Chapter 5
DISCUSSION

Diabetes Mellitus is characterizing as a metabolic disorder consisting beta cell dysfunction and decreased insulin sensitivity (Mackenzie and Elliott, 2014). Excessive generation of free radicals is proved and known for taking part in many human pathogenic conditions like diabetes, arthritis, cancer and other complications (Goyal et al., 2011). Several scientific studies indicated that natural products consisting antioxidants play a major role in avoidance of these free radicals (Sikora et al., 2008).

India specifically Northeast India has rich heritage of valuable medicinal plants. An average of 8% of the total global biodiversity is having approximately 49,000 plant species (Gireesha and Raju, 2013). There are a few or almost none studies on these plants that have been recorded although there are few plants such as *Alterathera ficoicka* leaf, *Amaranthus viridus* leaf, *Amaranthus viridus* stem, *Corriandrum sativm* leaf, *Cotus igneous* leaf, *Zea mays* silk, *Phyllanthus amarus* leaf, *Tridex procumbens* flower, *Lucas lucifera* leaf, *Syzigium cumnii* seed, *Punica granatum* peel (*var* dadim), *Moringa olifera* leaf, *Moringa olifera* stem and

Lucas lucifera stem were recorded for their antihyperglycemic property using *in-vivo* studies (Ranjbar et al., 2001; Aggarwal and Aggarwal, 2011; Middha et al., 2012). In their report WHO also indicated *S. cumini* seed and *M. charantia* fruit in traditional system of medicine to treat diabetes (WHO, 2008). Plants like *Bambusa tulda* are not much studied for its anti-hyperglycemic activity. Therefore less explored and not much literature found

on BT prompted us to choose BT to validate its therapeutic effect to substantiate its ethano-medical use. Natural products are known to be rich in alkaloids, anthroquinone, carbohydrates, coumarins, flavonoids, glycosides, reducing sugars, tannins, saponins, steroids, and tepenoids. *Bambusa tulda* extract also divulged the existence of theses phytochemicals. These compounds or molecules are well known to be bio-active in therapeutic plants (Al-Mustafa and Al-Thunibat, 2008), therefore accountable for the antioxidant activities. Several authors indicated the similar results in their studies (Ismail et al., 2012; Sultana et al., 2009; Aggarwal and Aggarwal, 2011).

Phenolic acids are the most imperative secondary metabolites in plants and proved to be excellent sources of accepted antioxidants in humans (Goyal et al., 2011). These natural antioxidants are capable of scavenging liberated superoxide radicals, protecting biological systems against the destructive effects of oxidative progressions on carbohydrates, proteins, lipids and DNA. Antioxidants are known to be protect cells from the injuries caused by unbalanced particles known as ROS (reactive oxygen species) and free radicals. Previous reports designated that plants/herbs are rich in flavonoids and polyphenols are known to be effectual in scavenging the free radicals (Singh et al., 2012).

These plant based antioxidants are isolated and purified by solvent/ distillation extraction methods. Though, extracting solvents are differing and depending on the nature of extracting compounds as well plant material. This is due to physical characteristics, solubility and polarities of various antioxidants (Sultana et al., 2009).

Polar solvents along with their aqueous combinations of methanol, ethanol, acetone, and ethyl acetate are used as most appropriate solvents (Al-Mustafa and Al-Thunibat, 2008; Peschel et al., 2006). In the current study methanol was used as solvent to make methanolic extract of *Bambusa tulda* (BT). BT has shown a good phenolic and flavonoid profile. Mostly herbs/ plants display their antioxidant activities due to the presence of phenyl propanoid derivatives (PPD), such as polyphenols.

Halvorsen et al., 2002 indicated the antioxidant results are not comparable because of the different methods used

by different reports. The antioxidant activities of the extract may be due to poly-phenolic content (Al-Mustafa and Al-Thunibat, 2008).

Polyphenols are the prime antioxidant components, known as terminators, which are potent free radical absorbers (Goyal et al., 2011).

They donate hydrogen ($H^{\cdot}$) to free radicals and break the effect of lipidoxidation at the beginning step (Florian et al., 2004; Gil et al., 1999; Vinson et al., 1995).

Proanthocynadins are majorly known as condensed tannins and BT was found to have considerable proanthocynadins. Prior studies have also specified the antiradical activity of these ubiquitous molecules (Muir, 1995; Amarowicz et al., 2000; Amarowicz and Troszyńska, 2004; Amarowicz and Pegg, 2006).

