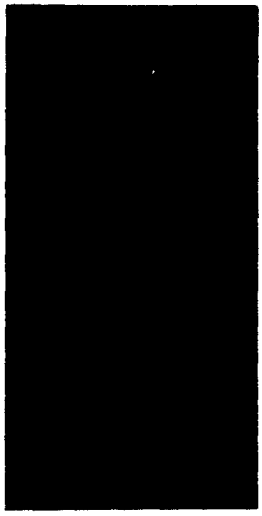
Chapter -III

RESULTS

TLC analysis of AETOF fraction

The thin layer chromatogram of AETOF fraction confirmed the presence of multiple compounds, when exposed to iodine vapor, as represented in Fig. 13. This technique is not suitable for the identification of constituents present in AETOF fraction. Therefore, we have gone for LC-ESI-MS/MS analysis.

Fig.13: Thin Layer Chromatogram of AETOF fraction.



LC-ESI-MS/MS analysis of AETOF fraction

In order to identify the phytochemical profile of AETOF fraction LC-Q-TOF-MS/MS was carried out. Both positive and negative modes were used for the detection of compounds present in total oligomeric flavonoid fraction of *A. echioides*. The positive mode of (+) ESI (Fig. 14a), negative mode of (-) ESI (Fig. 14b) and LC-DAD (Fig. 14c) chromatograms are illustrated in Fig. 14. LC-ESI-MS/MS chromatograms of individual compounds present in AETOF fraction of *Andrographis echioides* are shown in Fig.15. LC-ESI-MS/MS analysis resulted in the detection of 32 compounds (Table. 7) in AETOF fraction and they were identified by search with Agilent technologies Mass-hunter software by comparing its spectral data.

Fig. 14: (a) +ESI, (b) -ESI and (c) DAD chromatograms of AETOF fraction of *Andrographis echioides*.

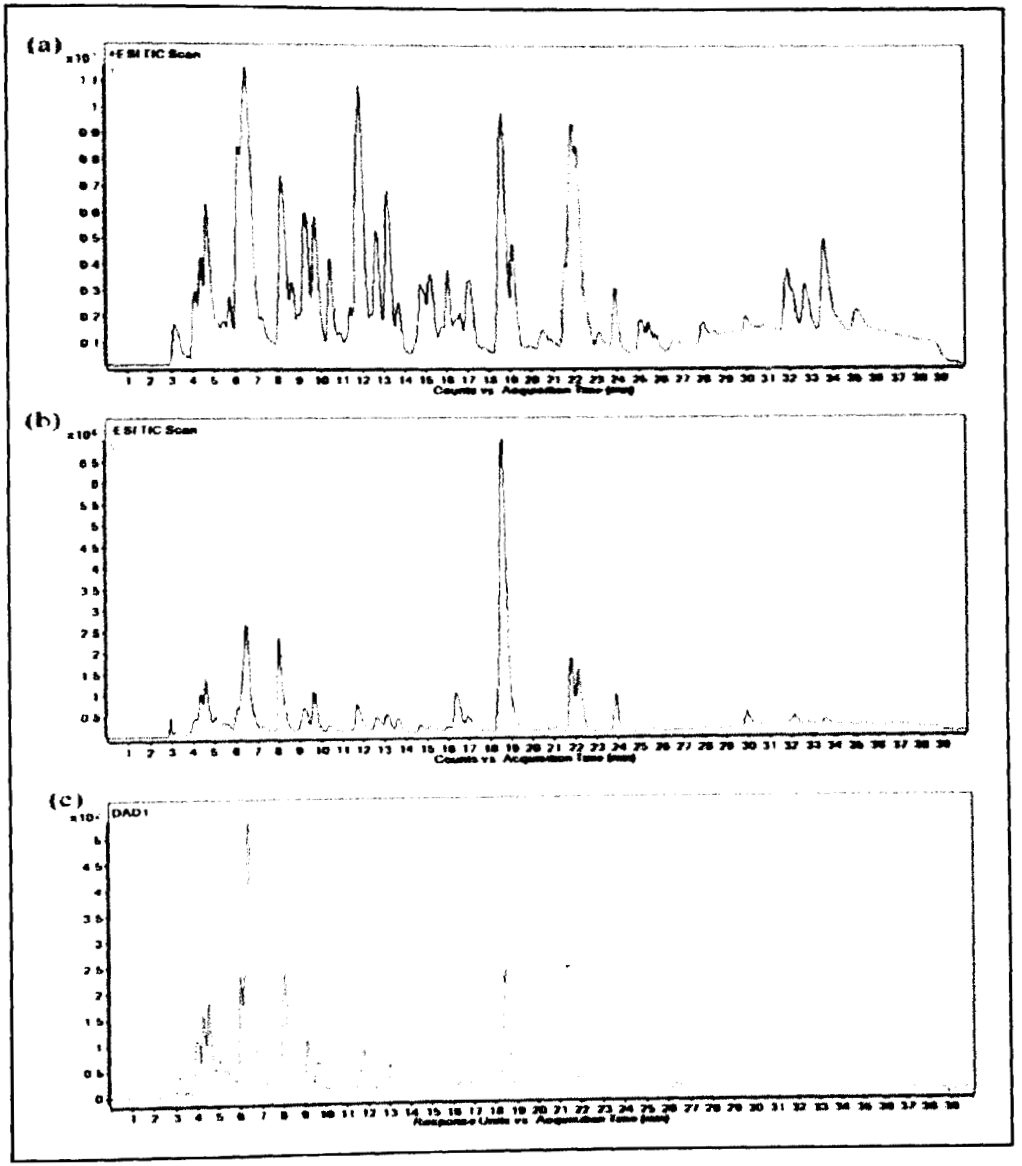
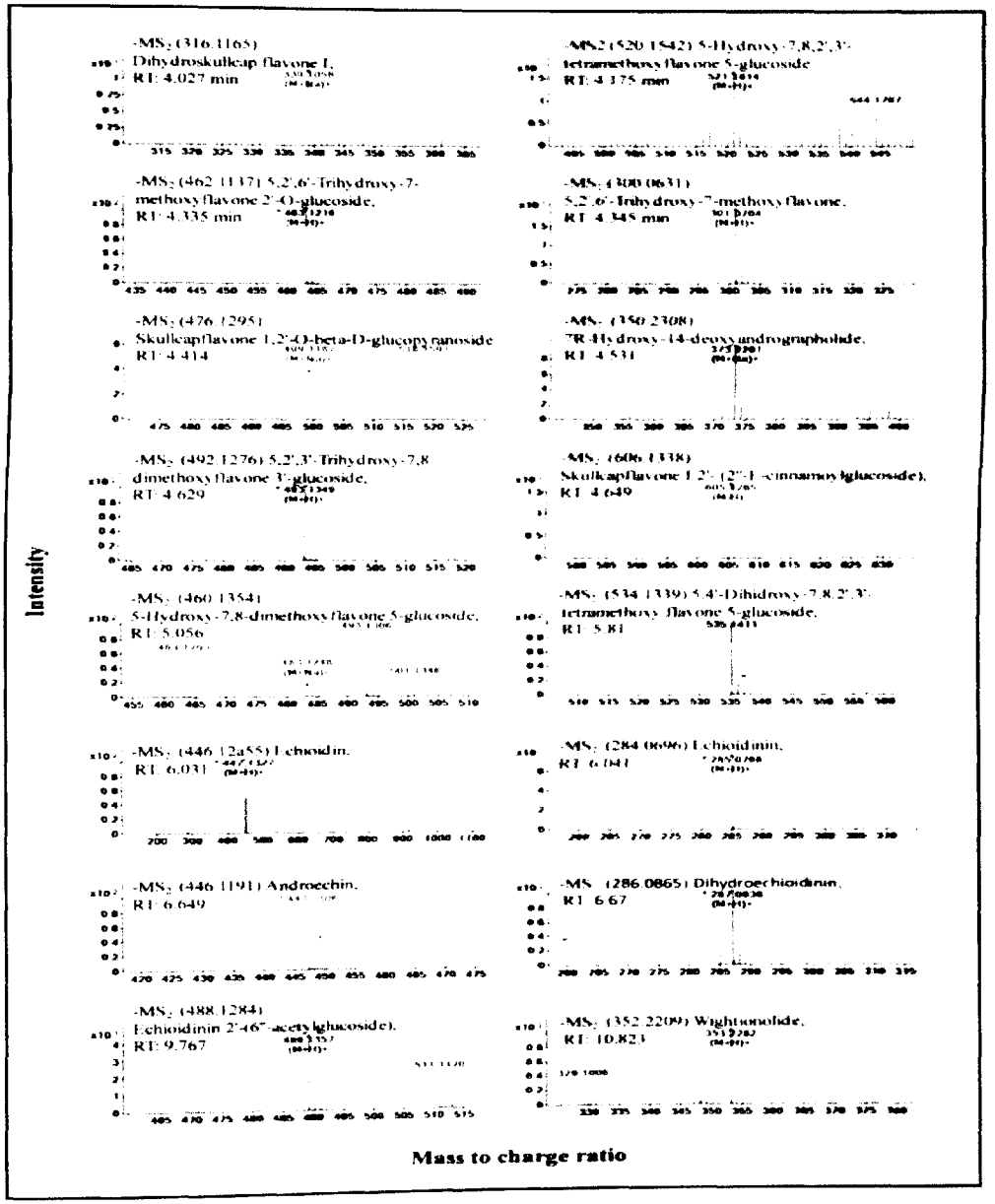


Fig. 15: LC-ESI-MS/MS chromatograms of individual compounds present in AETOF fraction of *Andrographis echioides*.



