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FREE RADICAL AND ANTI – OXIDANT STATUS IN DIABETIC MELLITUS – II CHEMICAL INDUCED DIABETIC MODEL

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The present investigations concluded that the ethanolic and aqueous extracts of aerial parts of *A. mexicana* endowed with potential antidiabetic activity which could be attributed by their possible multiple effects on both pancreatic and extra-pancreatic site by influencing either the metabolism and/or absorption of glucose, which in turn also influence the lipid metabolism. On the other hand, hydro-alcoholic extract, chloroform and aqueous fraction of *A. mexicana* also possess potential hypoglycaemic and antihyperglycaemic activity. Conversely the extracts and fractions of both plants exert very good potentials to scavenge toxic free radicals along with the inhibition of the liver lipid peroxidation products and activation of the enzymatic antioxidant defense mechanism in diabetic rats that might be due to the presence of high levels of sterols, phenolics, alkaloids and flavonoids, which may be responsible for the supporting properties of the extracts and fractions for their hypoglycaemic and antidiabetic activity. Stigmasterol, a plant sterol isolated from chloroform fraction of *A. mexicana.*

Key Words:*A. Mexicana,*hypoglycaemic and antihyperglycaemic activity.

Introduction: The inhibition of intracellular free radical formation would provide a therapeutic strategy to prevent oxidative stress and the related diabetic vascular complications. Antioxidants may act at different levels, inhibiting the formation of ROS or scavenge free radicals, or increase the antioxidants defense enzyme capabilities. Supplementation with antioxidants and/or factors essential to nitric oxide (NO) production may potentially improve endothelial dysfunction in T2DM by re-coupling eNOS and mitochondrial function, as well as decreasing vascular NAD(P)H oxidase activity[14]. However, in the case of macrovascular/microvascular complications, the antioxidant therapy is beneficial together with blood pressure control, management of dyslipidemia, and optimal glucose control[15].

Generally, the antioxidant pharmacotherapy can be divided in the use of antioxidant enzyme and substrates, biogenic elements, combined drugs, synthetic antioxidants, and drugs with antioxidant activity. There are also a large number of natural cellular defense mechanisms as the naturally existing antioxidant components, which neutralizes free radical damage. The enzymatic antioxidant systems, such as copper, zinc, manganese superoxide dismutase, gluthatione peroxidase, gluthathionereductase, and catalase may remove the ROS directly or sequentially,

preventing their excessive accumulation and consequent adverse effects. Non-enzymatic antioxidant systems consist of scavenging molecules that are endogenously produced such as glutathione, ubichinol, and uric acid or derivatives of the diet such as vitamins C and E, carotenoids, lipoic acid, selenium, etc[16].Exercise training results in an up-regulation of antioxidant defense mechanisms in various tissues, presumably due to increased levels of oxidative stress that occurs during exercise[17].

DPPH

The DPPH radicals are widely used to investigate the scavenging activity of some natural compounds. In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. The method helps to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH at 517 nm. Resulting a color change from purple to yellow, the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. (Matthaus 2002)

STEP – I (Hypoglycemic and Anti-diabetic study of extracts)

- Acute toxicity studies of different extracts
- Effect of the extracts on normoglycemic rats
- Oral Glucose Tolerance Test of the extracts
- Effect on blood glucose level of the extracts in alloxan induce hyper glycemic rats(Acute and sub-acute models)
- Effect of the extracts on serum biochemical parameters
- Effect onchange of body weight in sub-acute model

STEP– II (Hypoglycemic and Anti-diabetic study of fractions)

- Solvent-solvent fractionation of active extract
- Acute toxicity studies of different fractions
- Effect of the different fractions on normoglycemic rats
- Sub acute antidiabetic study of the fractions
- Effect of the fractions on serum biochemical parameters
- Effect of the fractions on serum lipid profile
- Effect on body weight
- Effect on food and water intake
- In vitro alpha amylase inhibitory effect of