Flavonoid display an outstanding potential to decrease neurodegenerative disorders, risk of heart diseases and possess anticancer properties (Oliveira et al., 2009). In this report, we have reported the DPPH radical scavenging activity (% inhibition). The activity was found 20 to 78% as increase in concentration respectively. Previous studies also indicated the methanolic extract is more suitable solvent for the extract preparation since it also inhibit the degradation of polyphenols present in the plants by neutralizing the activity of polyphenol oxidase (Goyal et al., 2010). Hu and his co-workers discussed the antioxidant activity of bamboo. In their study they have shown bamboo exhibits a good concentration dependent scavenging activity of the $DPPH^{\cdot}$ radical activity. Their study indicated bamboo as an effective natural antioxidant. DPPH accepts an electron or hydrogen radical to turn out to be a diamagnetic molecule. As the electron is established from free-radical scavenger the colour disappears and outcome follows discoloration coincides with the number of electrons accepted. DPPH reacts with specific antioxidant molecule which engages decline of DPPH, primary reaction engrossed in this respect is as follows

$Z^{\cdot}$ (DPPH) + AH (donor molecule) → ZH + $A^{\cdot}$

Free radicals have been reported to be dependable for the caustic effects on protein in different diseases such as cataract formation, oxidative damage of certain cancers and diabetes (Langseth, 1995; Alho and Leinonen, 1999).

DPPH radical scavenging activity of BT was significantly higher (p>0.05) at higher concentration. The reducing power of the BT was found to be notable, which augmented gradually with an increase in the concentration. Fe^{3+} was transformed to Fe^{2+} in the presence of BT and the standard compound BHT, which is measured as reductive capability. Any compound which is having redox potential lower than that of redox pair Fe (III)/Fe (II) can theoretically reduce Fe (III) to Fe (II) (Goyal et al., 2011). The antioxidants are directly linked with the occurrence of reductones. The action of reductones is considered to split radical chain by donating a hydrogen atom indicating with the development of reducing power. Therefore, the spotted antioxidant possessions of BT may be credited to its elevated reducing power.

This study has specified the BT extract's elevated phenolic content and antioxidant potential, free radical scavenging activity that is quite comparable with the reports by previous research (Lapornik et al., 2005; Othman et al., 2007; Al-Mustafa and Al-Thunibat, 2008).

Our study was confirmed by further co-relational studies. Correlation studies revealed a positive association between biochemical activity such as total phenol, flavonoids content, antioxidant activity of BT and free radical scavenging (DPPH) activity and reducing power assay. Misra et al., (2012) also indicated the same co-relational approach.

The MDA level reverses, when the experimental rats were fed with BT extract, the probability of this phenomena could be due to some antioxidant enzymes present in the extract such as SOD, CAT etc. (Badmus et al., 2011). This activity would be co-related with the high lipid peroxidation inhibition of BT, due to the presence of several phenolic compounds. (Al-Mustafa and Al-Thunibat, 2008).

In order to study the active molecules of BT, the LC-MS was performed. It revealed the existence of few foremost phenolic compounds. Table 3 indicated the phyto-constituents identified from BT with their IUPAC name, molecular formula, retention time (RT), chemical structure, molecular weight, concentration (peak

area %). The major compounds detected were 4-hydroxybenzoic acid, 2-hydroxybenzoic acid, (2E)-3-(2-hydroxyphenyl) prop-2-enoic acid,

(2E)-3-(4-hydroxyphenyl)-prop-2-enoic acid, 2,4-dihydroxybenzoic acid,

4-hydroxy-3-methoxybenzoic acid,

(2E)-3-(4-hydroxy-3-methoxy-phenyl) prop-2-enoic acid in BT extract using GC-MS.

p-Hydroxy benzoic acid was found to be effective in anti-diabetic study (Peungvicha et al., 1998). p-Hydroxybenzoic acid was also known to have anti-fungal, anti-mutagenic, anti-microbial estrogenic activities (Khadem and Marles, 2010). 2,4-dihydroxy benzoic acid has proven thyroid peroxidase inhibitory effect.

Vanillic acid or 4-hydroxy-3-methoxybenzoic acid,

(2E)-3-(4-hydroxy-3-methoxy-phenyl) prop-2-enoic acid possess astringent, antineoplastic and bacteriostatic activities (Khadem and Marles, 2010).

(2E)-3-(4-hydroxyphenyl)prop-2-enoic acid or Benzothiophene derevatives are known be estrogen receptor degraders (Faulds et al., 2011).

Gayathri et al, 2012 has indicated that 2-hydroxy-4-methoxy Benzoic acid isolated from *Hemidesmus indicus* as a promosing compound for diabetes. Gallic acid possesses antimelanogenic and antioxidant properties. (2El)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid or Ferrulic acid has known to have various therapeutic potentials such as cancer, diabetes, inflammatory diseases and in aging (Zhao and Moghadasian, 2008). All identified compounds are known to have various therapeutic or pharmacological activities. As there are no reports found in public databases in case of *Bambusa tulda*. Although Goyal et al. (2017) have reported the presence of gallic acid and few more compounds in *Bambusa balcooa*.

In-vitro analysis was followed by *in-vivo* study. This study the effect of BT on alloxan induced diabetic rats. Lethal

dose of BT (LD_{50}) was 6088.13mg/kg body weight. There were very none reports found in regards to toxicity studies of BT.

Current study explains that BT significantly reduced blood glucose level in the alloxan induced diabetic animals.

Investigating the antidiabetic mechanism by the *Bambusa tulda* extracts exhibited significant anti-hyperglycemic activities in alloxan induced hyperglycemic rats with an increase in body weight. Sumana and Suryawashi also indicated in increase in weight of alloxan induced diabetic rats.

Alloxan (150mg/kg) induced weight loss in untreated rats within 10 days of the experiment whereas a gain in body weight occurred in the treated rats. The administration of *B. tulda* extracts (100 and 200mg/kg) corrected the loss in body weight and restored their levels towards normal. This could be as a result of its ability to reduce hyperglyceamia by increased glucose metabolism and also due to its tissue protective effect of *B. tulda*. Viana et al. (2004) also indicated the same results previously. Alloxan is a well known diabetogenic agent that is used to induce type1 diabetes in experimental animals and previously reported by Szkudelski (2001). The underlying mechanism starts with the selective uptake of the compound due to its structural similarity to glucose as well as highly efficient uptake mechanism by Glut -2 transporter in the pancreatic beta-cells. In this regard, Alloxan, an analogue of urea selectively destroys the beta-cells. This toxic action involves oxidation of essential sulphydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis.

Low dose (100 mg/kg body weight) and high dose (200 mg/kg body weight) of BT indicated a decrease in the blood glucose level. The high dose showed more reduction than the low dose of BT, indicating that BT has

strong anti-diabetic activity and acted as an efficient dose.

Middha et al. (2011) showed the decrease in body weight after the alloxan introduced intraperitoneally. Administration of BT also recovered the body weight in the diabetic rats.

Weight loss was seen may be as a symptom of diabetes. Reversal of bodyweight might be because of the reduction of hyperglycaemia after supplementing BT. The possible mechanism by which BT brings down the hyperglycaemia may be by increasing either the pancreatic secretion of insulin from ß-cells of Islets of Langerhans. This was confirmed by the histo-pathological evaluation of the pancreas, indicated the recovery and improved percentage of ß-cells, of the rats that was supplemented with extract.

Comparison of histo-pathological slides specified the decrease in the pancreatic Islets numbers (β-cells) in the diabetic rats. As our results indicated, the Islets were asymmetrical in shape and relatively diminutive in diabetic control experimental animals.

The results of this investigation indicated that extracts of *Bambusa tulda* has antihyperglycemic activity and also has tissue protective properties. In this investigation, the damage (atrophy and necrosis) of pancreatic tissue in alloxan treated diabetic control rats and β-cells regeneration by 100mg/kg extract, 200mg/kg extract and glibenclamide respectively were seen. It was understood that the extract at high dose (200mg/kg) is more effective in reducing glucose level than at low dose (100mg/kg) after 42 days of treatment. Hence, the above discussion revealed that extract of *Bambusa tulda* at high dose (200mg/kg) showed similar curative effect as standard, glibenclamide.

The regeneration of the β-cells of the alloxan-destructed cells with high and low dose of BT. There might be probability that pancreas holds stable cells which have a capacity of restoration. Therefore, the surviving cells can propagate the lost cells (Govan et al., 1986; Kumar et al., 1992).