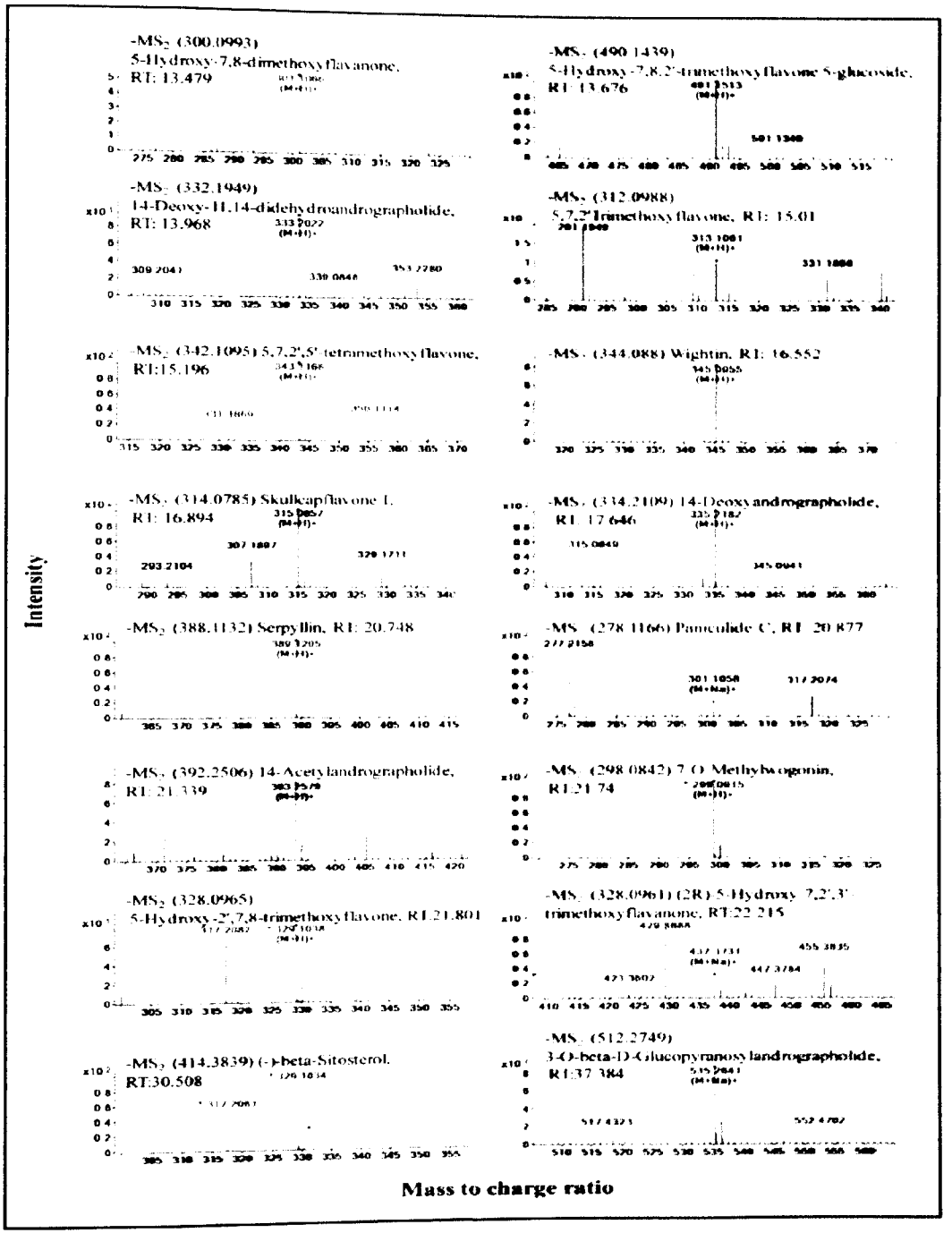

-MS2 (300.0993)
5-Hydroxy-7,8-dimethoxyflavanone,
RT: 13.479
-MS2 (490.1439)
5-Hydroxy-7,8,2'-trimethoxyflavone 5-glucoside,
RT: 13.676
-MS2 (332.1949)
14-Deoxy-11,14-didehydroandrographolide,
RT: 13.968
-MS2 (312.0988)
5,7,2'Trimethoxyflavone, RT: 15.01
-MS2 (342.1095) 5,7,2',5' tetramethoxyflavone,
RT:15.196
-MS2 (344.088) Wightin, RT: 16.552
-MS2 (314.0785) Skullcapflavone I,
RT: 16.894
-MS2 (334.2109) 14-Deoxyandrographolide,
RT: 17.646
-MS2 (388.1132) Serpyllin, RT: 20.748
-MS2 (278.1166) Pancuilide C, RT: 20.877
-MS2 (392.2506) 14-Acetylandrographolide,
RT: 21.339
-MS2 (298.0842) 7-O-Methylwogonin,
RT:21.74
-MS2 (328.0965)
5-Hydroxy-2",7,8-trimethoxyflavone, RT:21.801
-MS2 (328.0961) (2R)-5-Hydroxy-7,2',3'-trimethoxyflavanone, RT:22.215
-MS2 (414.3839) (-)-beta-Sitosterol,
RT:30.508
-MS2 (512.2749)
3-O-beta-D-Glucopyranosylandrographolide,
RT:37.384
Intensity
Mass to charge ratio

Table. 7: Compounds identified in AETOF fraction when subjected to LC-ESI-MS/MS analysis.

S.No	RT(mins)	Compounds	Formula	Mass
1	4.027	Dihydroskullcap flavone I	$C_{17}H_{16}O_6$	316.1165
2	4.175	5-Hydroxy-7,8,2',3'-tetramethoxyflavone 5-glucoside	$C_{25}H_{28}O_{12}$	520.1542
3	4.335	5,2',6'-Trihydroxy-7-methoxyflavone 2'-O-glucoside	$C_{22}H_{22}O_{11}$	462.1137
4	4.345	5,2',6'-Trihydroxy-7-methoxyflavone	$C_{16}H_{12}O_6$	300.0631
5	4.414	Skullcapflavone I,2'-O-beta-D-glucopyranoside	$C_{23}H_{24}O_{11}$	476.1295
6	4.531	7R-Hydroxy-14-deoxyandrographolide	$C_{20}H_{30}O_5$	350.2308
7	4.629	5,2',3'-Trihydroxy-7,8-dimethoxyflavone 3'-glucoside	$C_{23}H_{24}O_{12}$	492.1276
8	4.649	Skullcapflavone I 2'-(2"-E-cinnamoylglucoside)	$C_{32}H_{30}O_{12}$	606.1338
9	5.056	5-Hydroxy-7,8-dimethoxyflavone 5-glucoside	$C_{23}H_{24}O_{10}$	460.1354
10	5.81	5,4'-Dihidroxy-7,8,2',3'-tetramethoxy flavone 5-glucoside	$C_{25}H_{28}O_{13}$	534.1339
11	6.031	Echioidin	$C_{22}H_{22}O_{10}$	446.1255
12	6.041	Echioidinin	$C_{16}H_{12}O_5$	284.0696
13	6.649	Androechin	$C_{22}H_{24}O_{10}$	446.1191
14	6.67	Dihydroechioidinin	$C_{16}H_{14}O_5$	286.0865
15	9.767	Echioidinin 2'-(6"-acetylglucoside)	$C_{24}H_{24}O_{11}$	488.1284
16	10.823	Wightionolide	$C_{20}H_{32}O_5$	352.2209
17	13.479	5-Hydroxy-7,8-dimethoxyflavanone	$C_{17}H_{16}O_5$	300.0993
18	13.676	5-Hydroxy-7,8,2'-trimethoxyflavone 5-glucoside	$C_{24}H_{26}O_{11}$	490.1439
19	13.968	14-Deoxy-11,14-didehydroandrographolide	$C_{20}H_{28}O_4$	332.1949
20	15.01	5,7,2'-Trimethoxyflavone	$C_{18}H_{16}O_5$	312.0988
21	15.196	5,7,2',5'-tetramethoxyflavone	$C_{19}H_{18}O_6$	342.1095
22	16.552	Wightin	$C_{18}H_{16}O_7$	344.088
23	16.894	Skullcapflavone I	$C_{17}H_{14}O_6$	314.0785
24	17.646	14-Deoxyandrographolide	$C_{20}H_{30}O_4$	334.2109
25	20.748	Serpyllin	$C_{20}H_{20}O_8$	388.1132
26	20.877	Paniculide C	$C_{15}H_{18}O_5$	278.1166
27	21.339	14-Acetylandrographolide	$C_{22}H_{32}O_6$	392.2506
28	21.74	7-O-Methylwogonin	$C_{17}H_{14}O_5$	298.0842
29	21.801	5-Hydroxy-2',7,8-trimethoxyflavone	$C_{18}H_{16}O_6$	328.0965
30	22.215	(2R)-5-Hydroxy-7,2',3'-trimethoxyflavanone	$C_{18}H_{18}O_6$	328.0961
31	30.508	(-)-beta-Sitosterol	$C_{29}H_{50}O$	414.3839
32	37.384	3-O-beta-D-Glucopyranosylandrographolide	$C_{26}H_{40}O_{10}$	512.2749

Evaluation of antihyperglycemic activity of crude aqueous suspension of leaves of the powder of *A. echioides* (CASAE) in STZ-induced rats:

The effect of crude aqueous suspension of leaves powder of *A. echioides* on the fasting blood glucose levels of diabetic rats is given in Table. 8. Fasting blood glucose levels of diabetic rats (group 2 & group 3) were significantly higher than those in normal rats (group 1). A significant (63 %) decrease in fasting blood glucose levels were observed in diabetic treated (group 3), when compared to those of diabetic untreated (group 2) rats.

Table. 8: Evaluation of antihyperglycemic effect of crude aqueous suspension of *A. echioides* leaves (CASAE) in normal and STZ induced diabetic rats.

Groups	Fasting Blood Glucose (mg/dl) levels after treatment with CASAE.						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Normal	83±3.7	78±40	80±3.4	83±5.1	82±5.7	79±4.8	82±4.9
Diabetic	389±25.1†	379±22.5	390±23	382±19.5	377±21.8	373±18.2	379 ±19
Diabetic treated with CASAE	383±23.7†	318±21.3*	279±21.9** (27.1 %)	236±19.4** (38.3 %)	199±17** (48 %)	180±19.1** (53 %)	141±16.9** (63 %)

† P <0.0001 compared with the initial level of blood glucose (0 h) of normal rats.

** P <0.0001 compared with the initial level of blood glucose (0 h) in the respective group.

* P <0.001 compared with the initial level of blood glucose (0 h) in the respective group.

Numbers in parenthesis indicate the percentage of fall in (0 h) blood glucose.

Evaluation of antihyperglycemic activity of AETOF in normal and STZ- induced diabetic rats.

The antihyperglycemic activity of AETOF in normal and STZ- induced diabetic rats is given in Table. 9. The FBG levels of diabetic untreated rats were significantly higher than those of normal untreated rats. When different doses of AETOF were administrated for evaluating their antihyperglycemic activity, significant (68.4 %) decrease was observed in the fasting blood glucose levels of diabetic rats treated with

AETOF fraction at a dose of 50 mg/kg bw. 57.2% decrease in FBG was observed at a dosage of 30 mg/kg bw. Even though the blood glucose levels were decreased to a maximum fall of 83.8% with the AETOF fraction at a dosage of 70 mg/kg bw, it produced hypoglycemia in the diabetic rats. However none of the doses of the AETOF fraction exhibited hypoglycemic condition in normal treated rats. The antihyperglycemic activity of AETOF fraction was compared with that of glibenclamide a standard drug (20 mg/kg bw) and the effect of AETOF fraction was more prominent (68.4 %) when compared to that of glibenclamide (31.1%).

Table. 9: Antihyperglycemic activity of different doses of AETOF fraction in normal and STZ induced diabetic rats.

