CHAPTER 5
PHARMACOLOGICAL ACTIVITIES OF SYNTHETIC NUTRACEUTICALS

5.1. INTRODUCTION
Oxidative damage, inflicted by exceeding Reactive Oxygen Species (ROS) is considered as an important pathophysiologica l condition, promoting cell injury and death in a broad variety of disorders\textsuperscript{1}. Normally, the cell is protected by virtue of an intricate antioxidative system, consisting of enzymatic and non-enzymatic system to maintain redox status homeostasis. Enzymic superoxide dismutase (SOD), catalase (CAT) and non-enzymic reduced glutathione (GSH) play an important role during the process by scavenging reactive oxygen species (ROS) or preventing their formation\textsuperscript{1,2}. However, when the excessive ROS impairs antioxidant defenses or exceeds the scavenging ability of the antioxidant defense system, oxidative stress and injury may be unavoidable.

Alcohol abuse has been linked as a risk factor for various cardiovascular diseases, digestive tract disorder and cancers\textsuperscript{3}. The most extensively investigated aspect of ethanol on health is alcoholic liver disease (ALD), which is one of major causes of illness and death worldwide. In addition, chronic alcohol binging also includes pathological changes and dysfunction of multi-organs, including myocardium damage, renal inadequacy and spermatiferous disorder\textsuperscript{4-7}. 
As an intensive oxidative stressor and hyperosmoic micromolecule possessing both hydrophilicity and lipophilicity, alcohol produces excessive free radicals and ROS during metabolism and results in lipid peroxidation, protein oxidation, DNA damage and adduct formation, by which it induces injury to liver and other organs\textsuperscript{8,9}. However, the
experiments dealing with the influence of alcohol on some oxidative impairment markers have brought controversial results. More importantly, investigations of prophylactic effects of antioxidants on ethanol induce oxidative stress in liver tissues are still relatively rare.

The present study was undertaken to evaluate antioxidant activity of heteroarylhydrazinocurcumin (44a-d).

5.1.1. Antioxidant activity of curcumin

The antioxidant activities of curcumin (41) and derivatives have been investigated by a various in vivo assays. Sharma et al observed that phenolic hydroxyl groups are needed for antioxidant activity. The mechanistic aspects of curcumin antioxidant activity have been analysed by several groups by Wright, Sun et al, Priyadarsini et al, Ligeret et al, Suzuki et al, and Chen et al. The phenolic OH groups are important in the antioxidant activity. The enhancement of antioxidant activity offered by additional hydroxy substituents on the phenyl rings of curcumin-type compounds has been further demonstrated by Venkateswarlu et al. The antioxidant potential of curcumin complexes has been investigated by another approach. The manganese complexes of curcumin and its diacetyl derivative were found to show greater superoxide dismutase (SOD) activity, hydroxy radicals-scavenging activity and nitric oxide radical-scavenging activity than the parent molecules. The copper complex of curcumin also has been found to exhibit antioxidant, superoxide-scavenging, and SOD enzyme-mimicking activities superior to those of curcumin itself. Hydrazinocurcuminoids were also synthesised and investigated for its antioxidant activity by C. Selvam et al.
5.2. RESULTS AND DISCUSSION

5.2.1. General data

The present study was undertaken to assess the response of the antioxidant defense system and the extent of lipid peroxidation in liver of rat after chronic ethanol consumption and heteroarylhydrazinocurcumins (44a-d) prophylactically. Selection of doses of ethanol was based on the research of Husain et al. The dose over 2 g/kg ethanol given orally to rats could yield a plasma ethanol concentration exceeding the threshold level (0.1%) in human beings and have influence on the antioxidant system and lipid peroxidation. The dose exceeding 3.5 g/kg daily might lead to partial deaths with chronic treatment. All the rats survived during the experimental period until sacrifice. There was no statistical significance in weight gain or food and water intake of the rats after chronic administration of ethanol and heteroarylhydrazinocurcumins (44a-d) supplement with normal control.

