicity.