5. DISCUSSION AND CONCLUSION
Apoptosis is a genetically programmed cell death mechanism that can be activated by various stimuli, including the activation of specific pro-apoptotic receptors, and cellular stress or injury caused by loss of growth factor signals, heat shock, irradiation, cytotoxic drugs, bacteria, and viruses. Disturbances in mechanisms that direct abnormal cells to undergo apoptosis perilously contribute to tumorigenesis; yield a logical target for potential therapeutic intervention. The mechanism of action of many anticancer drugs is based on their ability to induce apoptosis. There are many mechanisms through which apoptosis can be enhanced in cells. Agents suppressing the proliferation of malignant cells by enhancing apoptosis may constitute a useful mechanistic approach to both cancer chemoprevention and chemotherapy.

In the present work phytochemical profile, cytotoxicity and chemopreventive potential of *Acacia catechu* heartwood and its bioactive constituent, (+)-catechin from aqueous extract of *Acacia catechu* heartwood were studied against multiorgan carcinoma, both *in-vitro* and *in-vivo*, models. The mechanism of chemotherapeutic drug mediated cell killing of (+)-catechin was also evaluated by studying different signalling pathways.

The cytotoxic effect of different catechu extracts on MCF-7, A431 and HepG2 cells were characterized by the trypan blue dye exclusion method and three different colorimetric assays (MTT, SRB and LDH release). A reduction in cell growth and induction of cell death are two major ways to inhibit tumour growth. The cytotoxicity assays employed revealed similar profiles, with the MTT, SRB and LDH assays being the most widely used cytotoxicity assays showing statistically significant IC$_{50}$ values. The results obtained from the three cytotoxicity assays were not in close agreement. This observation can be explained by the nature of each assay. The LDH leakage assay is based on the release of the enzyme into the culture medium after cell membrane damage, whereas the MTT assay is mainly based on the enzymatic conversion of MTT in the mitochondria. The SRB assay is a colorimetric assay measuring the uptake of the dye by metabolically active fixed cells. The inverse relationship between the MTT, SRB and the LDH responses adds credence to the
accuracy of the data. Overall, the aqueous extract of *Acacia catechu* heartwood (AQCE) showed the greatest activity against these cancer cell lines and was selected for further studies. The AQCE was also evaluated for its effect on normal cells. To check this, human peripheral lymphocytes were isolated and the effect of AQCE on the percent viability of normal lymphocytes was calculated. The results showed that AQCE did not show any toxic effect to lymphocytes which suggested that AQCE have no toxicity to normal cells.

As a test to confirm the cytotoxicity of AQCE, cells were incubated with different concentrations of AQCE and their morphological alterations were verified via a phase-contrast microscope. Results from the present study demonstrated that there were marked morphological changes in MCF-7, A431 and HepG2 cells following the treatment with AQCE which were indicative of cell apoptosis. In addition, marked morphological changes that occurred in apoptotic cells were also perceived through Hoechst 33258 (HO) and acridine orange/ethidium bromide (AO/EB) staining and this helped in deducing that the cell death observed was not due to necrosis, but due to apoptosis. It was possible to observe better the difference between early and late apoptosis, showing that AQCE exhibited a larger concentration response effect for late apoptosis and a smaller effect for early apoptosis, in both of the studied concentrations.

The chromatographic profile of aqueous extract of *Acacia catechu* heartwood showed the abundance of (+)-catechin. (+)-Catechin was also isolated from the aqueous extract of *Acacia catechu* heartwood and further evaluated against multiorgan carcinoma, *in-vitro* and *in-vivo*.

Imbalances in proliferation and apoptosis, by favoring the promotion of genetically altered cells in a tissue play an important role in cancer promotion [Arun and Hortobagyi 2002]. (+)-Catechin induced a marked concentration dependent inhibition of MCF-7, A431 and HepG2 cell proliferation in various cytotoxicity assays and results suggested that (+)-catechin induced cell death of these cells. As a test to confirm the cytotoxicity of (+)-catechin, these cells were incubated with different concentrations of (+)-catechin and their
morphological alterations were verified via a phase-contrast microscope. Results from the present study demonstrated that there were marked morphological changes in MCF-7, A431 and HepG2 cells following the treatment with (+)-catechin which were indicative of cell apoptosis. The morphological changes that occurred in apoptotic cells were also perceived through Hoechst 33258 (HO) and Acridine orange/ethidium bromide (AO/EB) staining and this helped in deducing that the cell death observed was not due to necrosis, but due to apoptosis. (+)-Catechin exhibited a larger concentration response effect for late apoptosis and a smaller effect for early apoptosis.

Formation of DNA fragmentation is one of the characteristic features observed in apoptotic cells and it is generally considered as the biochemical hallmark of apoptosis [Fink and Cookson, 2005]. The formation of a DNA ladder has been widely used as a distinctive marker of the apoptosis process [Ariffin et al., 2009]. The ladder-like appearance of DNA observed in the MCF-7, A431 and HepG2 cells treated with (+)-catechin, was also another indicator of the apoptosis inducing capability of the (+)-catechin. In addition, a noticeable phenomenon was that the percent DNA fragmentation was increased after treatment with (+)-catechin when analyzed by diphenylamine (DPA) assay. Data from an apoptosis assay showed that (+)-catechin induced obvious apoptosis in cancer cells, presenting a concentration-dependent manner of apoptosis-specific fragmentation.

Further to study the effect of AQCE and (+)-catechin on multiorgan carcinoma in-vivo, different cancer models were used. In breast cancer, the chemopreventive study in mice showed less gain in the body weight in the carcinogen-treated animals. This observation confirmed the fact that, during cancer progression, there is a decrease in body weight [Veena et al., 2006]. An increase in body weight of the animals treated with AQCE and (+)-catechin compared with DMBA-treated animals suggested their beneficial effect in breast cancer. The results indicated that both AQCE and (+)-catechin treatment caused a significant reduction in tumor multiplicity and final tumor mass. However, (+)-catechin showed more significant reduction in tumor multiplicity and final tumor mass in
comparison to AQCE. Cyst formation, ductal hyperplasia and cell proliferation were observed in carcinogen-treated animals. On treatment with AQCE and (+)-catechin, the histological examination showed the features of a normal mammary gland with normal ductular morphology. As ductal hyperplasia is closely associated with progression of carcinogenesis, a reversion of hyperplastic alterations suggested the chemopreventive efficacy of AQCE and (+)-catechin against DMBA-induced mammary carcinogenesis.

Results from skin cancer model showed a significant increase in tumor latency by administration of AQCE and (+)-catechin in Balb/c mice initiated by DMBA and promoted by TPA. This may be due to the delay in the promotion phase of carcinogenesis. There was decrease in mean tumor burden (84.40% by AQCE and 87.93 by (+)-catechin) by the end of the experiment (20 weeks) and significant reduction in the number of tumors formed per mouse was observed (55.66% by AQCE and 76.74 by (+)-catechin) until the end of week 20. A significant decrease in the final body weights of DMBA/TPA treated mice was observed when compared to their initial body weights indicated a decrease in body growth probably due to tumor burden. However, the administration of AQCE and (+)-catechin depicted a normal growth similar to vehicle-treated mice. The histopathological investigations showed a normal histological pattern in the skin of vehicle-treated animals. Whereas, in DMBA/TPA-treated mice, all the tumors were confirmed to be papillomas showed necrotic keratinised squamous pearls and islands of dysplastic squamous epithelial cells, suggested invasive squamous cell carcinoma. On the other hand, intact basal cell layer and dysplastic lesions characterized benign papillomas in DMBA/TPA/AQCE-treated animals were observed. Tumors of animals treated with DMBA/TPA/(+) catechin displayed intact basement membrane with the shrinkage of tumors. The direct evidence showed that both AQCE and (+)-catechin administration inhibited the carcinoma formation.

In the liver cancer model, the in-vivo chemopreventive study in mice showed that there was a significant decrease in tumor incidence (62.5% by AQCE and 77.78 by (+)-catechin) by the end of experiment (20 weeks) and a significant decrease was observed in
tumor multiplicity (62.14% by AQCE and 68.94% by (+)-catechin) until the end of 20 weeks. A significant decrease in the body weight was observed in the carcinogen-treated animals. However, the administration of AQCE and (+)-catechin increased the body weight in comparison to carcinogen-treated animals, which suggested its beneficial effect in carcinogenesis. Loss of cellular architecture with proliferation of Kupffer cells, severe centriloculobular necrosis, and nodular hyperplasia was observed in all the animals treated with carcinogen. The animals treated with AQCE and (+)-catechin showed a feature of a normal liver tissue with minimal cellular inflammation. A reversion of hyperplastic alterations suggested the chemopreventive efficacy of AQCE and (+)-catechin in HCC. Serum transaminases, ALT, AST, ALP and γ-GT are sensitive markers of liver function and their increased levels into serum indicates damage to the cells and thus injury to liver. In the present investigation, liver cell damage caused subsequent leakage of these enzymes into circulation was observed in carcinogen-treated animals. AQCE and (+)-catechin treatment reverted the enhanced level of these enzymes, which showed that AQCE and (+)-catechin aids in liver cell regeneration and thereby protecting membrane integrity by decreased enzyme leakage. Overall, (+)-catechin showed more significant chemopreventive potentiality against multiorgan carcinoma in comparison to AQCE.

Various studies have reported the significance of sialic acid as a tumor biomarker. Aberrant glycosylation process in tumor cells contribute to the biosynthesis of certain oligosaccharides, hence, malignant or transformed cells contain increased sialic acid residue on their surfaces [Yogeeswaran and Salk, 1981]. A significant increase in the total sialic acid (TSA) and lipid-associated sialic acid (LASA) levels were observed in different carcinogen-treated animals. The increase may be related to the enhanced activity of sialidase enzyme in the tumor formation that causes the cleavage of sialic acid. A marked reduction in the TSA and LASA levels in multiorgan carcinoma indicated the protective potential of AQCE and (+)-catechin. However, (+)-catechin showed reduction in these tumor biomarker more significantly in comparison to AQCE.
It is well documented that oxidative stress contributes to multiple physiological events including cell proliferation and inflammation, mediated by modifying redox sensitive AP-1 and NF-κB pathways. Oxidative stress occurs when the critical balance is disrupted due to excess production of ROS or depletion of antioxidants or both. During oxidative stress, malonaldehyde and other aldehydes are formed in the biological system as a result of lipid peroxidation. The products of LPO are considered mutagenic and carcinogenic as they cause damage to cellular macromolecules by generating ROS [Rao et al., 2006; Vásquez-Garzón et al., 2009].

Free radicals are involved in both the process of aging and the development of cancer; hence substances which possess antioxidant or free radical scavenging activity have an important role in the prevention of cancer. Reactive oxygen species (ROS) normally exist in all cells in balance with antioxidants. Oxidative stress results due to excess production of ROS or depletion of antioxidants or both. During oxidative stress, malonaldehyde and other aldehydes are formed in the biological system as a result of lipid peroxidation [Vásquez-Garzón, 2009]. The current study showed an elevated level of MDA in animals treated with carcinogens only. The increased level of lipid peroxidation may be due to the poor antioxidant defense or inactivation of antioxidant enzymes in cancerous tissues. Both AQCE and (+)-catechin caused a significant decrease in MDA levels, suggested their modulating effect on antioxidant system and ultimately protective potential against carcinogenesis. Nitric oxide radical (NO·) plays an important role in physiological and pathological processes. An increased nitrite level is generally associated with the process of carcinogenesis [Esme et al., 2008]. The present results showed that nitrite levels (an indicator of NO·), were significantly increased in carcinogen treated groups. This increase may be related to an increase in nitric oxide synthase (NOS) activity. A significant decrease in nitrite levels was observed in different tissues on AQCE and (+)-catechin treatment.
Body's antioxidant defense systems operate for scavenging ROS to prevent oxidative stress. The antioxidant enzymes (SOD and CAT) act as the first line of cellular defense against oxidative damage [Ferreccio et al., 1998]. Administration of different carcinogens decreased the activities of these antioxidant enzymes in different tissues, which may be related to saturation of SOD with superoxide radicals in tumor cells or a decrease or loss of expression of SUJ. A decrease in SUJ activity will result in decreased production of $H_2O_2$ which in turn affects CAT activity. The second line of cellular defense includes glutathione antioxidant system that plays an important role against free radicals [Bitensky, 1990]. Reduced glutathione and its dependent enzymes, GPx, GR and GST serve as reliable markers of chemoprevention [Dasgupta et al., 2004]. The present study showed significant decrease in the activities of GPx, GR, and GST after carcinogens exposure, resulting in considerable decline in the activities of these enzymes. In response to oxidative stress, GPx works in tandem with CAT to scavenge excess of $H_2O_2$ and lipid peroxides [Chen et al., 1995]. However, unlike CAT activity, GPx activity also depends on the balance between the levels of GSH and GSSG. Thus, decrease in GPx activity may be implicated in both free radical dependant inactivation of enzyme and depletion of its co-substrates, i.e., GSH and NADPH. The observed decrease in GPx activity may also be due to reduced availability of GSH. A significant decrease in GR activity may be attributed to the impaired conversion of GSSG into GSII and thus balancing GSII/GSSG ratio [Pollak et al., 2007]. At the same time, thiol groups have ability to act as reducing agents and thus play a central role in coordinating the antioxidant defense system [Sen, 1998]. The present findings revealed the alterations in the levels of T-SH, GSH, and Pr-SH in carcinogen-treated animals. The decrease in T-SH content in carcinogen treated animals might be contributed to reduction in GSH levels and/or change in Pr-SH groups. The observed decrease in GSH levels may be due to diminished activity of GR, which is a crucial enzyme for maintaining GSH/GSSG ratio in the cell. The restoration in the activities of these enzymes after AQCE and (+)-catechin administration indicated that both AQCE and (+)-catechin acts as an
effective antioxidant. However, (+)-catechin restored these enzymes more significantly in comparison to AQCE.

