CHAPTER 5: DISCUSSION

5.1. PHARMACOGNOSTIC STUDIES

The pharmacognostical study is a major and reliable criterion of identification of plant drugs. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of a crude drugs (Bhattacharya and Zaman, 2009). To ensure reproducible quality of herbal products, proper control of starting material is utmost essential (Venkatesh et al., 2004). Thus, in recent years there has been an emphasis on standardization of medicinal plants, and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. Pharmacognostic studies on different plants has been done by various workers (Khatoon et al., 2006; Abere et al., 2007; Dave et al., 2010; Essiett et al., 2010; Sandhya et al., 2010). According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (WHO, 2002). The flowers of *Woodfordia fruticosa* have three types of matured stomata viz. anomocytic, actinocytic and anisocytic. Stomata is the main factor responsible for the physiological activities of the plant, abnormal stomata is responsible for behavior and hormonal imbalance in plants (Kridemann et al., 2000). In calyx both rosette and cluster type of calcium oxalate crystals were found; these could be used to distinguish the species.

5.2. PHYSICOCHEMICAL ANALYSIS

The physical constant evaluation of the powder is an important parameter in detecting adulteration or improper handling of drugs. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. The moisture content of dry powder of *Woodfordia fruticosa* flowers was 8 % which is not very high, hence it would discourage bacteria fungi or yeast growth (Bhattacharya and Zaman, 2009). The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter
such as metallic salts and/or silica (Musa et al., 2006; Chanda et al., 2010). Low amount of total ash, acid insoluble ash and water soluble ash indicate that the inorganic matter and non-physiological matter such as silica is less in *Woodfordia fruticosa* flowers. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. The variation in extractable matter in various solvents is suggestive of the fact that the formation of the bioactive principle of the medicinal plants is influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive values reveal the presence of polar substance like phenols, tannins and glycosides, as reported by Sharma et al. (2009) and Baravalia et al. (2010).

Heavy metals like lead, chromium and cadmium were absent, while mercury and arsenic were present in very low amount, which was within the permissible limits of heavy metals (The Ayurvedic Pharmacopoeia of India, 2008). Therefore it can be stated that the extract was free from heavy metal contamination.

### 5.3. PHYTOCHEMICAL ANALYSIS

The phytoconstituents are known to play an important role in bioactivity of medicinal plants. In qualitative phytochemical analysis, tannins and alkaloids were present in high amount as compared to other phytoconstituents analyzed. In quantitative phytochemical analysis, phenolic content was much more than flavonoid content. The presence of alkaloids, phenolic compounds, tannins, flavonoids have been associated with various degrees of anti-inflammatory, analgesic (Wang JR et al., 2008) and antioxidant activities (Molina et al., 2003; Gholivand et al., 2010). Therefore, the anti-inflammatory, analgesic and hepatoprotective effects observed in this study may be due to the activity(s) of one or a combination of some of the classes of compounds present in *Woodfordia fruticosa* flowers.

### 5.4. ANTI-INFLAMMATORY STUDIES

It is believed that current anti-inflammatory drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases because of their side effects and...
low potency (Jaishree et al., 2009). As a result, search for other alternatives became necessary and imperative. Novel anti-inflammatory agents could be discovered from medicinal plants containing a wide variety of phytoconstituents. Traditional medicine for the treatment of various diseases is becoming more popular. Many medicinal plants provide relief of symptoms comparable to that of conventional medicinal agents. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *Woodfordia fruticosa* flowers using *in vivo* anti-inflammatory models.

### 5.4.1. Carrageenan induced rat paw edema

Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction, which was discernible within 30 min (John and Nodine, 1999; Marzouk et al., 2010). Carrageenan induced rat paw edema is a suitable *in vivo* model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation (Morebise et al., 2002). Carrageenan-induced hind paw edema in rat is a biphasic event. The early phase (90 - 180 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (270–360 min) is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome (Erdemoglu et al., 2009; Thomazzi et al., 2010). The methanol extract of *Woodfordia fruticosa* flowers inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX-dependent mechanism, thus, it is suggested that the WFM may possess arachidonate COX inhibitory property.