The food intake and weight gain of ethanol fed rats were slightly lower, this finding is consistent with other reports. Rajasekaran (2000) reported that Wistar rats (60-70 g) treated with ethanol (2 g/kg) showed induced reduction only in food intake but not in body weight gain. A compensation of calorific deficit by ethanol ingestion may account for the lack of reduction in body weight gain despite decreased food intake in these animals. Kumar et al (2002) found that exposure to ethanol (9.875 g/kg) resulted in a significant decrease in the net gain in body weight. This apparent discrepancy is mainly attributed to the different doses of ethanol exposure. Alcoholism, with high dose is associated with increase in metabolic rate and thermogenesis of brown fat but decrease in appetite which, in turn, may be responsible for the decrease in growth of the animals.
5.2.2. The effect of chronic ethanol administration and heteroarylhydrazinocurcumins (44a-d) prophylaxis on GSH level in rat liver

As a water soluble tripeptide, GSH is the most abundant intracellular small thiol molecule and predominant defense against ROS/free radicals in tissues of the body. GSH reacts directly with ROS/free radicals and electrophilic metabolites, protects essential thiol groups from oxidation, promotes the regeneration of $\alpha$-tocopherol and serves as a substrate for GSH – related enzymes GPx (Glutathione Peroxidase) and glutathione S-transferases. A deficiency of GSH puts the cell at risk of oxidative damage. It is not surprising that GSH plays a crucial role in both scavenging ROS and detoxification of drugs and an imbalance of GSH is observed in a wide range of pathologies including alcohol related diseases. Chronic ethanol administration at a dose of 2.4 g/kg for 28 days depleted GSH content of rat liver GSH content of ethanol fed rats decreased in liver as compared with the normal control. Similar results were found in other studies. The primary organ for metabolism of ethanol where free radicals / ROS are generated liver had a higher depletion in GSH which is in agreement with the research of Husain et al (2001). Importantly increased cholesterol deposition in the mitochondrial inner membrane resulting from alcohol – induced liver injury, decrease membrane fluidity and impairs the mitochondrial transport of GSH. Depletion of mitochondrial GSH is believed to further exacerbate hepatic sensitization to alcohol because mitochondria are the major source of ROS production.

In the present study, GSH depletion induced by ethanol is prohibited by compounds curcumin (41), (44b) and (44d) significantly but not by (44a) and (44c). Curcumin (41),
(44b) and (44d) pre administration significantly increased GSH content in liver compared with ethanol intake alone. Curcumin (41), (44b) and (44d) compound may partly suppress GSH deflection by scavenging ROS generated during ethanol metabolism.

5.2.3. The effect of chronic ethanol administration and various heteroarylhydrazinocurcumin (44a-d) prophylaxis on SOD activity in rat liver

SOD is considered as the first line of defense against oxygen toxicity and the control regulators of ROS levels by catalyzing the decomposition of superoxide, the first but most abundant ROS into hydrogen peroxide and water. Over expression of SOD reduced oxidative damage and extends life span while SOD mutation reduces oxidative stress resistance and cell viability with decrease of SOD expression or activity. Prolonged ethanol administration inactivated SOD in liver. Ethanol intake resulted in significant decrease in SOD activity in liver compared with normal control. This finding is supported by a number of reports on liver, heart or testes. Hepatic SOD was inactivated more than that of organs which is in agreement with hepatic GSH alteration induced by ethanol. Inhibition of SOD activity in tissues may be a consequence of decrease of de novo synthesis of SOD protein or irreversible inactivation resulting from overproduction of ROS – during ethanol metabolism. However Husain et al (2001) found that ethanol treatment (2 g/kg for 6.5 weeks) induced hepatic SOD. SOD can be activated to scavenge excessive superoxide in the presence of moderate oxidative stress with compensation. Hence, biphasic fluxes of SOD activity are common and an increase or decrease may relate to the presence of excessive superoxide. Curcumin (41), (44b) and (44d) medication
suppressed SOD inactivation in liver induced by ethanol, indicating that curcumin (41), (44b) and (44d) alleviates the SOD inactivation of ethanol fed rats owing to its antioxidant capacity.

