ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

EFFECT OF EXTRACTED ALOE VERA IN COMPARISON WITH MARKETED PREPARATION ON ALLOXAN INDUCED DIABETIC FOOT ULCERS PRODUCED BEHAVIOURAL DYSFUNCTIONS BY USING RODENTS.

Vivek Kahale, N. J. Gaikwad.

Department of Pharmaceutical Scinces , Rashtrasant Tukadoji Maharaj Nagpur University,NagpurM.S.India.

Manuscript Info

Manuscript History:

Received: 18 December 2015
Final Accepted: 22 January 2016
Published Online: February 2016

Key words:
Alloxan, Diabetic Foot Ulcers, Anxiety, Depression, Aloe Vera.

Corresponding Author

Vivek Kahale.

Abstract

The core finding of the present preclinical study demonstrated that, administration of prescribed oral and topical doses of Aloe Vera is remarkably protective effects in diabetic and its complication using male wistar rats against alloxan induced Diabetic Foot Ulcers (DFU). Diabetes mellitus is an endocrine disorder. Diabetes mellitus is now defined as a metabolic disorder in which the body's capacity to utilize glucose, fat and protein is disturbed due to insulin deficiency, insulin resistance or both. Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities such as DFU.The collagen content of the skin is decreased as a result of reduced biosynthesis and or accelerated degradation of newly synthesized collagen. These qualitative and quantitative abnormalities contribute to the impaired wound healing observed in diabetes.Aqueous extract of Aloe Vera (AGE, Oral) with ethanol extract of Aloe Vera (AG, Topical) act as diabetes and its complication such as DFU protective agent. Relevant doses of Aloe Vera treatment (Extracted& Marketed products) protects behavioral changes, significantly attenuated oxidative damage & improved anxiety and depression activities in different regions of central nervous system (CNS) of rat brain against alloxan induced connective tissue abnormality in diabetic wound. Subcutaneous administration of alloxan is known to produce insulin deficiency that resembles juvenile onset and advanced diabetic disease in rats. The results show that Aloe Vera treatment is effective in various DFU produced behavioral dysfunction; it could be used as an effective therapeutic agent in the management of Diabetic foot ulcers and related conditions comparison with marketed products containing Aloe Vera.

Introduction:-

Diabetes mellitus is an endocrine disorder which was first described in the ancient book of Chinese medicine 'Huangdi Neijing' in China around 500 BC and was called 'thirsty disease'. Diabetes mellitus is now defined as a metabolic disorder in which the body's capacity to utilize glucose, fat and protein is disturbed due to insulin deficiency, insulin resistance or both. According to the information of the International Diabetes Federation, there are currently at least 382 million diabetics in the world (IDF Diabetes Atlas I Sixth edition) and the World Health Organization estimates that there is 347 million people with diabetes mellitus (WHO Update). International Diabetes Federation states that people with diabetes are 25 times more likely to have a leg amputated than those without the condition. Dunnig(2003) The combination of peripheral neuropathy, peripheral arterial disease and infection would result in unhealing ulcers, gangrene and amputation. Levin E (1995)Amputation leads to significant morbidity and mortality (Moulik et al., 2003).

The lifetime incidence of the diabetic developing a foot ulcer has been estimated at 15 – 25% and even with aggressive care approximately 14-24% of diabetics with a foot ulcer ultimately require an amputation (Lavery et al., 2003), Reiber(1996), (Ramsey et al., 1999), (Trautner et al.,1996).Patients can undergo surgical interventions, peripheral bypass to improve vascular circulation, wound debridement to control the wound and limit infection, grafts and flaps for coverage of the wound, and if required, amputation. However, medical therapies for wound care are currently limited with a focus on minimizing weight bearing, antibiotics, and diligent dressing changes. Development of novel therapies to improve wound healing in diabetics is therefore an essential and emerging field of investigation.

Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities. The collagen content of the skin is decreased as a result of reduced biosynthesis and or accelerated degradation of newly synthesized collagen. These qualitative and quantitative (Spaheimer., 1988) abnormalities contribute to the impaired wound healing observed in diabetes (Goodson et al., 1979).