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bioactive fractions

STEP – III (Anti-oxidant activity study)

- *In vitro* antioxidant activity of different extracts of plants by using Reduction of 1, 1-diphenyl- 2- picryl hydrazyl (DPPH)
- *In vitro* antioxidant activity of potentfraction by using :
- Reduction of 1, 1- diphenyl- 2- picryl hydrazyl (DPPH)
- Nitric oxide scavenging activity
- ABTS scavenging activity
- Super oxide radical Scavenging

In vivo antioxidant activity of fractions by Determination of Enzymatic antioxidantsin pancreas, liver and kidney homogenates

- Superoxide dismutase (SOD)
- Catalase (CAT)
- Reduced Glutathione
- Glutathione-S-transferase (GST)
- Total Protein level

STEP – IV (Isolation, Characterizationstudies)

Isolation and Characterization of phytoconstituents from the most bio-potent fraction.

Effect of extracts of *A. mexicana* in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method

The DPPH is stable free radical which reacts with appropriate reducing agent (hydrogen), to become paired off (diamagnetic molecules) and solution be converted into colourless stoichometrically depending on the number of electron taken up (Shirwaikar et al., 2006).

The capabilities of ethanolic and aqueous extracts of aerial parts of *A. mexicana* to scavenge DPPH were measured *in-vitro* the related IC_{50} values and the % scavenging results are mentioned in table-4.20.

Both the extracts, *A. mexicana* scavenges DPPH radical in a concentration dependent way. The antioxidants react through DPPH, a purple colored stable free radical and convert it into a colorless α-α-diphenyl-β-picryl hydrazine. The quantity of DPPH reduced could be quantified by measuring a decrease in absorbance at 517 nm.The IC_{50} value was found to be 45.21and 40.11µg/ml for ethanolic extracts and aqueous extracts while the IC_{50} value of ascorbic acid was 18.53µg/ml significantly reduced DPPH radical by bleaching it. From the results, it may be postulated that ethanolic and aqueous extracts of aerial parts of *A. mexicana*have hydrogen donors thus, scavenging the free radical DPPH.

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Table- 4.20: Antioxidant activity of various extracts of *A. mexicana* in DPPH radical scavenging method

Sample	Concentration µg/ml	% inhibition	IC $_{50}$ value µg/ml
	20	29.21±2.14	
	40	47.53±1.85	
Ethanolic Extract	60	59.21±2.46	45.21
	80	74.72±2.68	
	100	87.17±1.45	
	20	25.21±2.04	
	40	50.53±1.65	
Aqueous extract	60	61.21±2.46	40.11
	80	78.72±2.49	
	100	90.17±1.35	
Ascorbic acid			18.53

Values are expressed in MEAN ± S.E.M (n =3).

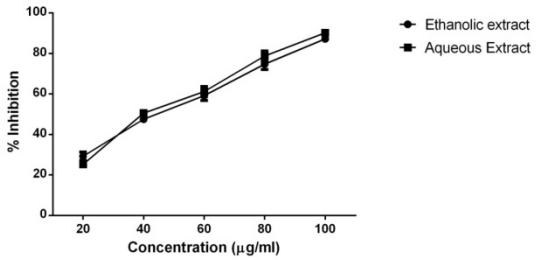

Fig.4.18: Effect of various extracts of *A. mexicana* in DPPH radical scavenging method

Effect of various extracts of *A. mexicana* in hydrogen peroxide (H_2O_2) radical scavenging method

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects.Scavenging of hydrogen peroxide of the both extracts of *A. mexicana* is presented in table 4.21 The IC_{50} value for *A. mexicana* was found to be 56.02and 56.24µg/ml for ethanolic extracts and aqueous extracts while the IC_{50} value of ascorbic acid was 21.41µg/ml significantly and concentration dependently scavenges H_2O_2 radical. Though, ethanolic and aqueous extracts of

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aerial parts of *A. mexicana*are having significant potential in scavenging the free radicals in the above experiments.