5.2.4. The effect of chronic ethanol administration and heteroarylhydrazinocurcumin (44a-d) prophylaxis on CAT activity in rat liver

CAT is an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes, mitochondria and to some extent in cytosol which catalyzes the reaction of hydrogen peroxide to water and molecular oxygen. By removing hydrogen peroxide, it indirectly detoxifies superoxide radicals which are turned into hydrogen peroxide by SOD. The enzyme also has peroxidase activity and reacts with organic peroxides donors to water and organic alcohols36. CAT is very effective in high level oxidative stress and protects cells from hydrogen peroxide produced within the cell. The enzyme is especially important in the case of limited glutathione content or reduced GPx (Glutathione Peroxidase) activity and plays a significant role in the development of tolerance to oxidative stress. CAT also has a secondary role in the metabolism of ethanol22. In the present study, CAT activity was inactivated in liver. Chronic ethanol intakes significantly decrease the CAT activity in liver compared with normal control. The finding is consistent with other reports24, 29, 37. The decreased level of CAT shows the highly reduced formation of hydrogen peroxide. In this study, curcumin (41), (44b) and (44d) medication supplement seemed to have a small role on reversion of inactivated CAT in liver tissues significantly than compounds (44a) and (44c).
5.2.5. The effect of chronic ethanol administration and heteroarylhydrazinocurcumins (44a-d) prophylaxis on MDA content in rat liver

Lipid peroxidation is used as an index for measuring the damage that occurs in membranes of tissues as a result of free radical infliction\textsuperscript{22}. MDA (Malonaldehyde) as a marker of lipid peroxidation was significantly increased in the liver after chronic ingestion of ethanol in the present study. In this study, MDA level increased significantly in liver, which equivalents, resulting from ethanol treatment have been found in extensive research\textsuperscript{29, 31, 34, 38}. The higher levels of hepatic lipid peroxidation may be linked to the fact that the majority of ethanol metabolism occurs in the liver, as discussed above. In addition, it is known that liver has relatively high concentrations of easily peroxidizable fatty acids and is highly enriched in iron, a metal that in its free form is catalytically involved in the production of damaging oxygen free radical species\textsuperscript{39}. Curcumin (41), (44b) and (44d) prophylaxis treatment decreased MDA level in liver, as compared with ethanol fed rats. Also curcumin (41), (44b) and (44d) prophylaxis decreased MDA level in liver significantly than compounds (44a) and (44c) group as compared with ethanol fed rats. The decreased MDA content in liver tissue indicates alleviated oxidative stress owing to chronic ethanol administration after curcumin (41), (44b) and (44d) pretreatment.

5.2.6. The effect of chronic ethanol administration and heteroarylhydrazinocurcumins (44a-d) prophylaxis on AST & ALT level in blood serum of rat

The hepatic injury induced by ethanol results in an increase in serum, AST and ALT levels. When cell membrane gets damaged, these enzymes which are normally
located in the cytosol leak to the blood stream thus manifesting damage affected to liver tissues. Curcumin (41), (44b) and (44d) prophylaxis decrease the elevation of serum enzymes due to the reduction in cell membrane disturbances.

5.3. CONCLUSION

The present study suggests that chronic ethanol intakes cause oxidative stress on liver of rats. On the other hand, curcumin (41), (44b) and (44d) supplement could partially contribute to the liver defense against oxidative stress. The detoxification action of curcumin (41), (44b) and (44d) were clearly beneficial for ethanol treated rats. And this result also shows that curcumin (41), (44b) and (44d) was significant as compared to the compounds (44a) and (44c). However, the detoxification mechanism at the pharmacological and biochemical level still needs to be elucidated.