Groups	Fasting Blood Glucose (mg/dl) levels after treatment with AETOF fraction.						
	0 h	1h	2 h	3 h	4 h	5 h	6 h
Normal	83± 5.6	90 ± 3.5	86 ± 7.7	89 ± 5.3	88 ± 4.7	87 ± 5.2	85 ± 4.5
Diabetic	397±18.2	396 ± 17.6	391± 21	390 ± 16.2	394±17.2	389± 18.5	374± 16
Normal + AETOF 30mg/kg bw	81± 3.9	85±5.5	83±3.5	86± 4.8	82 ±4.4	84.83±4.6	82±3.7
Normal + AETOF 50mg/kg bw	82± 4.1	82± 4.9	83±7.6	86±6.2	86±7.7	84± 6.8	82± 3.6
Normal + AETOF 70mg/kg bw	82 ± 3.3	84±5.2	81±4.1	82±4.1	82±3.4	80±3.1	84±7.9
Diabetic + AETOF 30mg/kg bw	384± 24†	318 ±21.3*	279±22.3**	236±15**	199±17**	183±18.4** (52.3 %)	164±15.1** (57.2%)
Diabetic + AETOF 50mg/kg bw	386±19.6†	310±25.7*	277± 25.9**	234±23**	188±19.4**	143±17.9** (62.9 %)	121 ± 14** (68.4%)
Diabetic + AETOF 70mg/kg bw	360±21.4†	230±22.8**	169±17**	119±9.4** (66.9 %)	63±9** (82.5 %)	61±8.6** (83%)	58±5.9** (83.8%)
Diabetic + 20mg Glibenclamide/kg bw	298±17.7†	276 ± 13.5	259±10.9	245±12.2*	225 ±8.8**	213 ±9.1**	201±8.8** (31.1%)

† P <0.0001 compared with the initial level of blood glucose (0h) of normal rats.

** P <0.0001 compared with the initial level of blood glucose (0 h) in the respective group.

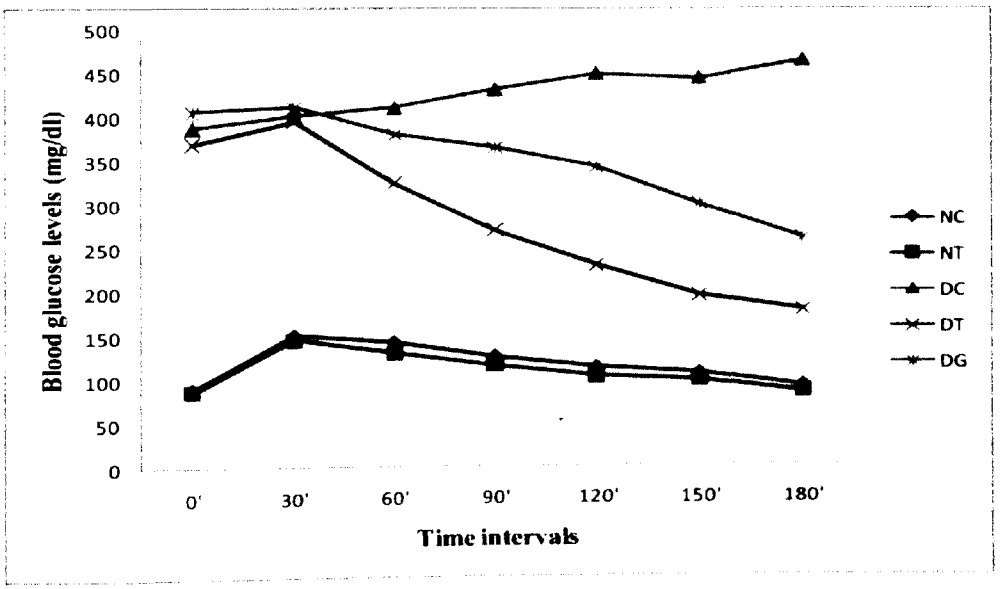
* P <0.001 compared with the initial level of blood glucose (0 h) in the respective group.

Numbers in parenthesis indicate the percentage of fall in (0 h) blood glucose.

Effect of AETOF fraction on oral glucose tolerance (OGT):

Treatment with AETOF fraction at a dose of 50 mg/kg bw along with 2 g glucose/kg bw has significantly enhanced the glucose tolerance in diabetic rats. In diabetic untreated rats the glucose levels remained higher without much change even at 180 min after glucose load, whereas in the AETOF fraction treated diabetic rats and glibenclamide administrated diabetic rats the glucose levels started falling from 30min after glucose load. There was a consistent decrease in the blood glucose levels with a maximum fall of 51.5 % after 180 min of treatment. The effect of AETOF fraction was much higher than that of glibenclamide. The blood glucose levels of normal and normal treated rats after 180 min of treatment are in normal condition. The results are depicted in Fig. 16.

Fig.16: Effect of AETOF fraction on oral glucose tolerance in normal and diabetic rats.



NC= Normal Control, NT= Normal Treated with AETOF Fraction, DC=Diabetic Control, DT= Diabetic Treated with AETOF Fraction, DG= Diabetic Treated with Glibenclamide.

Effect of long term treatment with the AETOF fraction on the blood glucose, plasma insulin, total Hb and glycosylated haemoglobin (HbA_{1c}) of normal and diabetic rats

Table.10: Shows the effect of long term treatment with the AETOF fraction on fasting blood glucose, (FBG) plasma insulin and change in body weights of normal and diabetic rats.

The FBG levels were significantly higher in the diabetic group than those in normal before starting the experiment. However, at the end of 40 days of treatment, there was 72.7 % decrease ($P < 0.001$) in the FBG levels of diabetic rats treated with AETOF fraction. The treatment with glibenclamide has produced only 42.6 % decrease in the fasting blood glucose levels in the 5^{th} group of diabetic rats, while there was a further increase in the blood glucose levels of untreated diabetic rats. There was no change in the FBG levels of normal rats after treatment with AETOF fraction. At the end of 40 days treatment, the body weights of normal, normal treated, diabetic rats treated with either AETOF fraction or glibenclamide, increased significantly by +34 g, +23 g, +15 g and +9 g respectively, whereas the body weights of diabetic untreated group decreased by -35 g.

Plasma insulin levels (14.4 ± 0.86 μU/ml) of diabetic untreated rats were significantly lower than those (26.3 ± 1.93 μU/ml) in normal rats and the insulin levels were further decreased (10.25 ± 1.22 μU/ml) in the untreated diabetic rats after 40 days. The diabetic rats treated with AETOF fraction have shown a significant increase in the insulin levels to 23.45 ± 0.67 μU/ml from an initial value of 15.31 ± 1.0 μU/ml. In the glibenclamide treated diabetic rats also there was a significant increase in the levels of insulin from 14.86 ± 0.94 μU/ml to 21.95 ± 1.42 μU/ml. In the normal treated rats there was no significant change in the plasma insulin levels.

The $HbA1_C$ levels of the diabetic untreated group were significantly higher than those in diabetic and normal control group (10.65 ± 1.22 % vs 6.166 ± 0.175 %). Treatment with the AETOF fraction in diabetic rats reduced the $HbA1_C$ to a significant lower level (7.13 ± 0.508%), indicating a significant improvement in glycemic control in diabetic rats upon treatment. Glibenclamide also caused a significant reduction in the $HbA1_C$ in the glibenclamide treated diabetic rats.

Table. 10: Effect of long term treatment with the AETOF fraction on the plasma glucose, insulin, total Hb and glycosylated haemoglobin ($HbA1_C$) and body weights of normal and diabetic rats.

Groups	Before treatment		After treatment		Hb (g/dl)	$HbA1_C$ (%)	Change in body weights (g)
	Glucose (mg/dl)	Insulin (µU/ml)	Glucose (mg/dl)	Insulin (µU/ml)			
Normal	94.83 ± 4.70^{a}	26.3 ± 1.93^{d}	87.66 ± 6.21^{a}	25.3 ± 2.49^{d}	12.083 ± 0.40^{c}	6.166 ± 0.175^{a}	+ 34
Normal + AETOF 50mg/kg bw	93.00 ± 5.79^{a}	25.9 ± 0.48^{d}	92.16 ± 7.250^{a}	26.5 ± 2.40^{cd}	11.75 ± 0.62^{d}	6.00 ± 0.178^{a}	+ 23
Diabetic control	388.0 ± 15.42^{c}	14.4 ± 0.86^{a}	456.83 ± 24.28^{d}	10.25 ± 1.22^{a}	7.55 ± 0.84^{a}	10.65 ± 1.22^{d}	-35
Diabetic + AETOF 50mg/kg bw	384.16 ± 30.54^{b}	15.31 ± 1.05^{c}	105.33 ± 8.95^{b}	23.45 ± 0.67^{c}	10.91 ± 0.65^{b}	7.13 ± 0.508^{b}	+ 15
Diabetic + 20mg Glibenclamide/kg bw	396.83 ± 38.80^{b}	14.86 ± 0.94^{b}	234.16 ± 36.79^{c}	21.95 ± 1.42^{b}	10.65 ± 0.72^{b}	7.81 ± 0.915^{c}	+ 9
F value	179.210	169.215	189.036	79.660	43.729	39.985	
Significance	0.000	0.000	0.000	0.000	0.000	0.000	

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Effect of long term treatment with the AETOF fraction on glycogen levels in liver, muscle & protein levels in plasma, liver, kidney of normal and diabetic rats.

Table. 11: shows the effect of long term treatment with the AETOF fraction on liver and muscle glycogen levels & plasma, hepatic and renal protein levels in different experimental groups of rats.

The glycogen levels in liver and muscle of diabetic rats were significantly lower than those in normal control group of rats. After treatment with the AETOF fraction, there was a significant increase in the liver and muscle glycogen levels. Glibenclamide treatment also resulted in a significant improvement in the glycogen levels of liver and muscle. The plasma, hepatic and renal protein levels in diabetic rats were lower than those in normal rats whereas on treatment with AETOF fraction/Glibenclamide in these respective groups, there was a significant increase in plasma and tissue protein levels.

Table. 11: Effect of long term treatment with the AETOF fraction on glycogen levels in liver, muscle & protein levels in plasma, liver, kidney of normal and diabetic rats.