A diabetic foot ulcer is an open sore or wound that most commonly occurs on the bottom of the foot in approximately 15-25% of patient with diabetes of those who develop a foot ulcer, six percent will be hospitalized due to infection or other ulcer related complication. In people with diabetes, prolonged high blood sugar levels are linked with damage to the nerves in the feet. Nerve damage can cause loss of sensation as well as deformities of the feet. This nerve problem is called **Peripheral Neuropathy** (Das, 1994). Neuropathy and peripheral arterial disease are the main etiologic factors making the diabetic foot vulnerable to minor trauma and ulceration. These ulceration will, often after infection, progress to deeper ulcerations and finally to gangrene (Moral et al., 2004).

Diabetes-induced behavioural and cognitive changes are related to several factors. Both diabetic complications and reduced central serotonin (5-hydroxytryptamine, 5-HT) synthesis and metabolism are thought to underlie behavioural and cognitive dysfunctions in patients with T1DM . It has become evident that insulin and C-peptide deficiencies, including perturbations of their signalling cascades, lead to cerebral dysmetabolism and interference with the regulation of neurotropic factors and their receptors. Ultimately, this cascade of events leads to neuronal loss, causing profound deficits in behavioural and cognitive functions . However, the specific mechanisms underlying these changes and whether they relate to the duration of hyperglycaemia are unknown (Ravishankar., 2011).

Cognitive dysfunctions are commonly seen in many stress-related disorders, including anxiety and depression the world's most common neuropsychiatric illnesses. Various genetic, pharmacological, and behavioral animal models have long been used to establish animal anxiety-like and depression-like phenotypes, as well as to assess their memory, learning, and other cognitive functions. Mounting clinical and animal evidences strongly supports the notion that disturbed cognitions represent an importantpathogenetic factor in anxiety and depression, and may also play a role inintegrating the two disorders within a common stressprecipitated developmental pathway (Allan et al.,2007).

Material and Method:-

Subjects:-

Adult male Wistar rats born and reared in the Animal House of the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur (India) was used in the present study. Young healthy male rats (250–300 g) were group housed (Six per cage) and maintained at 23±2 ◦C under 12:12 hrs light (08:00–20:00 h)/dark cycle with free access to rodent chow and tap water. The animal studies were approved by the Institutional Animal Ethics Committee, (sanction letter No. IAEC/UDPS -2014/39) constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India. The proposal for experimentation has been approved by IAEC of the Department (sanction letter No. IAEC/UDPS -2014/39).Animals were naive to drug treatments and experimentation at the beginning of all studies. All tests were conducted between 08:00 and13:00 h.

Drugs and solutions:-

Alloxan Monohydrare (Sigma-Aldrich Labs, Bangalore, India). Alloxan dissolved in double saline water. Drug solutions were prepared fresh and their doses are expressed in terms of free bases. Other chemicals used in the present investigation were of analytical grade.

Treatment schedule:-
The rats were divided in to 5 Groups and each group in the study consists of 6 rats. Diabetes Foot Ulcer model was induced by injecting alloxan monohydrate used as a2% solution in physiological saline subcutaneously at various dose level of body weight to overnight fasted animals. Control animal were injected with saline physiological solution.Diabetic foot ulcer treated with Aqueous extract of Aloe Vera (300 mg/kg, p.o.) and topical Aloe Vera gel (30 mg/kg) twice daily and comparison with marketed product containing Aloe Vera.