Table- 4.21:Effect of various extracts of *A. mexicana* in hydrogen peroxide (H_2O_2) radical scavenging method

Sample	Concentration µg/ml	% inhibition	IC $_{50}$ value µg/ml
	20	11.16±2.12	
	40	24.12±1.62	
Ethanolic Extract	60	56.25±1.12	56.02
	80	71.15±1.24	
	100	84.25±1.62	
	20	16.12±2.14	
	40	29.15±1.31	
Aqueous extract	60	55.21±2.42	56.24
	80	69.15±2.21	
	100	75.27±1.91	
Ascorbic acid			21.41

Values are expressed in MEAN ± S.E.M (n =3).

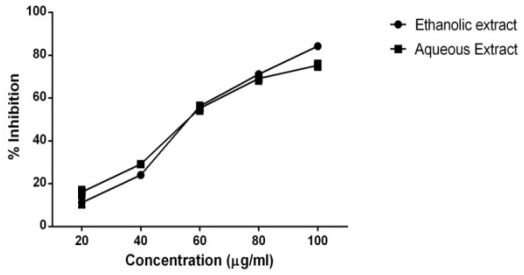

Fig.4.19: Effect of various extracts of *A. mexicana* in hydrogen peroxide (H_2O_2) radical scavenging method

Effect of chloroform fraction of *A. mexicana* in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, Super oxide radical, ABTS radical cation and Nitric oxide radical scavenging method

The capacity of chloroform fraction of *A. mexicana*to scavenge DPPH, super oxide radical, ABTS radical cation and nitric oxide radicalwere measured *in-vitro*; the related IC_{50} values and the % scavenging results are mentioned in table-4.22respectively.

DPPH is a stable free radical, which has been widely used in phytomedicine for the assessment of scavenging activities of

bioactive fractions.The DPPH radical is considered to be a model of a stable lipophilic radical. This reaction has been widely used to test the ability of compounds to act as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts/fractions. The scavenging activity of chloroform fraction of *A. mexicana* was determined using free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH). Results showed that chloroform fraction of *A. mexicana* (IC_{50}61.89 µg/ml) possessed the good antioxidant activity. While standard drug ascorbic acid have 10.05 µg/ml IC_{50} value.

Oxidation is life, but except of so many necessary processes of life, during normal metabolism of oxygen, various free radicals as well as superoxide are produced continuously. The high level of this superoxide radical is known to be harmful to cellular ingredients as, contributing to tissue damage and various diseases. The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm with the plant fraction and the reference compound indicates their abilities to quench superoxide radicals in the reaction mixture. The chloroform fraction of *A. mexicana* was found to be an effective superoxide anion scavenger to scavenge the superoxide anions as compared to ascorbic acid which is measured in terms of IC_{50} (87.76 µg/ml).

The 2, 2 azobis-(3-ethylbenzothiozoline-6-sulphonic acid (ABTS) assay is based on the inhibition of the absorbance of the radical cation $ABTS^{\bullet+}$ which is quantified at 734 nm. It is a decolorization assay, thus the radical cation is prepared prior to addition of the antioxidant test system, quite than the generation of the radical taking place repetitively in presence of antioxidants (Youdim and Joseph, 2001). These results, obtained that chloroform fraction of *A. mexicana* with IC_{50} of 115.29µg/ml, where ascorbic acid had IC_{50} of 7.71µg/ml, significantly showed activity which is either by inhibiting or scavenging the ABTS radicals in a concentration dependant manner.