Table 5.1. Effect of heteroarylhydrazinocurcumins (44a-d) on ethanol induced free radicals in rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SOD (U/mg of protein)</th>
<th>CATALASE (µM/Min/Mg of protein)</th>
<th>REDUCED GSH(µM/mg of protein)</th>
<th>LIPID PEROXIDATION (nM MDA/g of protein)</th>
<th>AST (U/L)</th>
<th>ALT(U/L)</th>
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<tbody>
<tr>
<td>Group-I</td>
<td>29.80 ± 0.18</td>
<td>284.60 ± 6.22</td>
<td>102.80 ± 1.45</td>
<td>172.87 ± 2.97</td>
<td>192.63± 1.93</td>
<td>84.80 ± 1.05</td>
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<tr>
<td>Group</td>
<td>Value 1 ± Standard Error</td>
<td>Value 2 ± Standard Error</td>
<td>Value 3 ± Standard Error</td>
<td>Value 4 ± Standard Error</td>
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<tr>
<td>Group-II</td>
<td>16.3 ± 0.09&lt;sup&gt;a*&lt;/sup&gt;</td>
<td>196.70 ± 4.67&lt;sup&gt;a*&lt;/sup&gt;</td>
<td>58.55 ± 0.95&lt;sup&gt;a*&lt;/sup&gt;</td>
<td>451.26 ± 6.94&lt;sup&gt;a*&lt;/sup&gt;</td>
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<tr>
<td>Group-III</td>
<td>20.36 ± 0.12</td>
<td>257.90 ± 4.38</td>
<td>90.35 ± 0.52</td>
<td>309.85 ± 4.40</td>
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<tr>
<td>Group-IV-Curcumin (41)</td>
<td>17.68 ± 0.11&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>212.86 ± 3.21&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>63.68 ± 0.55&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>346.71 ± 3.88&lt;sup&gt;b**&lt;/sup&gt;</td>
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<tr>
<td>Group-V-Compound (44a)</td>
<td>16.60 ± 0.08&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>199.93 ± 2.45&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>59.34 ± 0.61&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>435.54 ± 4.65&lt;sup&gt;b**&lt;/sup&gt;</td>
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<tr>
<td>Group-VI-Compound (44b)</td>
<td>17.86 ± 0.10&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>219.96 ± 3.30&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>64.28 ± 0.62&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>351.07 ± 2.69&lt;sup&gt;b**&lt;/sup&gt;</td>
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<tr>
<td>Group-VII-Compound (44c)</td>
<td>16.56 ± 0.10&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>201.90 ± 2.85&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>59.45 ± 0.56&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>425.25 ± 3.10&lt;sup&gt;b**&lt;/sup&gt;</td>
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<tr>
<td>Group-VIII-Compound (44d)</td>
<td>17.94 ± 0.11&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>222.13 ± 4.33&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>65.55 ± 0.75&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>358.70 ± 3.20&lt;sup&gt;b**&lt;/sup&gt;</td>
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• Values are expressed as mean ± SEM.

• No. of animals in each group (n) = 6

• Values were found by using one way ANOVA followed by Newman Keul’s multiple range test.

• (a*) values were significantly different from Normal control (G1) at (P < 0.01).

• (b **) values were significantly different from Ethanol group (G2) at (P < 0.01).

Where,

G1 - Normal control group

G2 - Toxic control group

G3 - Standard group

G4 - Treatment control (Curcumin (41))

G5 - Treatment control (Compound (44a))

G6 - Treatment control (Compound (44b))

G7 - Treatment control (Compound (44c))

G8 - Treatment control (Compound (44d))
5.4. MATERIALS AND METHODS

5.4.1. Chemicals

Thiobarbituric acid (TBA; Research-Lab fine chem. Industries, Mumbai, India), nitro blue tertazolium chloride (NBT; Himedia Laboratories Pvt.Ltd, Mumbai India), 5,5’-dithiobis (2-nitrobenzoic acid) (DTNB; Alfa Aesar, A Johnson Mathey company), 2,4 dinitrophenylhydrazine (2,4- DTNB) (Sigma, India), Hydrogen peroxide, Ellman’s reagent (Sigma India), LIV-52 (Himalaya, India), carboxy methyl cellulose (Research Lab, Mumbai, India) Ethylalchol (absolute ethanol), other chemicals and organic solvents were of analytical grade and purchased from a standard manufactures.

5.4.2. Animals

Male albino Wistar rats each weighing 180-220 were obtained from K.M. College of Pharmacy, Madurai, TamilNadu, India. Rodent laboratory chow was access and water ad libitum, and rats were maintained on a 12 hour light / dark cycle in a temperature regulated room (20 - 25°C) during the experimental procedures. The animals were cared for according to the guiding principles in the care & use of animals. The experiments were approved by the institutional animal ethics committee.

5.4.3. Ethanol ingestion and heteroarylhydrazinocurcumin (44a-d) administration

Rats were divided randomly into eight groups of six animals each and treated for four weeks i.e., for 28 days as follows:
Group - 1  Normal control group received normal saline in a dose of 10 ml/kg.

Group - 2  Toxic control group was administered by ethanol 2.4 g/Kg(30%V/V, 1-0 ml/100kg) orally.

Group - 3  Standard group was administered LIV-52 in a dose of 56 mg/kg orally.