The sensitivity of cells to any of the stimuli may vary depending on factors such as the expression of pro- and anti-apoptotic proteins. Experimental evidence suggests that apoptosis can be mediated by number of different ways and that there are numerous regulatory molecules associated with those paths. The mitochondrial apoptotic pathways and death receptor pathways are the two major pathways that have been characterized in mammalian cells. The mitochondria have a central role in regulating the caspase cascade and apoptosis [Hengartner, 2000]. Caspases have a central role in the apoptotic process in that they trigger a cascade of apoptotic pathways [Shah et al., 2003]. The release of cytochrome-c from mitochondria leads to the activation of procaspase-9 and then caspase-3 [Hengartner, 2000]. The activation of caspase-3 is an important downstream step in the apoptotic pathway [Earnshaw et al., 1999]. In addition, the effector caspase, caspase-3, and the initiator caspases, caspase-8 and -9, are the main executors of apoptosis [Riedl and Shi, 2004]. Caspase-8 is in the death receptor pathway whereas caspase-9 is in the mitochondrial pathway, and both pathways share caspase-3 [Pommier et al., 2004].

Catechin compounds including (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC) and epicatechin-3-gallate (ECG) have been shown to exhibit cytostatic properties in many tumor models [Nakachi et al., 1998; Zhang et al., 2009]. Most studies have investigated the different synergistic bioactivities of all compounds or have been focused mainly on the role of EGCG. EGCG conjugated with capric acid has been shown to be the catechin that causes a loss of mitochondrial membrane potential, release of cytochrome-c, activation of caspase-9 and -3 in U937 cells which eventually lead to apoptosis via intrinsic pathway. In addition, EGCG conjugated with capric acid also activates the extrinsic pathway as demonstrated by the time-dependent increase in Fas expression and caspase-8 activity [Ahmeda et al., 2010].
p53 is one of tumor suppressor genes in human cancers and both extrinsic and intrinsic apoptotic pathways are activated by p53. The p53 protein is normally present in minute amounts in cells but when cells are exposed to genotoxic stimuli, p53 levels rise rapidly and initiate a programme of cell death, probably by means of transcriptional regulation. This response is lost in many tumor cells as they have either inactivated their p53 genes by mutation or blocked the activity of p53 through the production of proteins that bind to it and neutralize it [Maximov and Maximov, 2008]. Many chemopreventive agents are known to exert their anticancer effects through the induction of apoptosis via p53 dependent mechanisms. In the present study, we observed the mRNA and protein expression levels of p53 in (+)-catechin treated MCF-7, HepG2 and A431 cells using RT-PCR and ELISA, respectively. We found that (+)-catechin markedly increased the mRNA and protein expression levels of p53 in MCF-7 and HepG2 cells in a concentration-dependent manner, whereas, on treatment with (+)-catechin the levels of p53 was decreased in A431 cells.

In *in-vivo* investigation a significant increase in the p53 mRNA and protein expression levels was seen in carcinogen treated animals. However, a significant decrease in mRNA and protein expression levels of p53 in (+)-catechin treated animals was observed. Similarly, immunohistochemistry studies indicate increased immunostaining in carcinogen treated animals. However, less immunohistochemical localization of p53 was observed in (+)-catechin-treated animals. The inactivation of p53 causes genetic instability leading to the accumulation of genetic alterations, which induce malignant transformation of cells. Mutant p53 proteins that frequently accumulate to high levels in many cancer cells and tumors facilitates tumor initiation and progression by a “gain of function” mechanism (Kaur *et al.*, 2010). (+)-Catechin could inhibit the mechanism by which mutant p53 accumulates in cancer cells.