Nitric oxide (NO) is a main chemical moderator generated by endothelial cells, macrophages, neurons etc. and regulates the various physiological processes. Reactive Oxygen Species like oxygen reacts with excess NO to generate RNS like NO_2, N_2O_4 and peroxynitrite, together ROS and RNS attack and damage cellular molecules including lipids, protein, nucleic acids and carbohydrates (Pacifici and Davies, 1991).The chloroform fraction of *A. mexicana*with IC_{50} value of 82.12µg/ml where ascorbic acid had IC_{50} value of 7.92µg/ml significantly scavenges RNS. The chloroform fraction of *A. mexicana*owing radical

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scavenging ability may provide protection against oxidative damage induced to the biomolecules. It may also scavenge free radicals due to presence of antioxidant principles which compete with oxygen to react with nitric oxide and thus inhibiting the generation of RNS.

Table- 4.22: Effect of chloroform fraction of *A. mexicana* in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, super oxide radical, ABTS radical cation and Nitric oxide radical scavenging method

Sl. No	Concentration (µg/ml)	DPPH (% inhibition)	Superoxide radical (% inhibition)	ABTS (% inhibition)	Nitric oxide radical (% inhibition)
1	5	15.58±0.33	11.75±0.19	8.01±0.30	12.11±0.64
2	10	24.58±0.12	19.52±0.15	14.81±0.28	22.08±0.47
3	20	37.98±0.19	28.11±0.02	21.22±0.11	29.16±0.29
4	40	47.55±0.25	34.56±0.03	32.10±0.22	38.14±0.26
5	80	67.63±0.28	58.43±0.16	47.47±0.12	58.48±0.64
6	160	83.52±0.16	71.03±0.16	58.57±0.14	73.56±1.75
IC_{50} (µg/ml)		61.89	87.76	115.29	82.12
Ascorbic Acid IC_{50} (µg/ml)		10.05	15.54	7.71	7.92

Values are expressed in MEAN ± S.E.M (n =3).

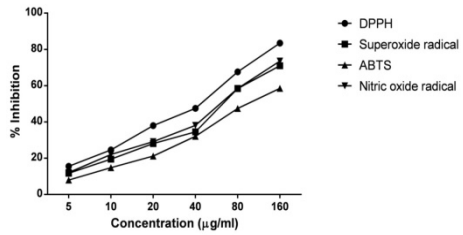

Fig.4.20: Effect of chloroform fraction of *A. mexicana* in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, super oxide radical, ABTS radical cation and Nitric oxide radical scavenging method

Effects of chloroform and aqueous fractions of *A. mexicana* on liver, kidney and pancreas antioxidant enzymes and total protein of 21-days treated diabetic rats

The antioxidant enzymes like reduced glutathione (GSH), Glutathione-S-Transferase (GST), Superoxide dismutase (SOD), Catalase (CAT) and total protein level in liver, kidney, and pancreas were determined after 21-days of treatment ofchloroform and aqueous fractions of hydro-alcoholic extract of *A. mexicana* in diabetic rats and the results are tabulated in table-4.23.The perusal of data, showed that the tested antioxidant enzymes and total protein level in liver, kidney and pancreasare lowered significantly (p<0.05) in diabetic rats as compared with normal control rats, whereas these values were significantly (p<0.05 to p<0.01) elevated towards normal level upon administration of chloroform and aqueous fractions when compared with diabetic control group.The level of SOD in liver, kidney and pancreas in chloroform and aqueous fraction treated group registered 224.16, 160.21; 134.29, 105.54; 116.92, 87.43 U/ml, respectively, whereas the standard drug glibenclamide possess 289.31, 139.53 U/ml and 129.83 U/ml. In a similar fashion the level of CAT in liver, kidney and pancreas in chloroform and the aqueous fraction treated group registered 45.83, 30.95; 49.08, 39.25; 26.26, 22.36 U/ml, respectively, whereas the standard drug glibenclamide possess 49.78, 52.86 U/ml and 29.31 U/ml. Same as the level of reduced glutathione in the liver, kidney and pancreas in chloroform and aqueous fraction treated group registered 522.61, 409.20; 436.76, 301.78; 348.83, 160.14 µM/ml, while the standard drug glibenclamide registered 560.45, 453.76 µM/ml and 395.25 µM/ml. Similarly the level of GST in liver, kidney and pancreas in chloroform and aqueous fraction treated group showed 55.20, 46.95; 43.58, 31.59; 24.13, 20.11 µM/ml, whereas the standard drug glibenclamide showed 57.38, 48.06 µM/ml and 29.27 µM/ml. Correspondingly the total protein level in liver, kidney and pancreas in chloroform and aqueous fraction treated group registered 51.37, 42.21; 36.41, 21.63; 21.97, 17.94 mg/dl respectively, though the glibenclamide standard drug showed 55.85, 41.75 mg/dl and 27.55 mg/dl.The extent of decrease in antioxidant enzymes and total protein levelin liver, kidney, and pancreas in the 21-days treated rats were glibenclamide followed by chloroform fraction, and aqueous fraction treated groups.