### 5.4.2. Histamine induced rat paw edema

Histamine is another pro-inflammatory mediator involved in exudation and cell chemotaxis (Jutel et al., 2005). The histamine is a basic amine related with
inflammatory and allergic process causing, among several effects, both vasodilatation and increase of vascular permeability (Rang et al., 2001). Edema was reduced by WFM in a dose dependent manner till the end of 5th hour. The antihistaminic activity may be related to the inhibition of inflammation mediator formation. The extract may also inhibit histamine release from mast cells and/or block histamine receptors.

5.4.3. Dextran induced rat paw edema

Dextran is a polysaccharide of high molecular weight that induces anaphylactic reaction after injection in rats extremities, which is characterized by extravasation and edema formation, as a consequence of liberation of histamine and serotonin from mast cells (Van Wauwe and Goossens, 1989; Prakash et al., 2009). Thus, carrageenan and dextran induced models are suitable test procedure to screen anti-inflammatory agents. In this study, WFM exhibited dose-dependent inhibitory effect in dextran induced paw edema and was capable to reduced the inflammation up to 5 h. The ability of the extract to reduce the edema volume suggests that the phytochemicals present in the extract may block or counteract the release of any of those mediators, alone or in combination.

5.4.4. Serotonin induced rat paw edema

The extract effectively suppressed the inflammation produced by serotonin. The extract of Woodfordia fruticosa was able to significantly reduce paw edema, and these effects were similar to those exhibited by the group of rats treated with diclofenac. So it may be suggested that its anti-inflammatory activity is possibly backed by its anti-serotonin activity.

5.4.5. Formaldehyde induced rat paw edema

It is well known that inhibition of formaldehyde induced paw edema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis (Greenwald, 1991). Thus formaldehyde induced paw edema is a model used for the evaluation of an agent with antiproliferative activity (Banerjee et al. 2000). Injection of formaldehyde
subcutaneously into hind paw of rats produces localized inflammation. In the present study, the methanol extract of *Woodfordia fruticosa* flowers significantly inhibited paw edema induced by formaldehyde. WFM showed significant decrease in paw volume till 48 h with both doses, which suggests its long duration of action.

**5.4.6. Cotton pellet induced granuloma in rats**

Cotton pellet-induced granuloma formation is a typical feature of an established chronic inflammatory reaction and can serve as a subchronic and chronic inflammatory test model for investigation of anti-arthritic substances (Panthong et al., 2004). This model has been employed to assess the transudative and proliferative components of chronic inflammation. The fluid adsorbed by the pellet greatly influences the wet weight of the granuloma whereas the dry weight correlates well with the amount of granulomatous tissue formed. The extract showed decrease in granuloma formation. This reflected its efficacy to reduce an increase in the number of fibroblasts and synthesis of collagen with mucopolysaccharide, which are natural proliferative events of granulation tissue formation.

At higher concentration of extract the protein level increased. Increase in protein level at higher concentration of methanol extract of *P. longifolia* was also reported by Tanna et al., (2009). The rise in protein and albumin levels at high doses suggests stabilization of the endoplasmic reticulum, leading to protein synthesis (Mondal et al., 2005).

**5.5. ANALGESIC STUDY**

**5.5.1. Formaldehyde induced paw licking test in rats**

The formaldehyde test has been described as a convenient method for producing and quantifying pain in rats (Dubuisson and Dennis, 1977). The test employs an adequate painful stimulus to which the animals show a spontaneous response and it is sensitive to commonly used analgesics. The pain stimulus, a continuous rather than a transient one, may have resemblance to some kinds of clinical pain and observations are made on animals which are restrained only lightly or not at all (Hunskaar et al., 1985;
Ghannadi et al., 2005). The advantage of the formaldehyde model of nociception was that it could discriminate between central and peripheral pain components. The test consists of two different phases which could be separated in time: the first one that occurs on the first 5 min after the formaldehyde injection was generated in the periphery through the activation of nociceptive neurons by the direct action of formaldehyde and the second phase that occurs between the 15th and 30th minute after formaldehyde injection, occurred through the activation of the ventral horn neurons at the spinal cord level (Tjolsen et al., 1992; Li et al., 2010). In the present study, WFM orally administered 1 h before formaldehyde injection, was capable of inhibiting the paw licking process when compared with the control group. It was observed that rats treated with WFM showed nociceptive reaction, which was dose-related and inhibited in second phase of formaldehyde test. Drugs that act primarily on the central nervous system inhibit both phases equally, while peripherally acting drugs inhibit the second phase only (Shibata et al., 1989). The second phase is an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs (Hunskaar and Hole, 1987; Rosland et al., 1990). The experimental results show that WFM produced better inhibitory effect during the second phase of the formaldehyde test. This experimental evidence suggests that the analgesic effect produced by WFM was involved in its peripheral action.