Group - 4  Treatment control group was administered by curcumin (41) in a dose of 10mg/kg dissolved with 0.5ml of DMSO intraperitoneally.

Group - 5  Treatment control group was administered by synthetic compound (44a) in a dose of 10mg/kg dissolved with 0.5ml of DMSO intraperitoneally.

Group - 6  Treatment control group was administered by synthetic compound (44b) in a dose of 10mg/kg dissolved with 0.5ml of DMSO intraperitoneally.

Group - 7  Treatment control group was administered by synthetic compound (44c) in a dose of 10mg/kg dissolved with 0.5ml of DMSO intraperitoneally.

Group - 8  Treatment control group was administered by synthetic compound (44d) in a dose of 10mg/kg dissolved with 0.5ml of DMSO intraperitoneally.
Group 4 to 8 were given the curcumin (41) and heteroarylyhydrazinocurcumins (44a-d) 1 hour prior to the administration of the ethanol. In the entire treatment period, the rats were weighed once in a week and consumption of food and water were monitored every day.

5.4.4. Biochemical analysis

Dissection and Homogenization – After 28 days, all animals were sacrificed by decapitation. Blood was collected and serum was separated for estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The liver was rapidly excised rinsed in ice-cold saline and a 10% w/v homogenate was prepared using (0.15M KCl) potassium chloride. Centrifuged at 800 rpm for 10 minutes at 4°C and the supernatant obtained was used for the estimation of catalase, and lipid per oxidation. Further the homogenate was centrifuged at 1000 rpm for 20 minutes at 4°C and the supernatant was used for estimation of SOD and Reduced glutathione.

5.4.5. Lipid per Oxidation Assay

Quantitative measurement of lipid peroxidation in liver was done by the method of Ohkawa\textsuperscript{41}. Malondialdehyde (MDA), a secondary product of lipid per oxidation reacts with thiobarbitoric acid at PH 3.5. The red pigment produced was extracted in n-butanol-pyridine mixture and estimated by measuring the absorbance at 532 nm.
5.4.6. Superoxide dismutase activity (SOD)

Superoxide dismutase activity was assayed according to the method of Kono\textsuperscript{42}; where in the reduction of nitroblue tetrazolium chloride (NBT) was inhibited by superoxide dismutase and measured at 560 nm spectrophotometer metrically. Briefly the reaction was initiated by addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of liver homogenate. The results were expressed as units per milligram of protein with one unit of enzyme defined as the amount of SOD required to inhibit the rate of reaction by 50%.

5.4.7. Catalase activity (CAT)

Catalase activity was assessed by the method of Luck\textsuperscript{43}, where the breakdown of H\textsubscript{2}O\textsubscript{2} was measured at 240 nm. Briefly the assay mixture consisted of 3 ml of H\textsubscript{2}O\textsubscript{2} phosphate buffer (0.0125M; H\textsubscript{2}O\textsubscript{2}) and 0.05 ml of supernatant of liver homogenate and the change in the absorbance was measured at 240 nm. The enzyme activity was calculated using the millimolar extension coefficient of H\textsubscript{2}O\textsubscript{2} (0.07). The results were expressed as micromole of H\textsubscript{2}O\textsubscript{2} decomposed per min per milligram of protein.

5.4.8. Reduced Glutathione

Reduced glutathione (GSH) in the liver was assayed according to the method of Ellman\textsuperscript{44}. Sample (0.75 ml) of homogenate was precipitated with 0.75 ml of 4% sulphosalicyclic acid and centrifuged at 1200g for 15 minutes at 4°C. The assay mixture contained 0.5 ml of supernatant and 4.5 ml of 0.01 M, DTNB. (5-5’-dithiobis (2-nitro
benzoic acid)) in 0.1 M, phosphate buffer (PH 8.0). The yellow colour developed was read immediately at 412 nm. The results were expressed as micromole of GSH per milligram of proteins.

5.4.9. Determination of AST and ALT

The activities of serum aspartate aminotransferase (AST) and of serum alanine aminotransferase (ALT) were estimated by the method of Reitman and Frankel\(^45\). The enzyme activity was expressed as U/l.