4.4.22.Effect of Aqueous and chloroform fractionof *A. mexicana*on Lipid peroxidation level (nanomole/mg protein) in alloxan induced diabetic rats.

Elevated lipid peroxidation can be due to augmented oxidative stress in the cell as a result of antioxidant scavenger system and it damage membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzymes and receptors (Arulselvan and Subramanian, 2007).Table-

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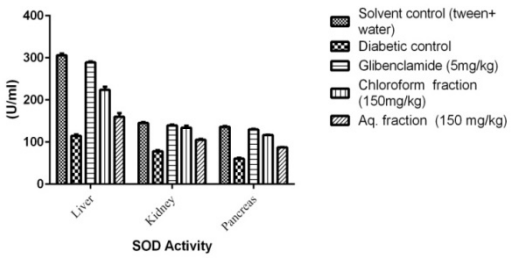
Table- 4.23:Effects of chloroform and aqueous fractions of *A. mexicana* on liver, kidney and pancreas antioxidant enzymes and total protein of 21-days treated diabetic rats.

Parameters	Organs	Solvent control r)	Diabetic	Glibenclamide (5mg/kg)	Chloroform (150mg/kg)	Aq. fraction (150 mg/kg)
SOD (U/ml)	Liver	306.00±3.94	114.99±3.14	289.31±2.11**	224.16±7.15*	160.21±8.63**
	Kidney	145.19±1.97	78.25±2.38	139.53±1.67**	134.29±4.13*	105.54±1.68*
	Pancreas	136.17±1.99	60.65±1.815	129.83±1.62**	116.92±0.64*	87.43±0.367**
CAT(U/ml)	Liver	52.83±0.23	22.57±1.21	49.78±0.89**	45.83±0.30**	30.95±0.62**
	Kidney	56.79±0.22	26.60±0.31	52.86±0.42**	49.08±0.50**	39.25±0.29**
	Pancreas	31.78±0.32	15.34±0.29	29.31±0.43**	26.26±0.09**	22.36 ±0.46**
GSH(µM/ml)	Liver	638.93±8.21	260.55±21.5	560.45±9.21**	522.61±11.6	409.20±8.03**
	Kidney	486.83±18.75	214.40±6.38	453.76±5.97**	436.76±13.7	301.78±18.88**
	Pancreas	424.25±19.25	79.19±5.98	395.25±7.43**	348.83±8.31*	160.14±17.14**
GST (µM/ml)	Liver	63.21±2.74	36.06±2.38	57.38±1.07**	55.20±0.51**	46.95±0.56**
	Kidney	53.01±2.64	22.71±2.67	48.06±1.46**	43.58±1.65**	31.59±1.95*
	Pancreas	34.05±2.59	14.83±1.89	29.27±1.95**	24.13±1.45**	20.11±0.85*
Total Protein(mg/ml)	Liver	60.27±4.78	33.05±0.94	54.85±1.34**	51.37±1.66**	42.21±0.73*
	Kidney	50.35±1.43	16.21±1.64	41.75±1.06**	36.41±2.84**	21.63±0.68*
	Pancreas	33.85±1.13	10.46±0.35	27.55±0.79**	21.97±0.36**	17.94±0.45**

Values are expressed in MEAN ± SEM of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at *p<0.05, **p<0.01 respectively, in comparison to diabetic control group).