Groups	Glycogen (mg glucose equivalents/ g wet tissue)		Protein		
	Liver	Muscle	Plasma (mg/dl)	Liver (mg/g wet tissue)	Kidney (mg/g wet tissue)
Normal	11.754 ± 0.191^{d}	7.075 ± 0.088^{c}	6.966 ± 0.475^{c}	130.74 ± 1.676^{d}	114.64 ± 0.696^{d}
Normal + AETOF 50mg/kg bw	12.097 ± 0.125^{d}	$7.831 \pm 0.211c$	7.345 ± 0.274^{c}	132.46 ± 1.576^{d}	125.87 ± 1.436^{c}
Diabetic control	6.688 ± 0.116^{a}	3.256 ± 0.192^{a}	4.218 ± 0.034^{a}	101.17 ± 1.001^{a}	82.564 ± 1.384^{a}
Diabetic + AETOF 50mg/kg bw	9.248 ± 0.305^{c}	5.937 ± 0.058^{b}	6.578 ± 0.025^{b}	128.82 ± 0.208^{c}	100.297 ± 1.704^{c}
Diabetic + 20mg Glibenclamide/kg bw	8.956 ± 0.117^{b}	5.23 ± 0.089^{b}	6.026 ± 0.037^{b}	122.52 ± 1.163^{b}	95.77 ± 1.039^{b}
F value	926.482	859.414	147.389	643.366	1005.345
Significance	0.000	0.000	0.000	0.000	0.000

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Effect of long term treatment on carbohydrate metabolism

The effect of AETOF fraction treatment on the activities of hepatic and renal hexokinase (HK), glucose-6-phosphate dehydrogenase (G-6-PDH), glucose-6-phosphatase and fructose-1,6-bisphosphatase in diabetic and normal rats are given in Table. 12 and 13 respectively. The activities of hexokinase and glucose- 6-phosphate dehydrogenase in liver and kidney were found to be decreased, while the activities of gluconeogenic enzymes, glucose-6-phosphatase and fructose-1, 6-bisphosphatase of both the tissues were significantly increased in diabetic rats compared to those in normal rats. Treatment of diabetic rats with AETOF fraction reverted the above changes significantly to near normal levels. Glibenclamide treated also significantly improved the activities of theses enzymes.

Table.12: Effect of long term treatment with the AETOF fraction on the activities of enzymes of carbohydrate metabolism in liver of different groups of experimental animals

Groups	Hexokinase (μ moles of glucose phosphorylated/hr/mg protein)	Glucose-6-phosphate dehydrogenase (U/ mg protein)	Fructose 1-6-bisphosphatase (μ moles of Pi liberated /hr/mg protein)	Glucose-6-phosphatase (μ moles of Pi liberated /hr/mg protein)
Normal	0.191 ± 0.099^{d}	0.345 ± 0.021^{a}	0.0712 ± 0.011^{a}	0.0356 ± 0.008^{d}
Normal + AETOF 50mg/kg bw	0.199 ± 0.201^{d}	0.372 ± 0.20^{b}	0.0601 ± 0.012^{a}	0.0361 ± 0.006^{d}
Diabetic control	0.090 ± 0.009^{a}	0.142 ± 0.102^{c}	0.154 ± 0.104^{d}	0.178 ± 0.06^{a}
Diabetic + AETOF 50mg/kg bw	0.154 ± 0.022^{c}	0.264 ± 0.201^{b}	0.0631 ± 0.001^{b}	0.0322 ± 0.005^{c}
Diabetic + 20mg Glibenclamide/kg bw	0.134 ± 0.006^{b}	0.209 ± 0.015^{c}	0.0614 ± 0.006^{c}	0.0294 ± 0.004^{b}
F value	34.162	99.356	184.866	35.027
Significance	0.000	0.000	0.000	0.000

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Table. 13: Effect of long term treatment with the AETOF fraction on the activities of enzymes of carbohydrate metabolism in kidney of different groups of experimental animals.

Groups	Hexokinase (µmoles of glucose phosphorylated/hr/mg protein)	Glucose-6-phosphate dehydrogenase (U/ mg protein)	Fructose 1-6-bisphosphatase (µ moles of Pi liberated /hr/mg protein)	Glucose-6-phosphatase (µ moles of Pi liberated /hr/mg protein)
Normal	0.178 ± 0.021^{a}	0.358 ± 0.001^{a}	0.721 ± 0.001^{a}	0.432 ± 0.015^{a}
Normal + AETOF 50mg/kg bw	0.166 ± 0.201^{b}	0.372 ± 0.021^{a}	0.756 ± 0.001^{a}	0.479 ± 0.005^{a}
Diabetic control	0.088 ± 0.001^{c}	0.136 ± 0.001^{b}	0.132 ± 0.003^{b}	0.129 ± 0.01^{b}
Diabetic + AETOF 50mg/kg bw	0.140 ± 0.022^{b}	0.274 ± 0.001^{c}	0.682 ± 0.001^{c}	0.381 ± 0.01^{c}
Diabetic + 20mg Glibenclamide/kg bw	0.125 ± 0.014^{c}	0.251 ± 0.003^{d}	0.649 ± 0.003^{d}	0.357 ± 0.004^{c}
F value	230.40	182.54	183.55	62.70
Significance	0.000	0.000	0.000	0.000

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Effect of long term treatment with AETOF fraction on Lipid and Lipoprotein profile

Table. 14 shows the effect of AETOF fraction on the levels of serum triglycerides, total cholesterol, LDL, VLDL and HDL-Cholesterol in different experimental groups of rats. The diabetic untreated group had significant elevation of triglycerides, LDL – Cholesterol, VLDL – Cholesterol and total cholesterol and reduction in HDL – Cholesterol levels compared to those in normal control rats. A significant reduction in levels of serum triglycerides, total cholesterol, LDL, VLDL – Cholesterol and increase in HDL – Cholesterol levels were observed in diabetic rats treated with either AETOF fraction or glibenclamide (Fig. 17). There were no significant changes in the levels of serum lipids and lipoproteins in the normal and normal treated rats.

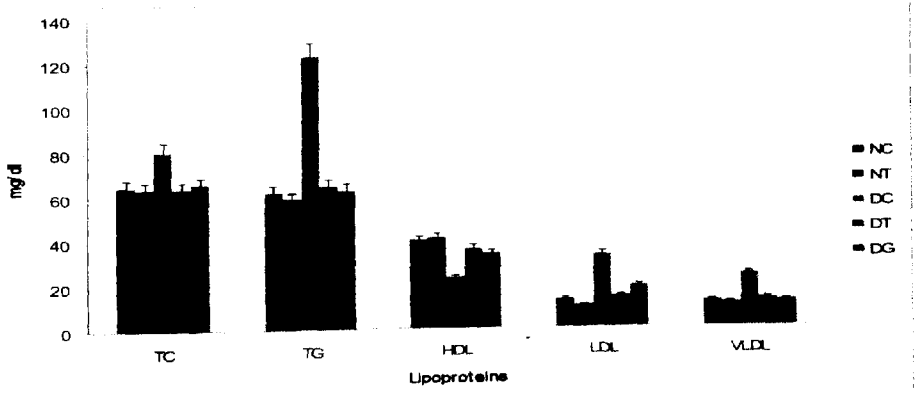
Table. 14: Effect of long term treatment with AETOF fraction on serum cholesterol, triglycerides, HDL, LDL, VLDL cholesterol in different groups of experimental animals

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	VLDL-Cholesterol (mg/dl)
Normal	65.18 ± 1.71^{a,b}	62.28 ± 1.12^{b}	40.14 ± 1.86^{d}	13.64 ± 1.93^{b}	12.42 ± 0.20^{a,b}
Normal + AETOF 50mg/kg bw	64.11 ± 1.19^{a}	59.80 ± 2.35^{a}	41.38 ± 1.99^{d}	10.78 ± 2.27^{a}	11.95 ± 0.47^{a}
Diabetic control	81.94 ± 1.90^{c}	124.67 ± 1.36^{e}	23.93 ± 0.94^{a}	33.09 ± 2.42^{d}	24.93 ± 0.27^{d}
Diabetic + AETOF 50mg/kg bw	64.33 ± 1.04^{a}	65.90 ± 2.41^{c}	36.79 ± 2.18^{c}	14.35 ± 2.79^{b}	13.17 ± 0.485^{c}
Diabetic + 20mg Glibenclamide/kg bw	66.27 ± 0.82^{b}	63.53 ± 2.19^{b}	34.14 ± 2.1^{b}	19.43 ± 2.48^{c}	12.70 ± 0.438^{c}
F value	179.175	119.279	118.08	81.751	120.157
Significance	0.000	0.000	0.000	0.000	0.000

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Fig. 17: Effect of AETOF fraction on serum lipid and lipoprotein profile:



Values are given as mean ± S.D from six rats in each group.

NC = Normal Control, NT = Normal Treated with AETOF Fraction, DC = Diabetic Control, DT + Diabetic Treated with AETOF Fraction, DG = Diabetic Treated with Glibenclamide.

Effect of long term treatment with AETOF fraction on HMG-CoA/Mevalonate ratio, Cholesterol, triglycerides in liver and Cholesterol and triglycerides in kidney of normal and experimental group of rats.

Table. 15 shows the effect of AETOF fraction on hepatic HMG-CoA/mevalonate ratio, cholesterol and triglycerides in liver and kidneys of different groups of experimental rats. Lower the ratio of HMG-CoA/mevalonate higher is the HMG-CoA reductase activity and vice-versa. The activity of hepatic HMG-CoA reductase was significantly increased in diabetic untreated rats when compared to normal control rats. Treatment with the AETOF fraction or Glibenclamide in diabetic rats significantly decreased the activity of HMG-CoA reductase in group 4 and 5 respectively. The levels of total cholesterol and triglycerides were significantly increased in diabetic untreated rats both in liver and kidney when compared to those in normal rats. Treatment with the AETOF fraction in diabetic rats significantly reverted the above changes.

Table.15: Effect of long term treatment with AETOF fraction on HMG-CoA/Mevalonate ratio, Cholesterol, triglycerides in liver and Cholesterol and triglycerides in kidney of normal and experimental group of rats.

Groups	Liver			Kidney	
	Hepatic HMG-CoA/ Mevalonate ratio	Cholesterol (mg/g tissue)	Triglycerides (mg/g tissue)	Cholesterol (mg/g tissue)	Triglycerides (mg/g tissue)
Normal	1.69±0.142^{c}	6.28±0.140^{a}	5.25±0.355^{a}	7.27±0.354^{a}	6.42±0.231^{a}
Normal + AETOF 50mg/kg bw	1.80± 0.240^{d}	6.10±0.421^{a}	5.15±0.357^{a}	7.31±0.201^{a}	6.29±0.067^{a}
Diabetic control	1.140±0.059^{a}	8.58±0.137^{c}	7.10±0.203^{d}	9.46±0.212^{c}	8.65±0.125^{c}
Diabetic + AETOF 50mg/kg bw	1.55±0.231^{a,b}	7.32±0.127^{a,b}	6.50±0.239^{b}	8.02±0.180^{b}	7.46±0.079b
Diabetic + 20mg Glibenclamide/kg bw	1.58±0.092^{a,b}	7.90±0.116^{b}	6.73±0.219^{c}	8.10±0.271^{b}	7.66±0.232b
F value	74.119	52.80	135.22	30.28	94.25
Significance	0.003	0.000	0.000	0.000	0.000

Values are given as mean ± S.D from six rats in each group.
Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Table. 16 and Table. 17 show the levels of TBARS, activities of SOD, CAT, GPx and GST in the liver and kidney respectively of normal and experimental groups of rats. There was a significant increase in the levels of TBARS, CAT activity and a significant decrease in the activities of SOD, GPx and GST in both tissues of diabetic rats. The treatment with AETOF fraction decreased the levels of TBARS, CAT activity and significantly increased the activities of SOD, GPx and GST in liver and kidney of diabetic rats. Similar effect was observed with glibenclamide but less in magnitude when compared to that of the AETOF fraction.