The anti-inflammatory activity and anti-nociceptive activity of WFM, justifies the traditional uses of \textit{Woodfordia fruticosa} for the treatment of pain and inflammatory related ailments. Previous studies have revealed that the NSAIDs were capable of inhibiting this test (Vinegar et al., 1969; Rosa et al., 1971); Therefore mechanism of anti-inflammation of WFM may be similar to the mechanism exerted by the NSAIDs. Since prostaglandins are known to take part in the inflammatory and nociceptive processes (Marieb, 2000; Katzung, 2005; Zakaria et al., 2010), the anti-inflammatory and anti-nociceptive activities of the WFM could be due to the modulation of the COX or prostaglandins actions.

5.6. HEPATOPROTECTIVE STUDIES

Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, etc. or chemical factors
such as CCl₄ administration, alcohol intake are known to contribute to liver fibrosis. The incidence of chronic fibrosis is high, but there are no satisfactory agents with ascertained effectiveness and with fewer side effects on liver. So, finding effective ways to inhibit liver fibrosis and prevent the development of cirrhosis are of great significance (Wang et al., 2009). The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxic agent is the index of its protective effect (Yadav and Dixit, 2003).

5.6.1. Diclofenac induced hepatotoxicity

Hepatotoxicity is currently a class warning for NSAIDs and infrequent hepatic injury has been observed for nearly all NSAIDs currently on the market. There are 3 drugs that have more commonly been associated with liver disease: diclofenac, sulindac, and aspirin (Purcell et al., 1991; Bjorkman, 1998).

Diclofenac, undergoes similar hepatic metabolism both in rat and in humans. Major metabolic pathways are the hydroxylation in position 4 and 5 and to a much lesser extent the formation of 3′-hydroxy- (humans) and 4′,5-dihydroxydiclofenac (rat and humans). Diclofenac and its metabolites undergo extensive conjugation with glucuronic acid and sulfate (Riess et al., 1978; Stierlin et al., 1979). The major constitutive P₄₅₀ form involved in diclofenac hydroxylation in man is cytochrome P₄₅₀2C9, the human orthologous form of rat 2C11. Diclofenac forms selective protein adducts in livers of treated mice (Pumford et al., 1993; Kretz-Rommel and Boelsterli, 1994). This is caused by a transacylation reaction of its glucuronide conjugate. This mechanism has been proposed to explain both the allergic and intrinsic hepatotoxicity of the drug.

Since unwanted side effects of diclofenac in man and other mammals was reported to occur particularly in the liver (Ramesh et al., 2002; Triebskorn et al., 2004) it was thought of interest to evaluate WFM for its hepatoprotective property in diclofenac induced hepatic damage in rats. In the present study, the administration of diclofenac to rats decreased the total protein and albumin level and increased the BUN level significantly. The pretreatment of WFM at two different dose levels restored the level
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of protein, albumin and BUN towards normalization. Hepatocellular injury from metabolic inhibition, oxygen radical toxicity, immunologically mediated damage, or some other mechanism results in predominant elevations of aminotransferase and alkaline phosphatase (Manoukian and Carson, 1996). The ALT, AST and ALP levels were significantly elevated when rats were administered with diclofenac indicating hepatocellular damage. The increased levels of these enzymes were significantly decreased by pretreatment with WFM in dose dependent manner. This is the indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by diclofenac. The hepatoprotective property of Polyalthia longifolia (Tanna et al., 2009), Curcuma longa, Glycyrrhiza glabra and Moringa oleifera (Hamza, 2007) in diclofenac induced hepatic damage in rats was reported.