Behavioral tests:-
Elevated plus maze (EPM)
The EPM is widely used for rodent neuropsychological assays, such as anxiety behavior

a. Anxiety protocol:-
The rats were placed on the central platform with their heads oriented towards an OA. The frequency of entries into the OAs and EAs were scored and time spent in the OAs was recorded for 5 minutes. The number of entries into the OAs of the maze and the time spent in those arms are the measures of anxiety, and decreases in these measures indicate an anxiogenic effect. The number of EA entries serves as the measure of locomotor activity in this test. An arm entry is defined as all 4 paws entering an arm, and an arm exit is defined as 2 paws leaving an arm.

b. Social Interaction Test:-
Social Interaction test was carried out by a method describe earlier (Egashira et al., 2007).Each mouse was subjected to a neutral cage (Opaque plastic box: 34×22×19cm), for 5 min to acclimatize. Two unfamiliar rat were placed together in neutral cage for 5 min,and their behavioral interaction was recorded by video camera placed 1.7m above the cage. The social interaction of treated animal with untreated one was evaluated in terms of cumulative time spent in behaviors such as sniffing, adjacent lying, following crawling under/over partner, and mutual grooming in 5 min. the experimenter remained outside the chamber during testing. After each test the fecal matter from the cage was removed and the cage was cleaned with damp cotton soaked with alcohol. The analysis of the recording was carried out by trained experimenter who was unaware about treatment identity. Increase in social interaction is considered as an anxiolytic effect.

c. Depression Protocol:-
Forced Swim Test (FST):-
The method used was essential as described by Porsolt et al. (1977). The investigator was blinded to the treatment groups. The forced swim test was carried out on mice individually forced to swim in an open cylindrical container (diameter 10cm, height 25cm), containing 15 cm of water at 25 ± 1^0C; the water depth was adjusted so that the animals must swim or float without their hind limbs or tail touching the bottom. The total duration of immobility during the 6min test was scored as described by Umathe et al (2008 b). Each rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The duration of immobility was recorded. Decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity

Statistical analysis:-
Results were expressed as mean ± S.E.M. The data were analyzed by two-way or one-way analysis of variance (ANOVA) followed by Bonferroni and Tukey's multiple comparison test respectively. Statistical significance was considered at $P<0.05$ in all the cases.

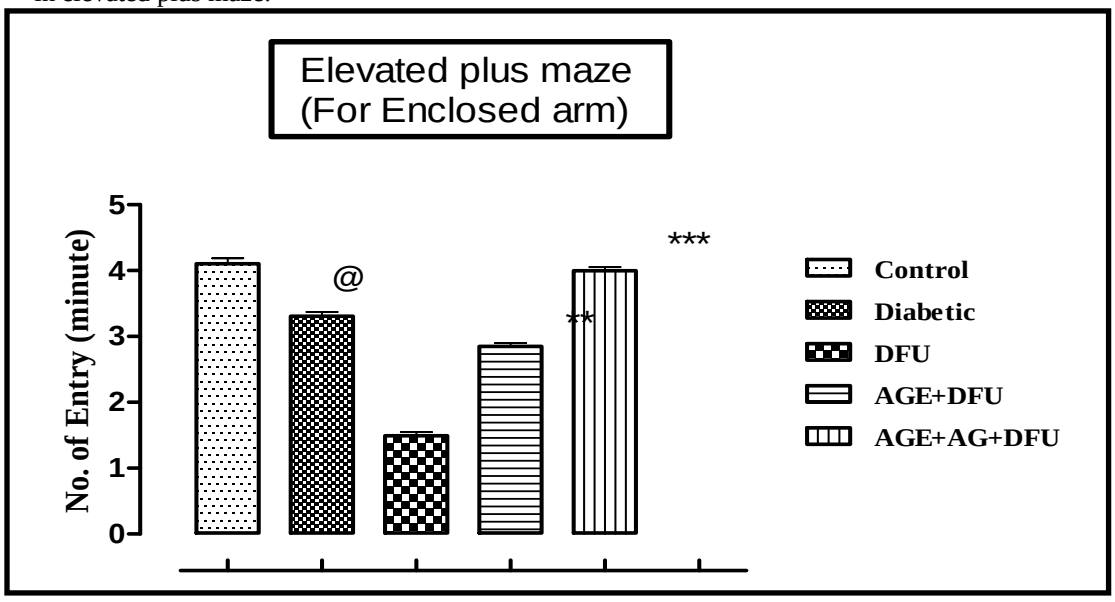
Result:-
We randomly assigned 74 rats to different experimental groups. There were 6 rats in each groups (N=6). The 48 rats were given Alloxan injections to induce diabetes. Out of these rats some for optimization to became diabetic; the diabetic rats achieved the required diabetic state (FBS greater than 200 mg/dL) and were included in the study as Diabetic . During the study period, 1 group ofthe diabetic rats was become DFU after surgical induction. Data collected from the 5 groups of rats; control,diabetic, dibetic foot ulcer (DFU) and treatment group 1 i.e extracted aloe vera (AGE; Oral& AGE; Oral + AG; Topical), treatment group 2 i.e marketed product containing aloe vera (MP,MP1+MP2) are summarised below.