Table. 16: Effect of long term treatment with AETOF fraction on TBARS levels and antioxidant enzyme activities in the liver of different experimental groups.

Groups	Lipid Peroxides (nmoles MDA/mg protein)	Catalase (U/mg Protein)	Glutathione Peroxidase (U/ mg protein)	Superoxide Dismutase (U/ mg protein)	Glutathione-S-Transferase (U/ mg protein)
Normal	0.110 ± 0.006^{a}	60.40 ± 1.70^{a}	0.273 ± 0.005^{c}	12.33 ± 0.50^{c}	15.12 ± 1.36^{d}
Normal + AETOF 50mg/kg bw	0.109 ± 0.004^{a}	58.83 ± 1.48^{a}	0.277 ± 0.006^{c}	12.37 ± 1.54^{c}	16.19 ± 1.16^{d}
Diabetic control	0.247 ± 0.003^{c}	109.64 ± 5.06^{d}	0168 ± 0.008^{a}	5.12 ± 0.91^{a}	6.99 ± 0.23^{a}
Diabetic + AETOF 50mg/kg bw	0.167 ± 0.004^{b}	86.68 ± 1.16^{b}	0.215 ± 0.005^{b}	11.56 ± 0.77^{c}	12.29 ± 0.65^{c}
Diabetic + 20mg Glibenclamide/kg bw	0.170 ± 0.002^{b}	90.21 ± 2.25^{b}	0.212 ± 0.009^{b}	9.41 ± 0.27^{b}	10.15 ± 0.85^{b}
F value	514.368	215.536	86.530	82.670	197.201
Significance	0.000	0.000	0.000	0.000	0.000

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Table. 17: Effect of long term treatment with AETOF fraction on TBARS levels and antioxidant enzyme activities in the Kidney of different experimental groups

Groups	Lipid Peroxides (nmoles MDA/mg protein)	Catalase (U/mg protein)	Glutathione Peroxidase (U/mg protein)	Superoxide Dismutase (U/ mg protein)	Glutathione-S-Transferase (U/ mg protein)
Normal	0.127 ± 0.002 [b]	40.72 ± 2.35 [b]	0.198 ± 0.015 [d]	11.52 ± 0.67 [d]	12.62 ± 0.63 [d]
Normal + AETOF 50mg/kg bw	0.124 ± 0.003 [a]	38.47 ± 1.16 [a]	0.194 ± 0.011 [d]	11.77 ± 0.51 [d]	13.15 ± 0.52 [d]
Diabetic control	0.247 ± 0.005 [c]	71.10 ± 1.93 [c]	0.0855 ± 0.007 [a]	4.29 ± 0.46 [a]	4.11 ± 0.67 [a]
Diabetic + AETOF 50mg/kg bw	0.173 ± 0.003 [c]	52.56 ± 1.52 [c]	0.154 ± 0.008 [c]	9.83 ± 0.77 [c]	8.22 ± 0.31 [c]
Diabetic + 20mg Glibenclamide/kg bw	0.199 ± 0.004 [d]	60.49 ± 1.24 [d]	0.129 ± 0.010 [b]	6.59 ± 0.68 [b]	6.8 ± 0.52 [b]
F value	326.108	63.639	69.204	58.230	158.604
Significance	0.000	0.000	0.000	0.000	0.000

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Effect of long term treatment with AETOF Fraction non-Enzymatic Antioxidants

Table. 18 shows the levels of vitamin C, vitamin E and GSH in plasma of normal and experimental groups of rats. The levels of vitamin C, vitamin E and GSH were significantly decreased in plasma of diabetic untreated rats compared to those in normal rats. The administration of AETOF fraction/glibenclamide in diabetic rats restored the normal level of non-enzymatic antioxidants.

Table. 19 shows the levels of non-enzymatic antioxidants vitamin C, vitamin E and GSH in liver and kidney of normal and experimental groups of rats. The levels of vitamin C and GSH were significantly decreased while the levels of vitamin E were significantly increased in both tissues of diabetic untreated rats compared to those of normal rats. The administration of AETOF fraction to the diabetic rats significantly restored the levels of Vitamin C and Vitamin E near to normal levels in both tissues. GSH levels were also improved after treatment with either AETOF fraction/glibenclamide.

Table. 18: Effect of long term treatment with AETOF fraction on non-enzymatic antioxidants vitamin C, vitamin E and GSH in plasma of normal and experimental groups.

Groups	Vitamin-C (mg/dl)	Vitamin-E (mg/dl)	Reduced glutathione (mg/dl)
Normal	1.53 ± 0.046^{d}	1.371 ± 0.029^{d}	21.06 ± 0.652^{d}
Normal + AETOF 50mg/kg bw	1.61 ± 0.33^{d}	1.362 ± 0.032^{d}	22.14 ± 1.425^{e}
Diabetic control	1.15 ± 0.050^{a}	0.820 ± 0.042^{a}	14.21 ± 0.101^{a}
Diabetic + AETOF 50mg/kg bw	1.45 ± 0.039^{c}	1.324 ± 0.023^{c}	19.45 ± 1.334^{c}
Diabetic + 20mg Glibenclamide/kg bw	1.39 ± 0.047^{b}	0.980 ± 0.046^{b}	17.74 ± 0.568^{b}
F value	42.78	67.37	18.83
Significance	0.000	0.000	0.000

Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Table. 19: Effect of long term treatment with AETOF fraction on non-enzymatic antioxidants Vitamin C, Vitamin E and GSH in liver and kidney of normal and experimental groups.

Groups	LIVER			KIDNEY		
	Vitamin-C (μ moles/mg tissue)	Vitamin-E (μ moles/mg tissue)	Reduced glutathione (μg/g tissue)	Vitamin-C (μ moles/mg tissue)	Vitamin-E (μ moles/mg tissue)	Reduced glutathione (μg/g tissue)
Normal	1.75 ± 0.070^{c}	0.512 ± 0.010^{b}	556.17 ± 29.8^{d}	1.27 ± 0.003^{e}	0.392 ± 0.019^{d}	445.78 ± 23^{d}
Normal + AETOF 50mg/kg bw	1.905 ± 0.050^{d}	0.503 ± 0.059^{a}	584.24 ± 23.6^{e}	1.156 ± 0.004^{d}	0.390 ± 0.023^{d}	459.27 ± 29.5^{e}
Diabetic control	0.763 ± 0.057^{a}	0.674 ± 0.012^{e}	178.80 ± 40.2^{a}	0.509 ± 0.016^{a}	0.591 ± 0.058^{a}	165.05 ± 30.3^{a}
Diabetic + AETOF 50mg/kg bw	1.26 ± 0.043^{b}	0.412 ± 0.080^{c}	345.71 ± 25.5^{c}	1.00 ± 0.003^{c}	0.302 ± 0.032^{c}	295.94 ± 27.5^{b}
Diabetic + 20mg Glibenclamide/kg bw	1.22 ± 0.073^{b}	0.398 ± 0.058^{d}	323.58 ± 27.042^{b}	0.99 ± 0.027^{b}	0.295 ± 0.029^{b}	360.00 ± 25.3^{c}
F value	32.24	14.56	229.09	17.05	15.23	321.7
Significance	0.000	0.000	0.000	0.000	0.000	0.000

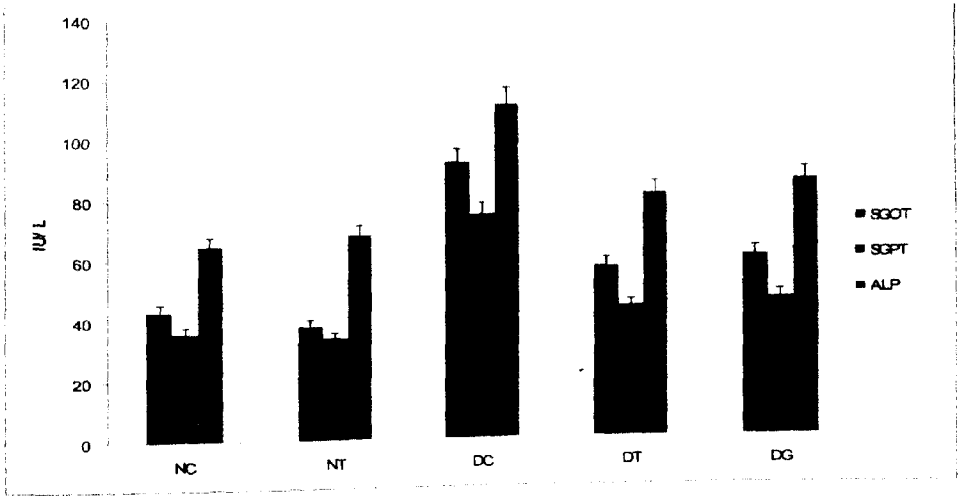
Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Effect of long term treatment with AETOF fraction on hepatic and renal function markers.

Fig. 18 and Table. 20 show the levels of hepatic and renal functional markers respectively in all the experimental groups of rats. Diabetic rats showed elevated activities of SGOT, SGPT and ALP. Renal function markers such as urea and creatinine in plasma were also increased in the diabetic rats when compared to normals. Treatment of diabetic rats with AETOF fraction at a dose of 50 mg /kg bw for 40 days resulted in a significant reduction in their blood urea and creatinine levels. In addition the treatment also brought back the activities of SGOT, SGPT and ALP to near normal levels. Similar effects were observed with glibenclamide, but they were less in magnitude when compared to AETOF fraction. There were no significant changes observed in levels of hepatic and renal function markers in the normal treated rats.

Fig. 18: Effect of AETOF fraction on serum SGOT, SGPT and ALP of normal and experimental animals



Values are given as mean ± S.D from six rats in each group.

NC= Normal Control, NT= Normal Treated with AETOF Fraction, DC=Diabetic Control, DT = Diabetic Treated with AETOF Fraction, DG = Diabetic Treated with Glibenclamide.