The significant increased liver weight of diclofenac exposed animals seems to be due to toxic potential of diclofenac. The significant increase in weight of liver was, however, found to be associated with concomitant increase of serum AST and ALT enzyme levels. It is important to note that the elevated activity of serum AST and ALT recorded in this study may be due to loss of enzymes of liver tissue. Pretreatment of WFM decreased the liver weight significantly indicating recovery of liver tissue from damage. Significant decrease in total protein of the liver contents is a reflection of hepatic toxicity (Gatsing et al., 2005; Adebayo et al., 2010). The significant reductions of protein in diclofenac intoxicated group indicate depletion in the protein reserve and thus suggest hepatic toxicity. WFM administration increased the total protein content leading to normalization. GSH is an extremely efficient intracellular buffer for oxidative stress and GSH acts as a non-enzymatic antioxidant that reduces $\text{H}_2\text{O}_2$, hydroperoxides (ROOH) and xenobiotic toxicity (Kadiiska et al., 2000). The level of GSH depleted when animals were injected with diclofenac. The depleted level of GSH raised with the pretreatment of WFM. The catalase and GPx are enzymatic antioxidants widely distributed in all animal tissues that decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Therefore, the reduction in the activity of these two enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. In the present study, WFM significantly restored the hepatic catalase and GPx activity, which indicated that WFM could scavenge reactive free radicals that eventually lessen the oxidative damage to the tissues and subsequently improved the
activities of these antioxidant enzymes. The preventive effect of WFM was also confirmed by the results of histopathological study, as evidenced by a dose related decrease in the incidence and severity of histopathological hepatic lesions.

*Woodfordia fruticosa* extract pretreatment prevented the reduction in the antioxidant enzyme activities and consequent oxidative damage to the liver. In fact, the multiple dose pretreatment of *W. fruticosa* extract alone significantly boosted the antioxidant enzyme activities. Molina et al. (2003), Srivastava and Shivanandappa (2010) also reported good hepatoprotective activity in their studies; and they suggested that the hepatoprotective activity of plant extract could be a result of boosting the antioxidant capacity of the liver.

5.6.2. Carbon tetrachloride induced hepatotoxicity

CCl$_4$ is a well-known hepatotoxic agent and the preventive action of liver damage by CCl$_4$ has been widely used as an indicator of liver protective activity of drugs in general (Clawson, 1989). Hepatotoxicity induced by CCl$_4$ is the most commonly used model system for the screening of hepatoprotective activity of plant extracts/drugs (Srivastava and Shivanandappa, 2010). The changes associated with CCl$_4$-induced liver damage are similar to that of acute viral hepatitis (Rubinstein, 1962). Toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structure (Recknagel, 1983).

CCl$_4$ is a xenobiotic that produces hepatotoxicity in various experimental animals. CCl$_4$ is metabolized by cytochrome P$_{450}$ to form a reactive trichloromethyl radical (CCl$_3$) and a trichloromethyl peroxy radical (CCl$_3$O$_2$). Both radicals are capable of binding to DNA, lipids, proteins or carbohydrates, leading to lipid peroxidation, cell necrosis, excessive deposition of collagen in liver, and liver fibrosis (Sheweita et al., 2001; Weber et al., 2003). The effect of CCl$_4$ is generally observed after 24 h of its administration. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24 h of CCl$_4$ intoxication (Sureshkumar and Mishra, 2006).

The total protein and albumin levels decreased due to the hepatotoxin intoxication. The reduction is attributed to the damage produced and localized in the endoplasmic
reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides. In the present study, CCl4 intoxication reduced the serum total protein and albumin levels. The pretreatment of WFM restored the total protein and albumin levels. The rise in protein and albumin level suggests the stabilization of endoplasmic reticulum leading to protein synthesis (Suresskumar and Mishra, 2006).

The liver marker enzymes (AST, ALT and ALP) are cytoplasmic in nature; upon liver injury these enzymes enter into the circulatory system due to altered permeability of membrane (Zimmerman and Seeff, 1970). In this study, significant increase in AST and ALT levels in the serum was observed after administration of CCl4. ALP level also increased after CCl4 administration. The increased levels of these enzymes significantly decreased by pretreatment with WFM extract. Reduction in the levels of AST, ALT and ALP towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl4 (Ranawat et al., 2010).