At the same time, Mdm2 is an important negative regulator of the p53. Mdm2 may impart some of its tumorigenic properties by increasing the degradation of multiple cellular
proteins [Brekman et al., 2011]. Targeting the Mdm2-p53 interaction is a striking cancer therapeutic approach [Shangary and Wang, 2008]. In this study, a significant increase in the mdm2 mRNA expression levels was seen in both cell in-vitro and in-vivo models. However, a significant decrease in mdm2 expression levels was observed on (+)-catechin treatment significantly decreased elevated mRNA expression level of mdm2 in both in-vitro and in-vivo models. Therefore, the data appear to imply that (+)-catechin down-regulated the expression of Mdm2, thus up-regulated p53 mRNA and protein expression levels.

One of the major gene groups that regulate apoptosis is the bcl-2 family and the genes consist of the anti-apoptotic and pro-apoptotic members such as bcl-2 and bax, respectively [Ashkenazi and Herbst, 2008]. Bcl-2 as a key regulator of apoptosis, promotes cell survival either by inhibiting factors for the activation of caspases or by regulation of apoptosis through functional antagonism through the formation of heterodimers with other bcl-2 family members. Bax, on the other hand, binds to the anti-apoptotic bcl-2 protein and thus acts by antagonizing the function of bcl-2 to abrogate apoptosis, indicating that the bcl-2 family regulates a common cell death pathway and functions at a point where various signals converge [Hengartner, 2000]. Induction of bax is also reported to promote cytochrome-c release from the mitochondria, which in turn, activates caspases-9 and -3 which eventually leads to apoptosis [Thomas et al., 2000]. Thus, it has been suggested that the ratio between the levels of pro-apoptotic bax and the antiapoptotic factor bcl-2 determines whether a cell responds to an apoptotic signal. In this study, an apparent increase in the mRNA and protein expression levels of bax and a concomitant decrease in bcl-2 mRNA and protein expression levels, as determined by RT-PCR and ELISA, respectively, were observed in the (+)-catechin-treated cells and animals. Thus, the results suggested that an up-regulation of bax and the corresponding down-regulation of bcl-2 proteins observed in this study may be one of the critical mechanisms through which (+)-catechin induces apoptosis. As the cytochrome-c is released by the induction of bax, which
in turn cause activation of caspases-9 and -3 which ultimately leads to apoptosis. In this study, an obvious increase in the mRNA expression level of cytochrome-c was observed in (+)-catechin-treated cells and animals. Thus, the results suggested that an up-regulation of Bax and the corresponding down-regulation of bcl-2 observed in this study may be one of the critical mechanisms through which (+)-catechin induces apoptosis.

The induction of apoptosis is almost always associated with the activation of caspases; a conserved family of enzymes that irreversibly commit a cell to die. They are cysteine proteases that cleave substrates after specific aspartate residues. The release of cytochrome-c from mitochondria to cytosol after being induced by a variety of apoptosis-inducing agents leads to the formation of apoptosome which forms a platform for the efficient processing and activation of caspase-9. Activation of caspase-9, in turn, cleaves effector caspases such as caspase-3 and 7 which eventually lead to apoptosis [Ghavami et al., 2009]. In the next series of experiment, we assessed the effect of (+)-catechin on the cascade of caspases. To investigate the effect of (+)-catechin on the caspase cascade, mRNA expression levels of caspase-3, -7, -8, and -9 and protein level of cleaved caspase-3 were determined in our experiments. Results from the present study demonstrated that mRNA expression levels of caspase-3, -7, -8 and -9 and protein level of cleaved caspase-3 were increased in both in-vitro and in-vivo models on (+)-catechin treatment. The activation of caspases-3, -7 and -9 is a result of the induction of the intrinsic pathway, while activation caspase-8 and then caspase-3 and -7 may be the result of the induction of the extrinsic pathway. In both pathways, the initiator caspase cleaves and activates downstream effector caspases, such as caspase-3 and -7. It could be concluded that (+)-catechin produced its anticancer effect by inducing apoptosis through the mitochondrial pathway of apoptosis. However, increased expression of caspase-8 with (+)-catechin suggested that extrinsic pathway may in part have contributed to the (+)-catechin-induced apoptosis.