Table 20: Effect of the AETOF fraction on kidney function in diabetic rats after 40 days of treatment

Group	Blood Urea (mg/dl)	Serum Creatinine (mg/dl)
Normal	41.23±4.79[a]	0.418±0.054[a]
Normal + AETOF 50mg/kg bw	42.33±3.16[a]	0.436±0.03[a]
Diabetic control	68.55±4.4[c]	0.912±0.028[b]
Diabetic + AETOF 50mg/kg bw	47.85±3.73[a, b]	0.566±0.10[c]
Diabetic + 20mg Glibenclamide/kg bw	49.07±4.0[b]	0.603±0.025[b]
F value	45.550	70.879
Significance	0.000	0.000

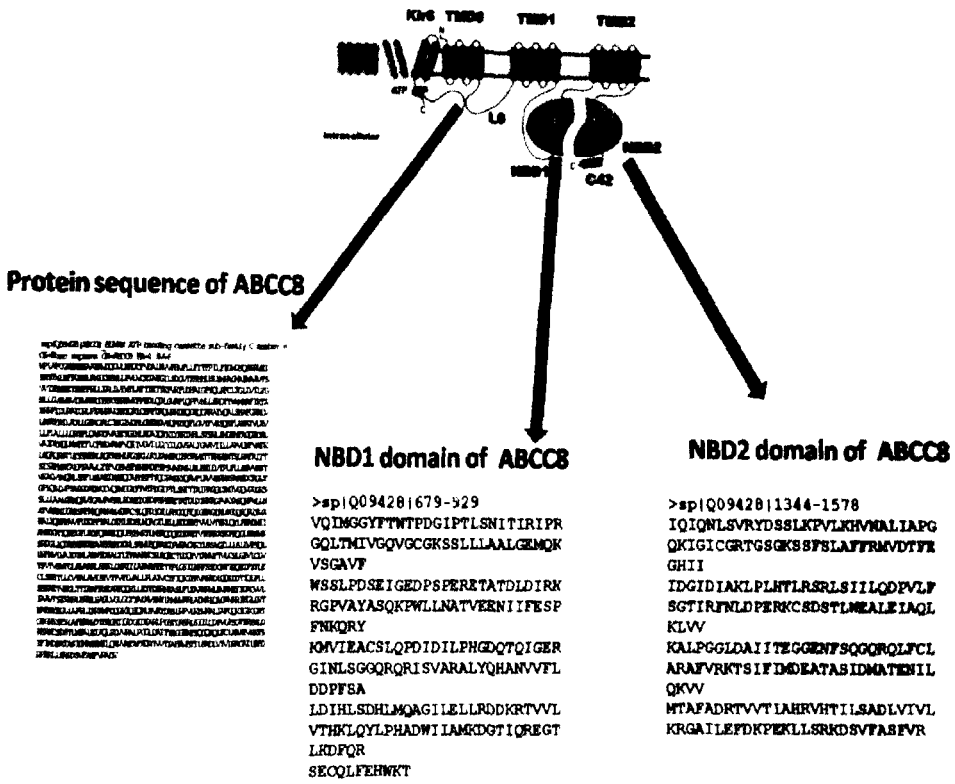
Values are given as mean ± S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.01$ (DMRT).

Homology modelling of ATP binding domain of Sur1 receptor

Sur1 receptor is a huge complex structure which possesses 12 trans membrane helices which are aligned in the lipid bilayer and two ATP binding domains are located at the cytoplasmic region (Fig. 19). In the present study, two ATP domains of Sur1 were modeled that protein sequences were retrieved from the SWISSPROT such as ATP-1 domain (Uniprot ID: Q09428) which consists of 250 residues (679-929) and ATP-2 domain (ABC transporter) (Uniprot ID: Q09428) which consists of 242 residues (1344-1578).

Fig.19: ATP binding domain of Sur1 receptor



>sp|Q09428|679-929 (ATP-1)

VQIMGGYFTWTPDGIPTLSNITIRIPRGQLTMIVGQVGCGKSSLLLAALGEMQKVS
GAVFWSSLPDSEIGEDPSPERETATDLDIRKRGPVAYASQKPWLLNATVEENIIFE
SPFNKQRYKMVIEACSLQPDIDILPHGDQTQIGERGINLSGGQRQRISVARALYQH
ANVVFLDDPFSALDIHLSDHLMQAGILELLRDDKRTVVLVTHKLQYLPHADWIIA
MKDGTIQREGTLKDFQRSECQLFEHWKT

>sp|Q09428|1344-1578 (ATP-2)

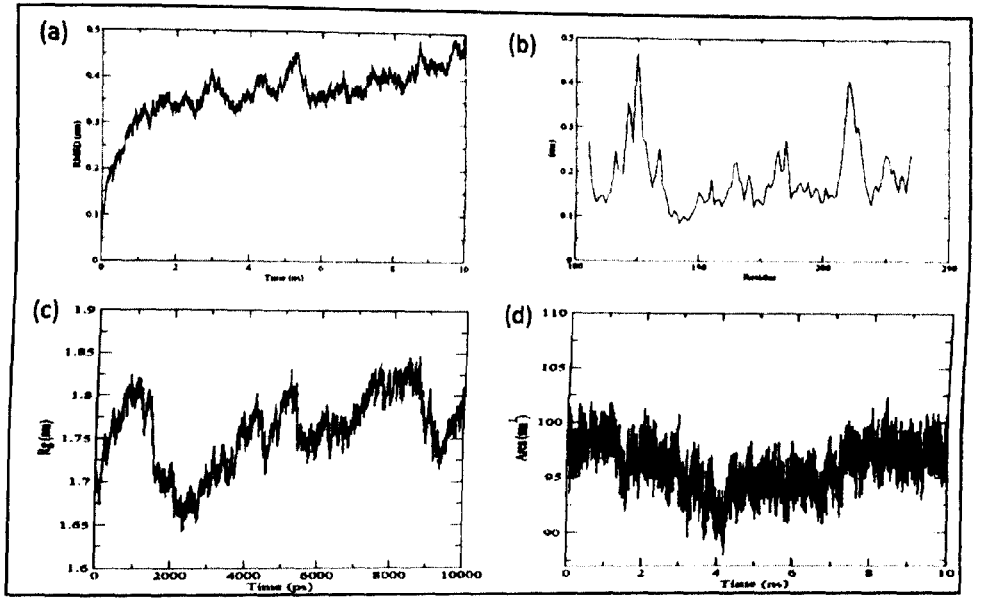
IQIQNLSVRYDSSLKPVLKHVNALIAPGQKIGICGRTGSGKSSFSLAFFRMVDTFE
GHIIIDGIDIAKLPLHTLRSRLSIILQDPVLFSGTIRFNLDPERKCSDSTLWEALEIAQ
LKLVVKALPGGLDAIITEGGENFSQGQRQLFCLARAFVRKTSIFIMDEATASIDMA
TENILQKVVMTAFADRTVVTIAHRVHTILSADLVIVLKRGAILEFDKPEKLLSRKD
SVFASFVR

In order to resolve the structures of these functional domains, BLASTp search was carried out against Protein Data Bank which furnishes that (PDBID) 2cbz has shown highest homology with ATP-1 and (PDBID) 3qf4 has shown highest homology with ATP-2 domains respectively. Subsequently, these structures were used as template on the basis of sequence identity, high score, less e-value, utmost resolution and R-factor. The coordinates for the query structure were assigned from template structure by means of pairwise sequence alignment using ClustalX. Thus, numerous 3D models of two domains were generated by MODELLER v9.14 and the least DOPE score modeller objective was subjected to molecular dynamics simulations.

Molecular dynamics

The protein structure was subjected to molecular dynamics simulation for the period of 10 ns in production phase. In order to understand the dynamic behavior of protein, we have plotted trajectory graphs for Root Mean Square Deviations (RMSD), Root Mean Square Fluctuations (RMSF), Radius of gyration (Rg) and Solvent Accessible Surface Area (SASA). Primarily, RMSD graph has been plotted for backbone and Cα atoms of protein was shown in Fig. 20a. During the 10ns simulation, Cα RMSD of the protein showed sharp increase of 2ns followed by equilibrium around 10ns. Apart from this, dynamic behavior of protein residues, Cα–root mean square fluctuations (RMSF) plot was drawn and shown in Fig. 20b.The standard deviation of most the residues have RMSF value more than 0.1 nm (>0.1nm) and fluctuation of 5.0Å indicating a higher level of fluctuations. On the other hand, resolution of the protein structure was analyzed by the parameter of radius of gyration (Rg). Rg plot for Cα atoms and protein was shown in Fig. 20c; indicating compactness of the protein structure is properly folded with the period of 10ns simulation phase. In addition, solvent accessible surface area (SASA) measures contacts between the amino acids with its environment (solvent and protein). SASA showed down fall of protein structure during a period of 10ns simulation in production phase (Fig. 20d).

Fig. 20: (a) Root mean square deviation (RMSD) profile, (b) Root mean square fluctuation, (c) Radius of gyration of Cα atoms and (d) Solvent accessible surface area of ATP-2 domainare depicted for the entire 10ns simulation.

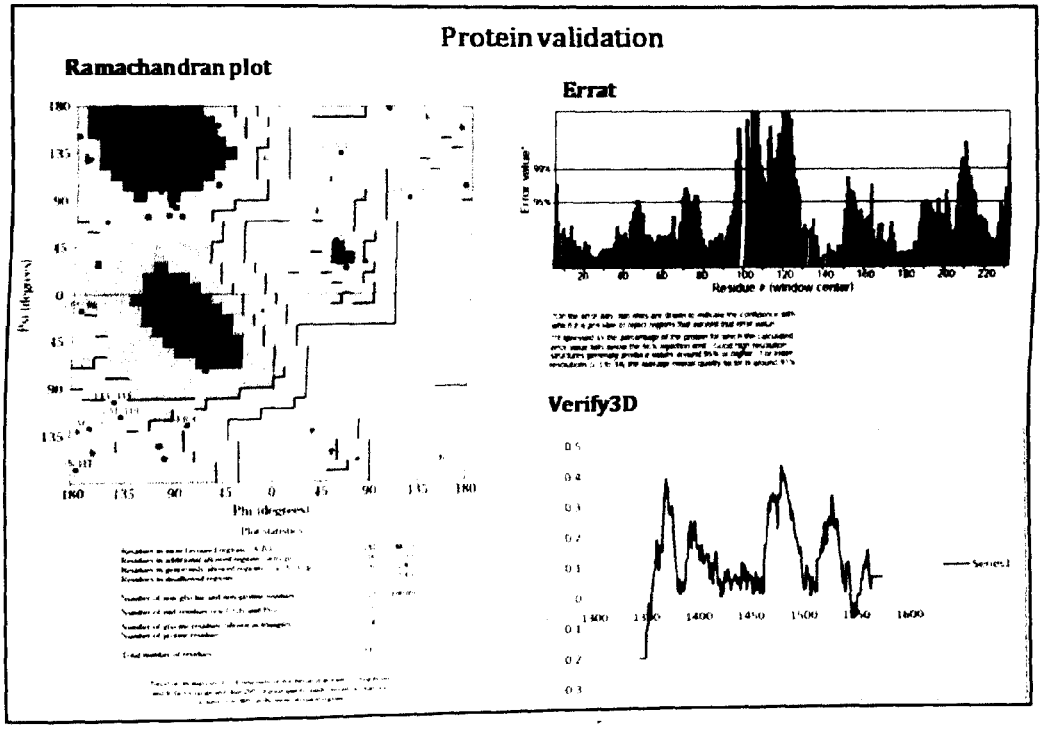


Model assessment

The final refined model was corroborated using standard bioinformatics utensils and shown in Fig. 21. Ramachandran plot calculations using PROCHECK has revealed that 88.2% with 187 residues were aligned within the most favored region, 7.5% with 16 residues were located within the additionally allowed regions, 2.5% with 6 residues were plotted within the generously allowed region and 3 (1.4%) residues were aligned within the disallowed region. The environment profile of Verify3D-1D value was found to be mostly above zero. The non-bonded interactions between various types of atoms were computed with ERRAT program which showed overall quality factor of 80.0. WHATCHECK program calculated quality indicators such as 2nd generation packing quality, Ramachandran plot appearance and Chi-1/Chi-2 rotamer normality were found to be 2.6, 0.325, 2.0 and RMS Z-scores like bond lengths, bond angles, omega angle