Many studies have demonstrated that the hepatoprotective effect of plant extracts may be related to its antioxidant capacity to scavenge reactive oxygen species (Naik and Panda, 2007; Tsai et al., 2009). CCl4 intoxication reduced the total protein level in liver homogenate, which restored significantly with the pretreatment of WFM. Liver cells possess antioxidant defense system consisting of antioxidants such as GSH and antioxidant enzymes such as catalase and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death. GSH is widely distributed among living cells and is involved in many biological functions, acting as an essential intracellular reducing agent for maintenance of intracellular redox status. It is also the most important biomolecule protecting against chemically induced cytotoxicity, by participating in the elimination of reactive intermediates by conjugation and hydroperoxide reduction, or by direct quenching of free radicals (Jewell et al., 1986; Wang et al., 2000). CCl4 intoxication slightly reduced the level of GSH, which was significantly restored in WFM treated (higher dose) rats. Trichloromethyl peroxy radical, the metabolic product of CCl4 binds covalently to the macromolecules and causes peroxidative degradation of cellular membrane leading to the necrosis of hepatocytes (Brattin et al., 1985; Ranawat et al., 2010). The hepatic
antioxidant enzymatic activity of catalase and GPx significantly decreased in CCl₄-intoxicated rats as compared with control rats. The decreased enzymatic activity would result in an increased steady-state level of oxidants, contributing to cell injury. The catalase level was elevated by administration of WFM to CCl₄ intoxicated rats suggesting that it has the ability to restore the enzyme activity towards normalization in CCl₄ damaged liver. However, administration of WFM to CCl₄ intoxicated rats had no effect in hepatic GPx activity and relative liver weight as compared to the CCl₄ treated toxin control group. This result suggests that WFM markedly inhibited CCl₄ induced liver damage by elevated hepatic antioxidant enzymatic system such as catalase and GSH.

The rise in marker enzymes level in CCl₄ treated animals has been attributed to damaged structural integrity of the liver. Administration of the WFM preserved the structural integrity of the hepatocellular membrane as evidenced from attenuation of the marker enzymes level when compared to CCl₄ treated animals. It was further confirmed by the histopathological assessment of the liver tissue.

5.6.3. Acetaminophen induced hepatotoxicity

Acetaminophen (APAP), a frequently used analgesic and antipyretic drug, is known to be hepatotoxic in higher doses, which is primarily metabolized by sulfation and glucuronidation to unreactive metabolites, and then activated by the cytochrome P₄₅₀ system to produce liver injury. It is established that acetaminophen is bioactivated to a toxic electrophile, N-acetyl p-benzoquinone imine (NAPQI), which binds covalently to tissue macromolecules, and probably also oxidizes lipids, or the critical sulphydryl groups (protein thiols) and alters the homeostasis of calcium (Lin et al., 1997). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes (Aldridge, 1981; Gilani et al., 2005). The experimental evidence suggests that during metabolism of this type of drug, different reactive metabolites are produced that covalently modify proteins (Bernareggi, 1998), impose oxidative stress (Berson et al., 1991; Ritter and Malejka-Giganti, 1998) and causes mitochondrial injury (Mingatto et al., 2000).
In the present study, a reduction in total serum protein including albumin levels observed in the APAP treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein. But the treatment with WFM did not have protective effects in regards to total protein and albumin levels. Blood urea nitrogen (BUN) is also a marker of liver and renal functions, which is used to diagnose acute and chronic diseases related to liver and kidney. APAP administration to the rats increased the BUN level. The increase in BUN after APAP administration was prevented by WFM.

The hepatic cells consist of higher concentrations of AST, ALT and ALP in cytoplasm and AST in particular exists in mitochondria (Wells, 1988). Due to the damage caused to hepatic cells, the leakage of plasma cause an increased level of hepatospecific enzymes in serum (Zimmerman and Seef, 1970). The elevated serum enzyme levels are indicative of cellular leakage and functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978; Satheesh Kumar et al., 2009). The hepatoprotective index of a drug can be evaluated by its capability to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been induced by a hepatotoxin. The measurement of serum AST, ALT and ALP levels serve as a means for the indirect assessment of condition of liver. The level of these enzymes significantly increased in serum, when animals were administered with APAP. The pre-treatment of the animals with WFM with respect to intoxication with APAP controlled the AST, ALT and ALP levels when compared with the toxic group.

The relative liver weight significantly increased in APAP intoxicated animals indicating toxic effect of APAP. Pretreatment of WFM decreased the liver weight significantly indicating recovery of liver tissue from damage. GSH, a major known protein thiol in living organisms plays a central role in coordinating the body’s antioxidant defense process (Boyd et al., 1981). Excessive peroxidation causes increased GSH consumption. GSH is a scavenger of toxic metabolites, including NAPQI, which is a metabolite of APAP (Hwang et al., 2008; Yuan et al., 2010). GSH, plays a major protective role as a scavenger of free radicals that combine with non-protein thiols at the GSH reactive center to abolish free radical toxicity (Vane et al., 1994; Swierkosz et al., 1995). Anti-oxidation by GSH protects the body from many diseases and conditions such as damage by \( \text{H}_2\text{O}_2 \), ethanol and numerous other toxins.
(Choi et al., 2009). Because GSH plays an important role in the antioxidant defense system (Hsu et al., 2008), it becomes the key determinant in the APAP-induced hepatotoxicity. In the present study, the contents of liver protein and GSH in the APAP group decreased significantly after APAP administration, when compared with the control group. Pretreatment with WFM restored the total protein and GSH levels towards normalization. Administration of APAP as well as WFM did not have any effects in catalase and GPx activity.