Activated protein-1 (AP-1) and nuclear factor-κB (NF-κB), two of important transcription factors, play important roles in signal transduction pathways of cell
differentiation, proliferation and apoptosis in response to a variety of physiological and pathological stimuli [Angel and Karin, 1991; Bellas et al., 1997]. AP-1 activation requires Jun and Fos through the formation of homo and hetero-dimers, and regulates transcription of a broad range of genes involved cell differentiation and proliferation. Expression of the various AP-1 factors is differentially regulated during cell-cycle progression and in response to many stimuli [Angel and Karin, 1991]. In the present study, we observed the mRNA expression levels of c-jun and c-fos (homodimers of AP-1) and protein expression level of c-jun in (+)-catechin treated cells and animals using RT-PCR and ELISA, respectively. We found that (+)-catechin markedly decreased the mRNA expression levels of c-jun and c-fos and protein expression level of c-jun in both cells and animals. These results were also supported by immunohistochemistry studies which had shown the immunohistochemical localization of c-jun which was found to be increased in carcinogen treated animals. However, less immunohistochemical localization of c-jun was observed in (+)-catechin treated animals. Considering the important role of AP-1 in tumor promotion, this inhibition may be functionally related to its chemopreventive effect.

NF-κB plays a pivotal role in initiation and progression of cancer and is thought to upregulate expression of genes that cause suppression of the apoptotic response in cancer cells [Bellas et al., 1997]. Several genes involved in cellular transformation, proliferation, invasion and angiogenesis are regulated by NF-κB. Oxidant stress can result in degradation of cytoplasmic NF-κB inhibitor, IκB and its translocation to the nucleus [Bellas, 1997].

In the present study, a significant increase in the mRNA and protein expression levels of NF-κB (p65) was observed in multiorgan carcinoma. This indicated the activation of NF-κB, which is probably due to inhibition of IκB protein that resides in the cytoplasm and hence, increased level of p65 expression in the nuclear fraction. The constitutive activation of NF-κB also appears to have role in cell proliferation. However, a significant decrease in the mRNA and protein expression levels of NF-κB (p65) was observed in (+)-catechin treated cells and animals. Similarly, immunohistochemistry studies indicated intense
localization of p65 in carcinogen-treated animals. However, weak immunohistochemical localization of p65 was observed in (+)-catechin treated animals. This may be due to suppression of NF-κB by (+)-catechin. Several chemopreventive agents are inhibitors of NF-κB activation. These inhibitors can block any one or more steps in the NF-κB signaling cascade, the translocation of NF-κB into the nucleus, DNA binding of the dimers and interactions with the basal transcription activation. Thus, blockers of NF-κB should be beneficial not only in prevention but also in the treatment of cancer. Thus, these findings suggested that down-regulation of AP-1 (c-jun and c-fos) and NF-κB (p65) may be one of the critical mechanisms through which (+)-catechin control cell proliferation in these cancer cells.

In recent years, the number of flavonoids has acquired a lot of attention because of their chemopreventive ability against various types of cancers. (+)-Catechin [3',4',5,7-tetrahydroxyflavan-3-ol] is the most abundant polyphenolic flavonoid in the Acacia catechu Willd. heartwood and previous studies of the biological effects of Acacia catechu and (+)-catechin in cell culture and in-vivo models indicated that this compound can inhibit lipid peroxidation. (+)-Catechin is reported to produce antimutagenic and anti-angiogenesis effects in-vivo. However, there is no report on the effect of Acacia catechu crude extracts and its most abundant constituent i.e. (+)-catechin on these studied cancers. Our data demonstrated that both AQCE and (+)-catechin could inhibit the proliferation of different cancer cells in-vitro and suppresses tumor growth in-vivo. However, (+)-catechin showed more promising results against multiorgan carcinoma in comparison to AQCE. (+)-Catechin exhibited its anticancer effects by inducing caspase dependent apoptosis via up-regulation of p53, bax and down-regulation of bcl-2, AP-1 and NF-κB. Therefore, the results from this study provided critically important experimental facts to suggest that (+)-catechin may be a potential therapeutic agent for treating cancer. The proposed mechanism of (+)-catechin against multiorgan carcinoma is shown in figure 5.1.
Figure 5.1: Proposed mechanism of (+)-catechin against multiorgan carcinoma.