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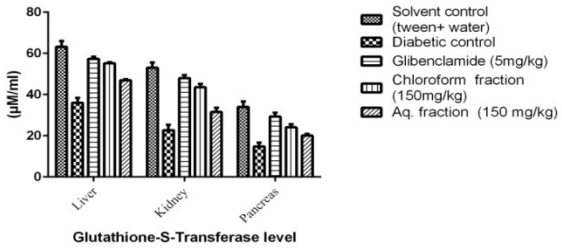

Fig.4.21: Effects of chloroform and aqueous fractions of *A. mexicana* on liver, kidney and pancreas antioxidant enzymes and total protein of 21-days treated diabetic rats.

4.24showed the estimated concentrations of liver Thiobarbituric acid reactive substances (TBARS), on 21st day of the study of aqueous and chloroform fractionof *A. mexicana* and standard drug. The study involves that normal concentrations of MDA were significantly increased in diabetic rats when compared with the control group. Treatment with chloroform and aqueous fractionof *A. mexicana* significantly decreased MDA (80.31 ± 0.74 and 130.61 ± 3.17nmol mg-1) in the pancreas and this indicates decreased oxidative stress.

Table- 4.24: Effect of Aqueous and chloroform fractionof *A. mexicana*on Lipid peroxidation level (nmole/ MDA mg protein) in alloxan induced diabetic rats.

Groups	Treatment and dose	Liver TBARS (nmol MDA/mg protein)
I	Solvent control	36.41±3.21
II	Diabetic control	185.39±7.05
III	Glibenclamide (5mg/kg)	57.73±1.65
IV	Chloroform fraction (150mg/kg)	80.31±0.74**
V	Aq. fraction (150 mg/kg)	130.61±3.17*

Values are expressed in MEAN ± SEM of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at *p<0.05, **p<0.01 respectively, in comparison to diabetic control group).

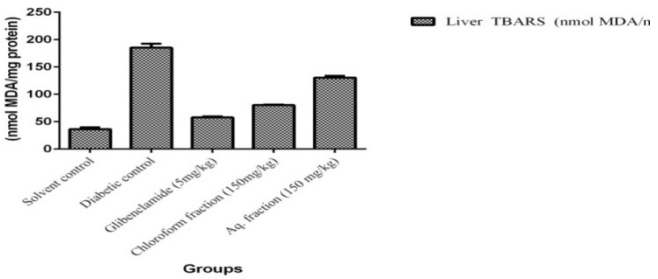

Fig.4.22: Effect of Aqueous and chloroform fractionof *A. mexicana*on Lipid peroxidation level (nmole/ MDA mg protein) in alloxan induced diabetic rats

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Effect of chloroform fraction of *A. mexicana* on α – amylase activity

It is well known that amylase inhibitors prevent dietary starches from being digested and absorbed by the body. This could be useful for treating diabetes mellitus (McEwan et al, 2010). The α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates and potentially useful in the control of obesity and diabetes. Acarbose is complex Oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in the breakdown of starch. Synthetic inhibitor causes side effect such as abdominal pain, diarrhoea and soft faces in the colon. The percent salivary alpha-amylase inhibitory activity of chloroform fraction of *A. mexicana* at different concentration was shown (Table-4.25). The observed IC_{50} value for *A. mexicana*chloroform fraction i.e. the concentration of the fraction, containing the alpha-amylase inhibitor that inhibited 50% of the enzyme activity was 48.45µg/ml /ml in comparison with acarbose.