Histopathological observations after APAP administration showed severe damage in hepatocytes, which basically supported the alterations observed in biochemical analysis. Hepatocellular necrosis, infiltration of periportal mononuclear cell of liver cells were characteristic alterations occurred due to acetaminophen intoxication. Treatment of WFM decreased focal necrosis, vacuolation and reduced the lymphocytic infiltration in liver and presented regenerative effects. This can be considered as an expression of the functional improvement of hepatocytes, which might be due to accelerated regeneration of parenchymal cells or little damage of cells.

5.7. ACUTE TOXICITY STUDY

Toxicology is a science to study adverse-effects of chemicals or physical agents on biological system and preclinical toxicology is a science to evaluate safety of a drug (mostly) in animals to decide if the drug is safe for human use or not. Plants, vegetables and herbs used as food and in the folk treatment have been accepted currently as one of the main source of drug discovery and development, but only a few of them have been scientifically investigated, especially regarding their toxic aspects (Pereira et al., 2010).

Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity and occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for Phase 1 human studies, and provide
information relevant to acute overdosing in humans. It could also be used to estimate the therapeutic index (LD$_{50}$/ED$_{50}$) of drugs (Rang et al., 2001; Maikai et al., 2008).

In the present study, acute toxicity test was done to establish if any adverse effects of the administration of the methanol extract of *Woodfordia fruticosa* on some observable and hematological parameters. The results indicate no abnormal symptoms and no death of the rats. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Hilaly et al., 2004; Mukinda and Eagles, 2010). In the present study, no significant changes were observed in the general behavior, body weight, feed and water intake of rats in the treated groups as compared to the control group, suggesting that at single oral doses administered, methanol extract of *Woodfordia fruticosa* flowers had no effect on the normal growth of rats.

Organ weight changes have long been accepted as a sensitive indicator of chemically induced changes to organs and in toxicological experiments, comparison of organ weights between control and treated groups have conventionally been used to predict toxic effect of a test material (Pfeiffer, 1968; Nisha et al., 2009). In acute toxicity study in male rats at lower dose kidney weight increased while in female rats at higher dose kidney weight decreased significantly. The weight of lung in acute toxicity study increased in both male and female rats; but this was not associated with morphological changes and no evidence of toxicity was found. Increased testis weight and decreased uterus weight in treatment groups cannot be considered as a manifestation of toxicity due to the variability attributable to its small size and physiological factors unrelated to treatment like estrus cycle and relative infrequency of these organs as target organs of toxicity. The absence of significant changes in other organs in the present study points to the fact that ingestion of *Woodfordia fruticosa* methanol extract did not induce any anomalous growth or inflammation to these organs which would otherwise have resulted in higher relative organ weights in the treatment groups.

The hematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (Mukinda and Syce, 2007). The various hematological parameters investigated in this study are useful indices of evaluating the toxicity of plant extract in animals (Toyin et
Assessment of hematological parameters are not only used to determine the extent of deleterious effect of extracts on the blood of animals, but it can also be used to explain blood relating functions of a plant extract or its products (Toyin et al., 2007). Analysis of blood parameters is relevant in risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies (Olson et al., 2000; Adebayo et al., 2010).

The hematological studies revealed increase in RBC count and PCV ($P < 0.05$) at higher dose in male rats and Hb ($P < 0.05$) in both male in female rats during acute toxicity study. It also revealed a fall ($P < 0.05$) in WBC level in lower dose during acute toxicity study in male rats. The general decrease in the values of these hematological parameters can be due to direct destruction of mature circulating cells or loss of cells from the circulation by hemorrhage, or leakage through capillary walls and reduced cell production (Nunia et al., 2007). However, this was not considered to be adverse or related to exposure to the WFM because similar differences were not observed in both sexes.