Results for extracted Aloe Vera preparation:-

Table 1**.** Effect of Duration on anxiety level in control, Diabetes, DFU and Treatment group 1 rats in elevatedplus maze.

Group	Number of Entries	
	Enclosed arm (EA)	**Open arm (OA)**
Control- 10 Days	4.1±0.98	1.83±0.16
Diabetic -10 Days	3.3±0.33	0.83±0.75
DFU-10 Days	1.50±0.84	0.42±0.36
AGE + DFU-10 days	2.86±0.45	1.00±.084
AGE + AG+ DFU-10 Days	3.98±0.96	1.8±0.36
Control- 20 Days	6.6±0.61	4.3±1.23
Diabetic -20 Days	5.6±0.33	1.23±0.35
DFU- 20 Days	4.50±0.84	0.82±0.36
AGE + DFU- 20 days	5.86±0.44	1.00±.084
AGE + AG+ DFU- 20 Days	6.08±0.98	3.88±0.66

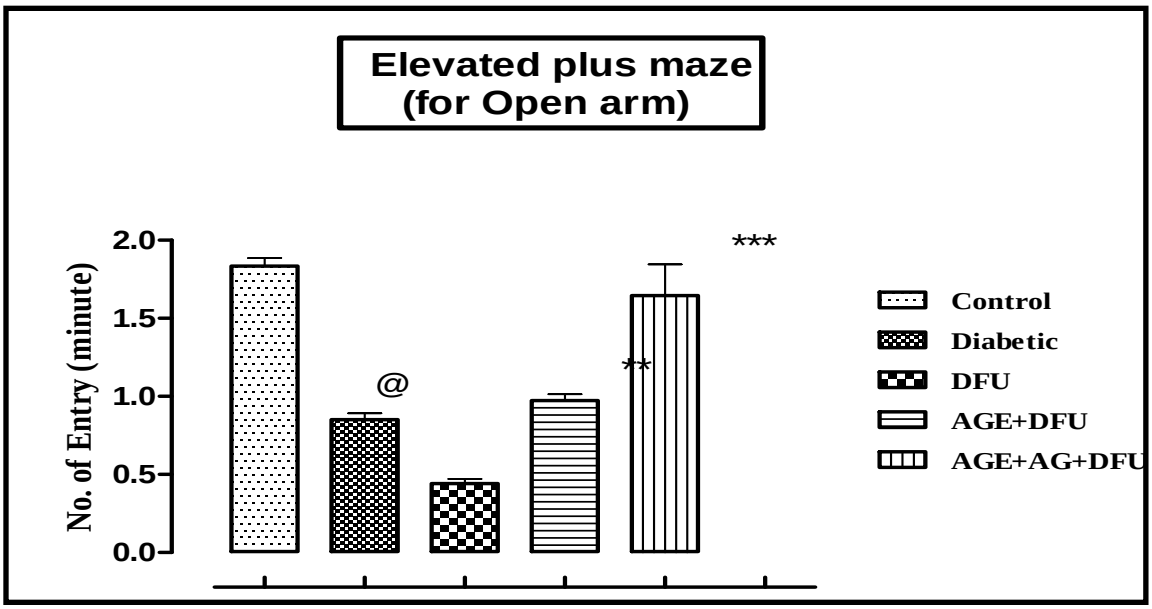
Fig.1. Influence of extracted Aloe Vera treatment on No. of entries & time spent in enclosed arm in elevated plus maze.