Table- 4.25: Effect of chloroform fraction of *A. mexicana* on α – amylase activity

Sample	Concentration (µg/ml)	% Reduction	IC_{50} value µg/ml
Chloroform fraction	20	36.48±1.02	41.45
	40	48.23±0.97	
	60	65.82±1.61	
	80	71.55±1.32	
	100	75.22±0.99	
Acarbose	20	22.41±1.14	88.33
	40	29.06±1.09	
	60	38.74±1.24	
	80	45.27±0.99	
	100	55.45±1.12	

Values are expressed in MEAN ± S.E.M (n =3).

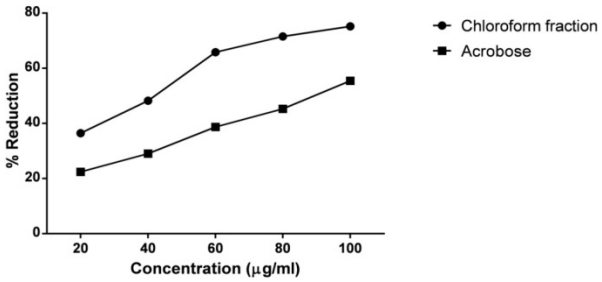

Fig.4.23: Effect of chloroform fraction of *A. mexicana* on α – amylase activity

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Anti-oxidant activity study

- High blood glucose level induces the production of free radicals which affect antioxidant defense mechanism, leading to the distraction of cellular functions, oxidative damage to cell membranes and increased susceptibility to lipid peroxidation.
- The experimental results found a marked quantity of phenolics and flavonoids in ethanol and aqueous extracts of aerial parts of *A. mexicana* contributing their antioxidant potential of the extracts which in turn responsible for antidiabetic activity. The total phenolic content of alcoholic and aqueous extracts of aerial parts of *A. mexicana* isfound to 56.75mg and 113.12mg with respect to gallic acid equivalent (GAE)/g respectively, while total flavonoids content is 6.86mg and 8.34mg in relation to, equivalent of quercetin/gm of the dry weight basis. Further, ethanol and aqueous extracts ofboth plants significantly scavenge DPPH and Hydrogen peroxide free radicals in a concentration dependent manner. The 50% inhibition (IC_{50}) value for test extracts of *A. mexicana* was found to 45.21and 40.11µg/ml for ethanolic and aqueous extracts respectively, while the IC_{50} value of ascorbic acid was 18.53µg/ml, which significantly reduces DPPH radical by bleaching.
- Whereas, ethanol and aqueous extracts of aerial parts of*A. mexicana* significantly **scavenge hydrogen peroxide radical**. The IC_{50} value for *A. mexicana* was found to 56.02and 56.24µg/ml for ethanolic extracts and aqueous extracts while the IC_{50} value of ascorbic acid was 21.41µg/ml significantly and concentration dependently scavenges H_2O_2 radical.
- While chloroform fraction of *A. mexicana* significantly scavenge DPPH, Superoxide, ABTS and Nitric oxide free radicals in a concentration dependent manner. The scavenging activity of chloroform fraction of *A. mexicana* was determined using **free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH)**. Results shows that chloroform fraction of both tested plants (IC_{50}61.89 µg/ml and 57.51 µg/ml) possessed the good antioxidant activity. Standard drug ascorbic acid has 10.05 µg/ml IC_{50} value. In ABTS free scavenging model results obtained that chloroform fraction of both tested plantswith IC_{50} of 115.29µg/ml and 93.16µg/ml, where ascorbic acid had IC_{50} of 7.71µg/mlwhich is by inhibiting or scavenging the ABTS radicals in a concentration dependant manner. The chloroform fraction of both tested plantswith IC_{50} value of 82.12µg/ml and 76.3µg/ml where ascorbic acid had IC_{50} value of 7.92µg/ml significantly scavenges nitric oxide free radicals. Similarly, the chloroform fraction of both tested plantswas found to an effective superoxide anion scavenger to scavenge the superoxide anions as compared to ascorbic acid which is measured in terms of IC_{50} (87.76 and 61.36 µg/ml).Which might

contribute to the reported antioxidant and antidiabetic potential of the chloroform fraction which in turn responsible for antidiabetic activity.