restraints, side chain planarity, improper dihedral distribution and inside/outside distribution were found to be 0.98, 1.203, 0.764, 0.08 and 1.13 respectively. Superimposition of NorA and GlpT has recorded RMSD of 0.86Å for Cα atoms.

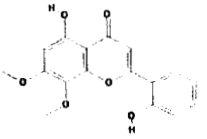
Fig. 21: Ramachandran plot calculations of Sur1 was assessed by using PROCHECK, 3D profile was verified using ERRAT server indicates that overall quality factor of Sur1 is reasonably folded and compatibility of an atomic model with its own amino acid sequences (1D) using verify3D.



Molecular docking

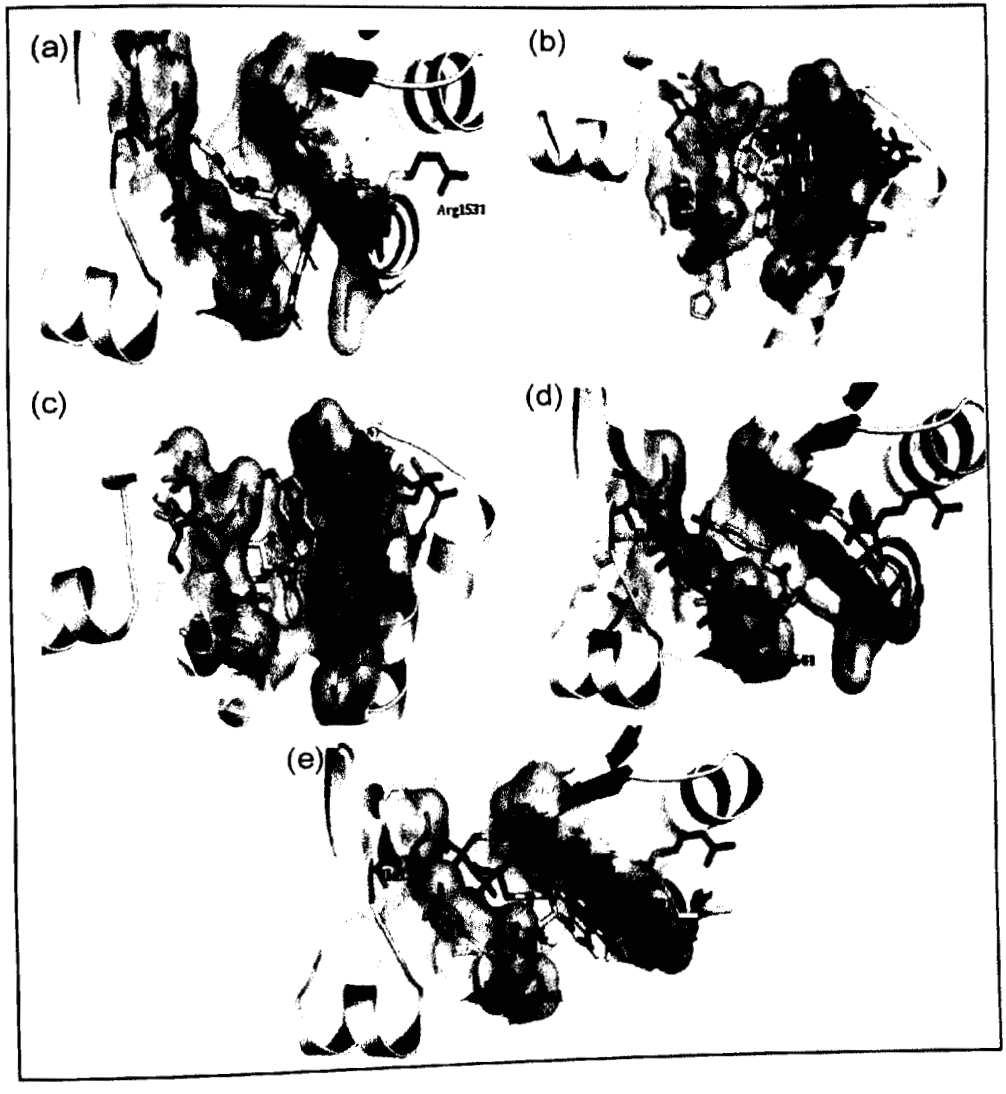
In the AETOF fraction, molecular docking of flavonoids on ATP binding domain of Sur1 receptor reveals that three flavonoids namely, Skullcap flavone I, Echioidin and Echioidinin were shown to have best binding energies of -10.0, -9.5, and -9.3 kcal/mol respectively (Table. 21).

Table. 21: Binding interactions, bond lengths, bond angle and binding energy of flavonoids with ATP2 domain of Sur1.

S. No	Compounds	2D Structures	Protein***ligand	Distance	Binding energy
1	Skullcap flavone I		Arg1531***OC35 Thr1532***OC35 Val1533***OC35 Val1533***OC40 Thr1535***OC33 Thr1535***OC34 Thr1535***OC40 Thr1535***OC40 Thr1543***OC42	3.4 2.8 3.5 2.1 3.2 3.6 3.2 3.0 3.0	-10.0
2	Echioidin		Asp1530***OC29 Val1533***OC29 Thr1535***OC25 Thr1535***OC24 Thr1535***OC23	3.5 3.3 2.9 3.4 3.5	-9.5
3	Echioidinin		Arg1531***OC35 Phe1528***HO36 Thr1536***HO37	3.4 2.2 1.9	-9.3
4	Glibenclamide		Ser1541***OC30 Ile1546***OP28	3.2 3.2	-8.8
5	ATP		Arg1531***HN41 Val1533***HN40 Val1534***OP18 Thr1535***OC16 Thr1535***OP18 Thr1535***OP19 Thr1535***HO32	2.6 2.5 3.3 3.2 3.4 3.3 2.1	-7.8

Apart from this, natural substrate ATP and reference drug glibenclamide were docked and exerting binding energies of -8.8 and -7.8 kcal/mol respectively. Skullcapflavone I was interacted with nine hydrogen bond interactions, one hydrogen bonding interaction with Arg1531, one with the Val1533, one hydrogen bond with the Val1534, four hydrogen bonding interactions with Thr1535 and one with the Thr1543 respectively (Figure 22a). Echioidin displayed five hydrogen bonding interactions, one H-bond with the residue of Phe1528, one with the residue of Arg1531 and three with the residue of Thr1536 respectively (Figure 22b). Echioidinin interact with three H-bonding interactions, one H-bond with the residue of Arg1531 and two H-bonds with the residue of Thr1536 respectively (Figure 22c). Standard drug glibenclamide was bound with H-bonds, one with the residue of Ser1541 and one with the residue of Ile1546 respectively (Figure 22d) Adenosine triphosphate (ATP) displayed seven hydrogen bonding interactions, one H-bond with the residue of Arg1531, two H-bonds with the residue of Val1533 and four H-bonds with the residue of Thr1535 respectively (Figure 22e).

Fig. 22: Binding mode of (a) Skullcapflavone I (b) Echioidin (c) Echioidinin (d) Glibenclamide and (e) Adenosine triphospate with in the ATP binding pocket of sur1.



Histopathology

Histological changes in liver, kidney and pancreas in normal rats, diabetic rats and diabetic rats treated with AETOF fraction and glibenclamide shown in 10x magnification and their are given below.

PANCREAS

Fig 23.a. is the photomicrographs of the pancreas of a normal rat showing the normal architecture and normal islets of Langerhans. Fig 23.b. is the photomicrographs of the pancreas of a normal rat treated with AETOF fraction, which shows the normal architecture as in the normal rats. Fig 23.c. is the photomicrographs of the pancreas of diabetic untreated rats. Fig 23.d. & 23.e. are the photomicrographs of the pancreas of diabetic rats treated with AETOF fraction and Glibenclamide respectively. In diabetic untreated rat pancreas there was insulitis with lymphocytic infiltrations. Atrophy and destruction of β-cells were marked. The regenerative changes in tissue architecture of pancreas were observed in the pancreas of diabetic rats treated with AETOF fraction and Glibenclamide.