5.4.10. Statistical analysis

The results are expressed as a mean of \(\pm\) SEM. Data was evaluated using one way ANOVA followed by Newman-Keuls multiple range test. Probability values less than (P < 0.01) were considered significant.
5.5. REFERENCES


44. Ellman, G. L. Tissue sulphydryl groups. *Arch. Biochemistry Biophys.* (1959); 80, 70.

SUMMARY

Most of the nutraceuticals have multiple bioactive compounds, which are used to prevent or control the disease or symptom of the disease. Various nutraceutical have proven health benefits against cancer, hepatitis, cardiovascular protective, asthma, anti-inflammatory etc. In the recent years, the nutraceutical based drug designing becomes popular due to the increased success rate and cheaper investment profile. Curcumin is a vital nutraceutical and the main yellow compound of *cucuma longa* rhizome. Curcumin is the main component of curry powder is the mostly modified compound due to its important biological properties. Curcumin has various biological properties which includes antioxidant, antiangiogenic, Alzheimer diseases, nephrotoxicity, arthritis, anti-inflammatory, cardiotoxicity, antifertility, antifungal, antibacterial, cardiovascular diseases, lung fibrosis, antidiabetes, wound healing and HIV replication.

In **Chapter 2**, the five carbon piperidone analogues of curcumin were synthesized and coupled with various sulfonyl chloride, amino acids and dipeptides. In total, thirty novel 3,5-diarylidene derivatives (21a-w), (22a-c), (23a, b) and (24a, b) were synthesized. Curcuminoids namely curcumin (41), Demethoxycurcumin (42) and Bisdemethoxycurcumin were (43) isolated from the dry powder of *cucuma longa* rhizome. The BOC-val-Glycine (32) was prepared by amide coupling and BOC chemistry. This dipeptide was coupled with compound (20b) to get compounds (23b, 24b) by T3P® amide coupling method. These compounds were characterised by using 1H NMR, 13C NMR spectra and also with LCMS.
In Chapter 3, 3,5-diarylidene derivatives (21a-w), (22a-c), (23a, b) and (24a, b) were screened against four bacteria namely *S.typhi*, *V.cholera*, *E.Coli* and *S.aureus*. The compound (21f) was identified as a Lead Compound (LC). The Lead Compound was further optimized with suitable bioisostere based structural modification. The compound (21v) was observed as a potential compound in this series where as the compounds (21s), (21w) and (24b) shows equivalent activity compared to the lead compound (21f).

In Chapter 4, curcuminoids namely Curcumin (41), DemethoxyCurcumin (42) and BisdemethoxyCurcumin were (43) isolated from the dry turmeric powder of cucuma longa rhizome. microwave assisted cyclisation of curcumin leads to ten novel compounds (44a-d) and (45c-h) (Scheme 4.1.). Microwave assisted synthesis improves the yield and drastically reduced the reaction time also. The aryldiazines (48c-h) were prepared by the usual amine to hydrazines via. diazonisation reaction path. The heteroaryl hydrazines (50a-d) were prepared by chloro displacement with hydrazines with mild reaction conditions. Microwave assisted synthesis also allows to acces the new class of curcumin derivatives namely heteroarylhydrazinocurcumins (44a-d) in a convenient manner. The reaction time of microwave assisted heteroarylhydrazinocurcumins (44a-d) was optimized by carrying out a model reaction by synthesizing the compound (44a). This model reaction was monitored by HPLC and the reaction time was optimized. These compounds were characterised by using $^1$H NMR, $^{13}$C NMR spectra and also with LCMS.

In Chapter 5, the heteroarylhydrazinocurcumins (44a-d) were investigated for antioxidant activity *in-vivo* against the ethanol induced free radicals in male albino Wistar rats. The heteroarylhydrazinocurcumins (44b, 44d) were observed as a good antioxidant in
six *in-vivo* assays GSH, SOD activity, CAT activity, MDA content, AST and ALT level. The compounds (44b, 44d) were shown promising antioxidant properties compared to curcumin (41). The compounds (44a, 44c) were shown low antioxidant properties compared to curcumin (41).

In summary, the quality profile, phytochemical profile and bioactive profile of curcumin (41), its derivatives and analogues have been carried out with most modern tools such as LCMS, $^1$H NMR and $^{13}$C NMR. The present investigation on nutraceutical curcumin and its synthetic analogues is a justification to rewrite the monograph of curcumin (41).