- The significant potential registered by chloroform and aqueous fraction of *A. mexicana* in reduction of the Liver lipid peroxidation products and elevation of the Liver, kidney and pancreas antioxidant enzymes in the diabetic rats, indicate that, both the fractions of plants are having very good potential to inhibit the oxidative damage of liver, kidney and pancreas tissues in diabetes. The increase in the liver, kidney and pancreas enzymatic antioxidant status might be due to decreased oxidative stress as evidenced by decreased lipid peroxidation in the fractions treated animals.
- The test extracts/fractionsof *A. mexicana* possess **radical scavenging ability** which in response provides protection against oxidative damage caused by diabetes mellitus. Overall, it is concluded that ethanolic, aqueous and hydro-alcoholic extracts and chloroform, aqueous fractions of hydro-alcoholic extracts of *A. mexicana* has potent antioxidant activity, contributedbythe phyto-constituents like phytosterol, polyphenols and flavonoids found in the test substances.

Isolation, Characterization and *In silico* study of the isolated compounds

- Since the test report embodied in the thesis evidence that chloroform fraction of *A. mexicana* shows comparatively better activity than aqueous fraction, hence it enforced us to isolate compound present in the chloroform fraction.
- Chloroform fraction of aerial parts of *A. mexicana* is done by column chromatographic techniques using different non-polar to polar solvents in different proportions and finally examined using TLC plate to get a single spot. The single spot containing solvent extract was eluted and dried to get the isolate product. The so obtained isolate product was analyzed by UV, IR, ^{1}H NMR, ^{13}C NMR and MASS spectroscopic methods, subsequently the spectral data were interpreted by referring previously reported information of similar spectral data and the isolate product is characterized as stigmasterol containing two double bond at 5 and 22-position along with a alcoholic group at 3-position of cyclopentanoperhydrophenanthrene in the same of using…….led to the isolation of (3S,8S,9S,10R,13R,14S,17R)-17[(E,2R,5S)-5ethyl-6methylhept-3-2-yl]-10,13-dimethyl,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (stigmasterol).All the inferences drawn are on the basis of characterization of these compounds through UV, IR, ^{1}H NMR, ^{13}C NMR and MASS spectroscopic techniques supported by the assessment based on the comparison

with the reported literatures on similar structures.This compoundwas isolated for the first time from aerial parts of *A. mexicana*.Various literatures revealed that stigmasterol have a significant potency in the control of BGL in diabetic rats in response to its antioxidant potential.

DISCUSSION

Conventionally, insulin dependent diabetes is treated with exogenous insulin and noninsulin dependent diabetes with synthetic oral hypoglycaemic agents like sulphonylureas and biguanides. However, hormone fails as a curative agent for complications of diabetes and the major drawbacks of insulin therapy are the side effects like insulin allergy, lipodystrophy and lipoatropy, insulin antibodies, altered metabolic control, autoimmunity and other late complications like morphological changes in the kidneys and severe vascular complications. Similarly, oral hypoglycaemic drugs have many side effects such as nausea, vomiting, cholestatic jaundice, aplastic and haemolytic anemias, generalised hypersensitivity reactions, dermatological reaction etc (Gupta et al, 2008; Mallick et al, 2007).

The collection of scientific and systematic approach for the biological assessment of plant products based on their exploit in the conventional systems of medicine forms the basis for an ideal approach in the development of new drugs from plants. Plant-based medicinal products have been known since ancient times, and several medicinal plants and their products (active natural principles and crude extracts) have been used to control diabetes in the traditional medicinal systems of many cultures worldwide. Several medicinal plants have found potential use as hypoglycemic in the Indian system of medicines. Several oral hypoglycaemic agents are the primary forms of treatment for diabetes. However, prominent side-effects of such drugs are the main reason for an increasing number of people seeking alternative therapies that may have less severe or no side effects.

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