In the current study, SOD and GPx have been evaluated. SOD is an

significant antioxidant defence enzyme which defends tissue alongside oxygen free radicals (Chis et al., 2009). This reactive oxygen species will be detoxified by another enzyme GPx. It converts the hydrogen peroxide (H_2O_2) produced by SOD (Halliwell, 2012), thus defends the outer membrane from lipid peroxidation.

In diabetic rats, the SOD activity was significantly lowered in the tissue (Fig 4.9). Oral supplementation of BT extract revealed an augmentation in the SOD activity in the liver tissue. A similar trend was observed in standard drug glibenclamide treated rats. Equally, GPx activity was diminished in diabetic control rats. Increase in GPx activity was seen in rats treated with BT extract. These study highlights that these enzymes are interrelated and lowering of their enzymatic action directing increases in oxidative stress in the diabetic rats. Treatments with BT extract amplified the activity of these enzymes significantly and might assist in get rid of the free radicals spawned during diabetes mellitus. This study underlines that these enzymes are inter-related and lowering their enzymatic activity can directly amplify in oxidative stress in the alloxan treated diabetic animals. The BT extract treatment can improved the activity of these enzymes radically and might assist in getting rid of these free radicals spawned during diabetes mellitus. Lv et al. (2012) indicated the boost in antioxidant enzymes such as SOD and GPx activity in hepatic tissue (liver), when the STZ induced diabetic rats were supplemented with *Dendrocalamopsis oldhamii* extract. Ying et al. (2017) proved that supplementing bamboo could reduce weight and glucose level in hyperglycemic rats. The glycosylated hemoglobin A1c (HbAlc), BUN and T-SOD activity were also observed increasing in their study.

The concentrations of MDA in the tissue of experimental rats are portrayed in figure 4.10. MDA is known to be the end product of poly-unsaturated fatty acid peroxidation whose fabrication augments with the amplification in free radicals (Gawel et al., 2004). In this study, diabetic control rats demonstrated a noteworthy increase in MDA as compared to normal rats. Supplementation of LB extract lowered the lipid peroxidation. Though, the effect of glibenclamide (standard drug) treatment was seen more significant than the rats treated with BT.

The results indicated in the study are in accordance with the previous reports on the bamboo's activity (Goyal et al., 2017).

Ying et al. (2012) also indicated that Bamboo leaf supplementation could improves diabetic nephropathy condition by triggering AKT pathway in investigational animals.

Nzreen and his co-workers (2011) indicated that the anti-hyperglycaemic activity of *Bambusa sp.* in streptozotocin (STZ) diabetic rats. Their study confirmed a significant reduction in FBS in experimentally treated animals, followed by the reduction in the glutathione (GPx) level and thus elevating enzyme activity. Another report by Lv et al. (2012) on *Dendrocalamopsis oldhamii* extract, observed significant reduction in the antioxidative enzymes like SOD and GPx, in the liver after the rats were treated with the same. Their study also proposed that the diabetic animals are in depiction to oxidative stress and the leaf extract of *B. balcooa* can partially decrease the inequities between the generation of reactive oxygen species (ROS) and the foraging enzyme activity. Goyal et al. (2017) also confirmed the antidiabetic and antioxidant potential of *Bambusa balcooa* in alloxan induced diabetic rats. Lenzen, investigated the antidiabetic mechanism of Alloxan and Streptozotocin by evaluating their effects on the histology of the pancreas and livers of normal and diabetic rats and reported that the enzyme glucokinase which helps in phosphorylating glucose to G-6-P in the synthesis of glycogen for storage is being inhibited by alloxan. This inhibition reduces glucose oxidation and ATP generation thereby suppressing the signal generating metabolic flux and signal generation of ATP for glucose-induced -insulin-secretion. This could be the cause of hyperglycemia and production of lesions. Thus, it can be inferred from the above study that BT could be a supplement as an antioxidant therapy due to its resemblance in activity with insulin and may prove to be beneficial in eliminating the hypergylcemia and preventing diabetic complications occurring due to lipid peroxidation and free radicals. Yang et al. (2014), previously reported that bamboo extract may exert anti-diabetic activity because of its helping in improvement of insulin sensitivity though not much changes were indicated HbA1C values in STZ animal model. Though, there

was nothing reported about the dosage toxicity and pancreatic anatomy. In this context, the current research may offer a promising strategy to understand the acute toxicity, *in vivo* anti-hyperglycemic and antioxidative activity of hydro-methanolic extract of *B. tulda* leaf native to Bodoland, India.