Influence of treatment of extracted Aloe Vera on the number of entries & time spend in enclosed arm for the time intervals, for the elevated plus maze test. Each value represents mean ± S.E.M. of 6 observations

ƐP<0.0001 vs. Diabetic group; *P<0.0001 vs. DFU test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); Alloxan (150mg/kg);AGE (300mg/kgp.o): AGE + AG (30 mg/kg): treated

Fig.2. Influence of extracted Aloe Vera treatment on No of Entry & time spent in open arm in elevated plus maze.

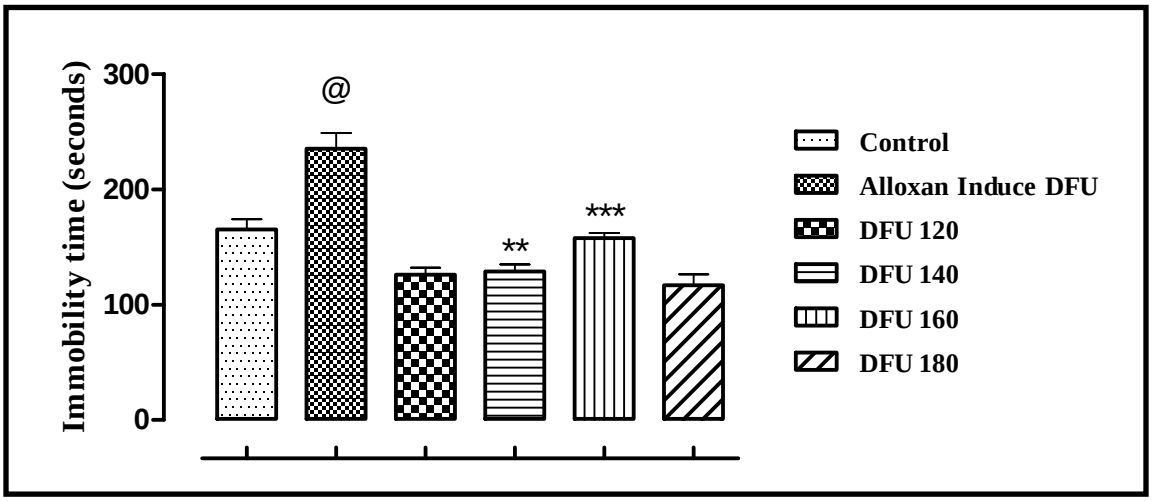


Influence of treatment of extracted Aloe Vera on the number of entries & time spend in open arm for the time intervals, for the elevated plus maze test. Each value represents mean ± S.E.M. of 6 observations
ƐP<0.0001 vs. Diabetic group; *P<0.0001 vs. DFU test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); Alloxan (150mg/kg);AGE; oral extracted aloe vera (300mg/kgp.o): AGE + AG; topical aloe vera (30 mg/kg): treated

Table 2.Effect of acute treatment with extracted Sample on DFU Depression and Anxiety.