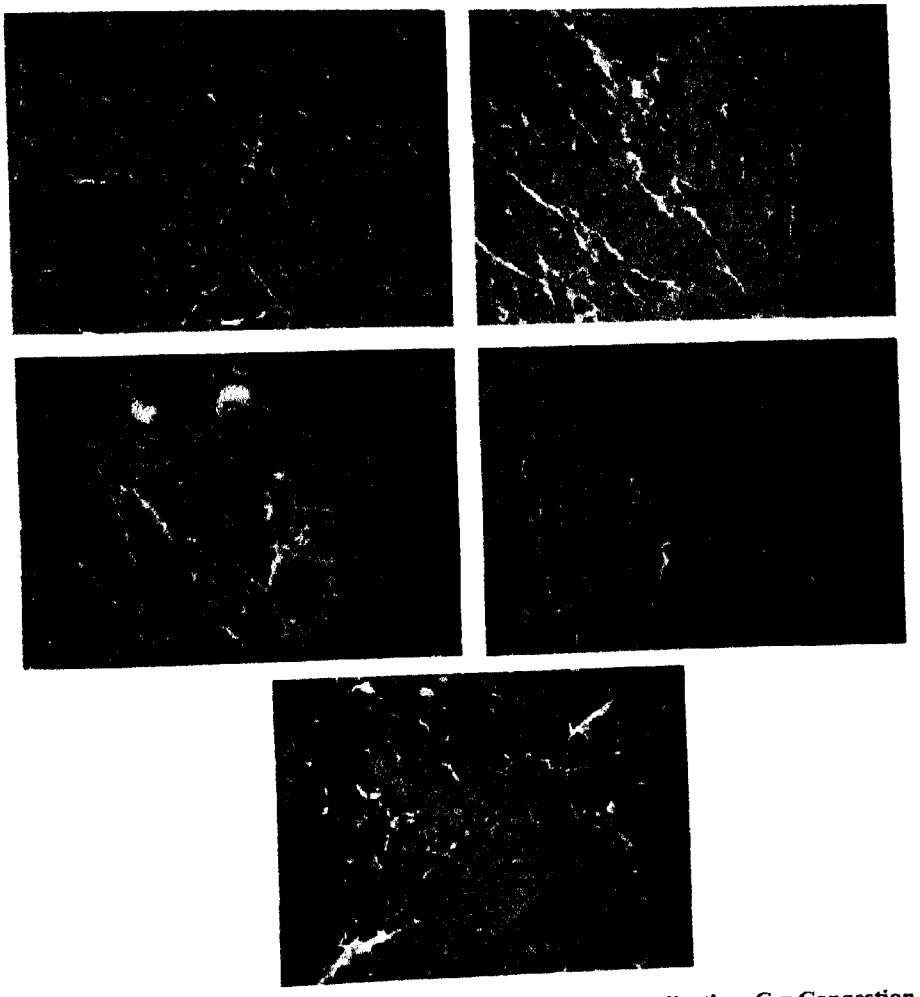
LIVER

Fig 24.a. is the photomicrographs of the liver of a normal rat showing the normal hepatic architecture with normal central vein, prominent nucleus and normal hepatocytes. Fig 24.b. is the photomicrographs of the liver of a normal rat treated with AETOF fraction, which show the normal architecture similar to normal rats. Fig 24.c. is the photomicrographs of the liver of diabetic untreated rats, which show degenerative liver with severe congestion of central vein, hemorrhages in the sinusoidal spaces and granular appearance of the hepatocytes (degenerative change) with cloudy swelling (hazzy nucleus). Fig 24.d. & 24.e. are the photomicrographs of the liver of diabetic rats treated with AETOF fraction and Glibenclamide respectively, showing normal liver architecture with slight congestions in central vein, normal sinusoidal spaces and normal hepatocytes.

KIDNEY

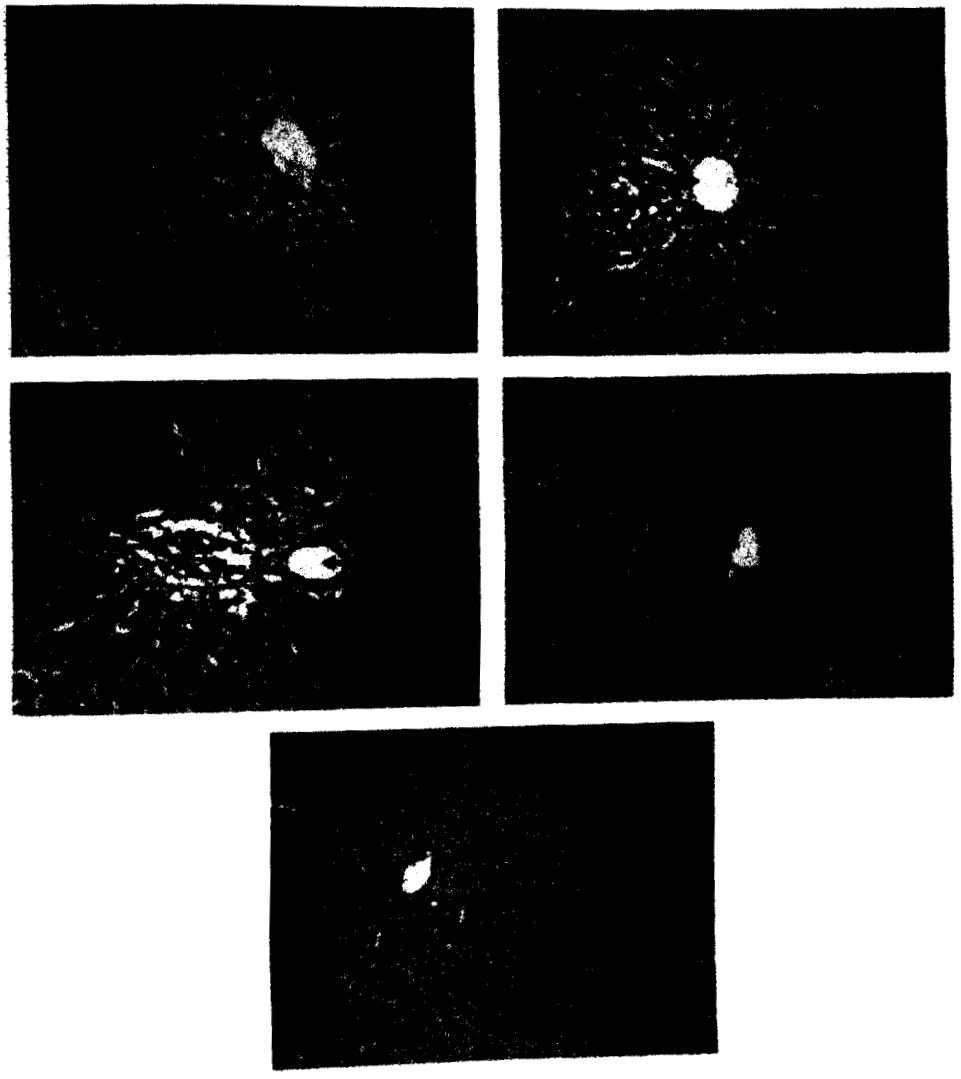
Fig 25.a. is the photomicrographs of the kidney of a normal rat showing normal architecture of kidney with normal glomeruli and normal tubular epithelial cells. Fig 25.b. is the photomicrographs of the kidney of a normal rat treated with AETOF fraction, which show the normal architecture similar to normal rats. Fig 25.c. is the photomicrographs of the kidney of diabetic untreated rats showing atrophy of the glomeruli, necrotic tubular epithelial cells and dark pyknotic nuclei. Fig 25.d. & 25.e. are the photomicrographs of the kidney of diabetic rats treated with AETOF fraction and Glibenclamide respectively, which show normal glomeruli, normal intertubular vessels and tubular epithelial cells indicating regenerative changes.

Fig: 23: Histopathology of Pancreas(10x), (a) Normal, (b) Normal treated with AETOF fraction, (c) Diabetic control (d) Diabetic treated with AETOF fraction (e) Diabetic treated with glibenclamide.



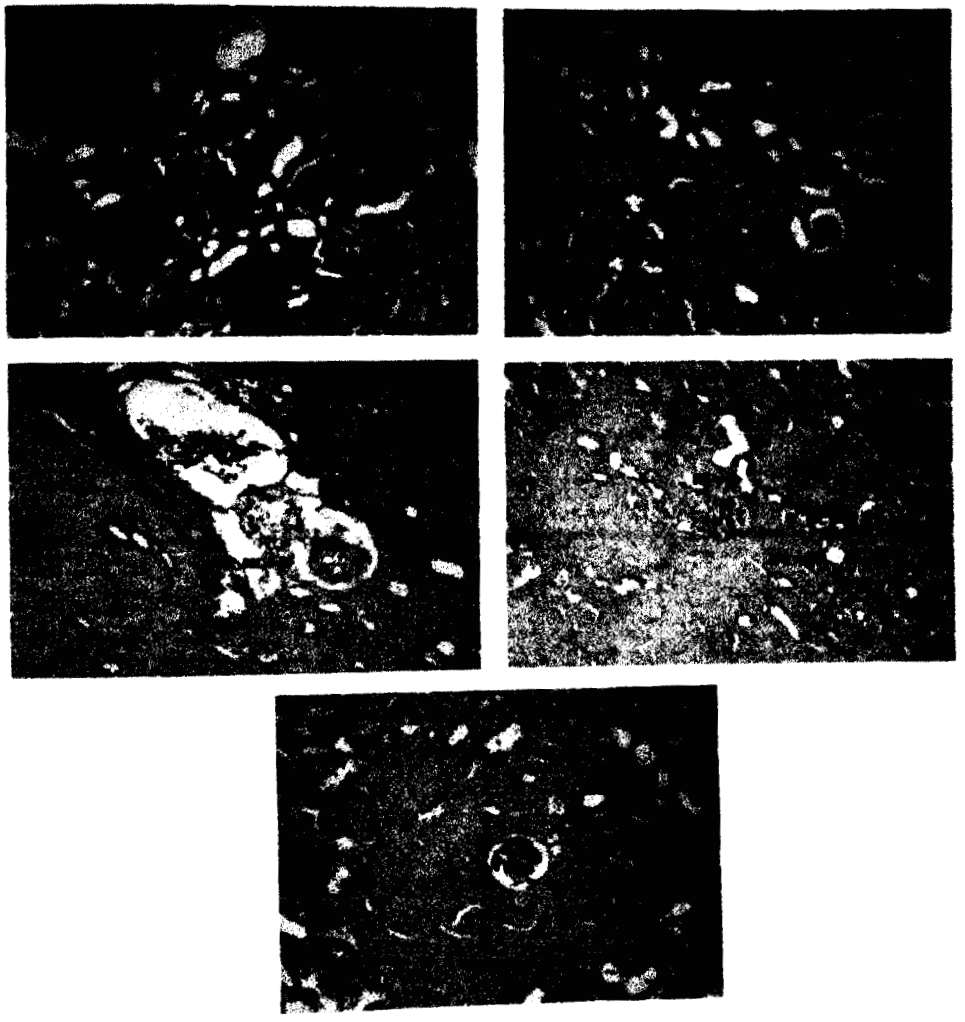
EC = Endocrine, EX = Exocrine, AC = Acinar cells, V = Vacuolization, C = Congestion.

Fig 24: Histopathology of Liver(10x) (a) Normal (b) Normal treated with AETOF fraction (c) Diabetic control (d) Diabetic treated with AETOF fraction, (e) Diabetic treated with glibenclamide



H = Hepatocyte, CV = Central vein, V = Vein, C = Congestion.

Fig 25: Histopathology of Kidney(10x) (a) Normal (b) Normal treated with AETOF fraction (c) Diabetic control (d) Diabetic treated with AETOF fraction (e) Diabetic treated with glibenclamide.



RT= Renal Tubule, BC= Bowman's capsule, V = Vein, C = Congestion, PT=Picnotic tubule, NC= Necrotic change.