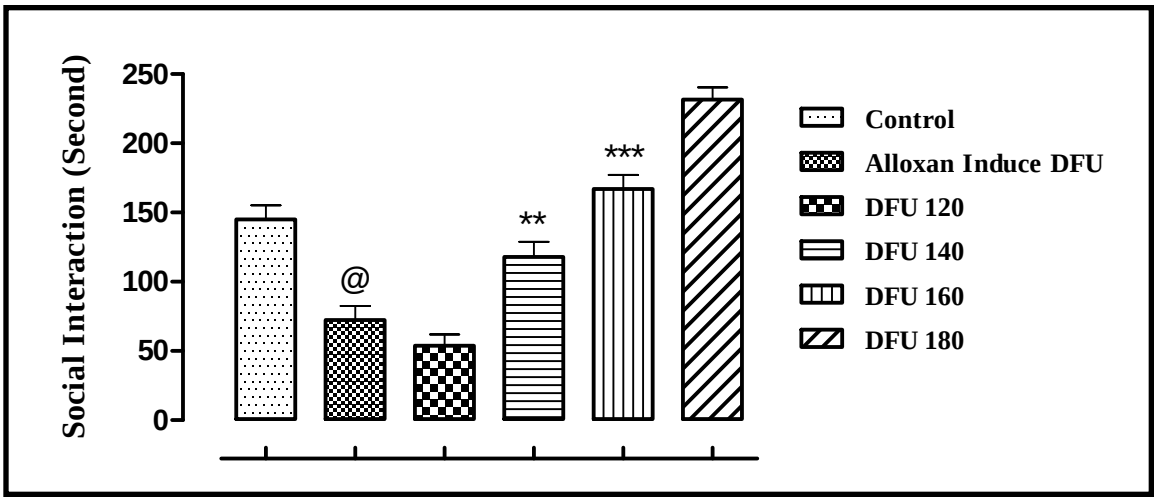
Duration	**Control**	**Alloxan Induce DFU**	**Extracted sample in DFU**			
			Optimization Doses (Mg / Kg)			
			120	**140**	**160**	**180**
Immobility Time (In Seconds)	180	230	125	129	146	140
	175	240	130	144	155	121
	178	238	132	114	168	111
	185	242	128	128	161	95
Social Interaction Time (In Seconds)	152	50	75	60	157	217
	122	99	80	112	124	189
	135	75	72	118	137	220
	168	65	86	92	170	230

Fig. 3. Influence of Extracted Aloe Vera treatment on immobility time in forced swim test.



Influence of treatment of extracted Aloe Vera on Immobility time spends in Forced Swim Test. Each value represents mean ± S.E.M. of 6 observations
ƐP<0.0001 vs. Diabetic group; *P<0.0001 vs. DFU test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); Alloxan (150mg/kg) ; Alloxan (120 mg/kg); alloxan (140mg/kg) ;alloxan (160mg/kg); alloxan (180mg/kg)AGE; oral extracted aloe vera (300mg/kgp.o): + AG; topical aloe vera (30 mg/kg): treated

Fig. 4. Influence of Extracted Aloe Vera treatment on Interaction time in social interaction test.



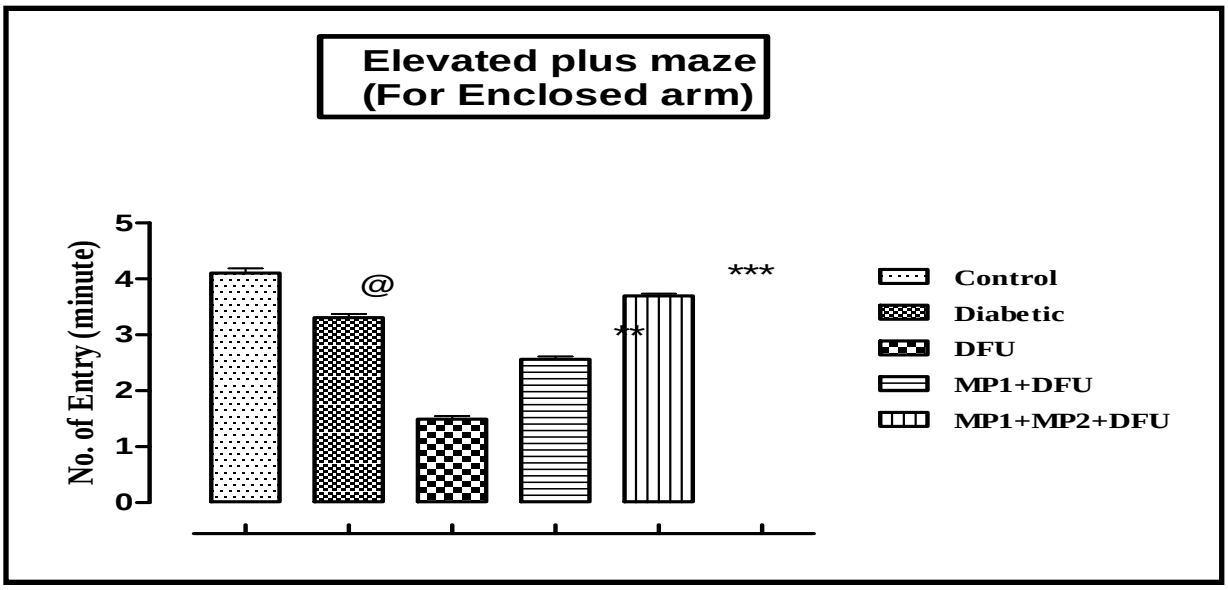
Influence of treatment of extracted Aloe Vera on social interaction time spends in social interaction test in anxiety protocol. Each value represents mean ± S.E.M. of 6 observations
ƐP<0.0001 vs. Diabetic group; *P<0.0001 vs. DFU test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); Alloxan (150mg/kg) ; Alloxan (120 mg/kg); alloxan (140mg/kg) ;alloxan (160mg/kg); alloxan (180mg/kg)AGE; oral extracted aloe vera (300mg/kgp.o): + AG; topical aloe vera (30 mg/kg): treated

Results for Marketed Aloe Vera preparation:-

Table 3**.** Effect of Duration on anxiety level in control, Diabetes, DFU and Treatment group 2 rats in elevatedplus maze .

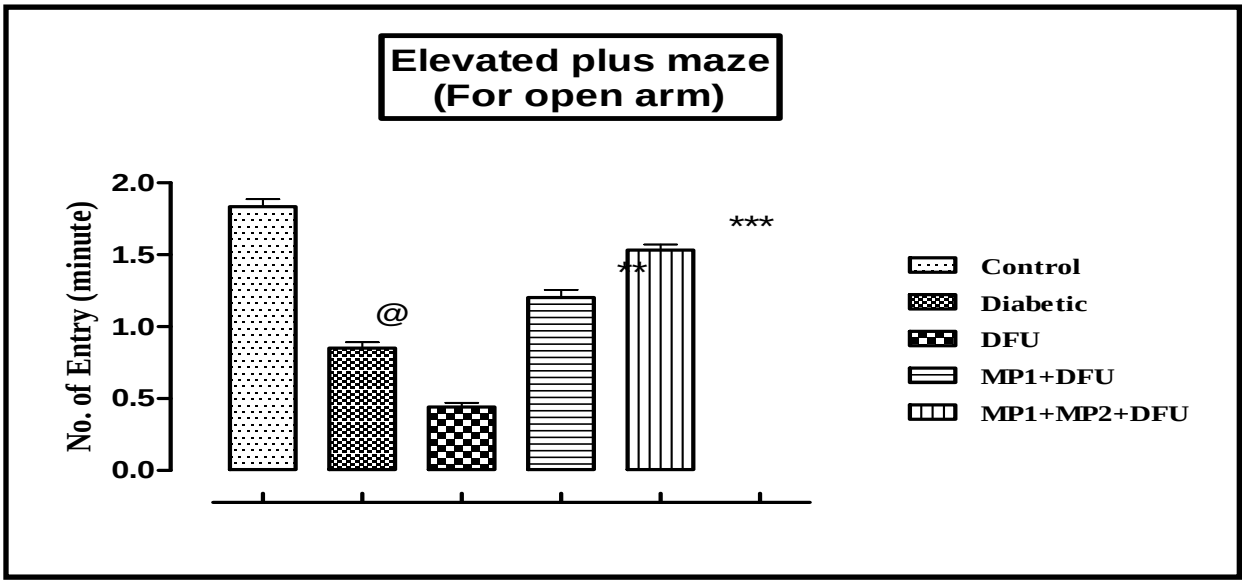
Group	**Number of Entries**	
	Enclosed arm (EA)	**Open arm (OA)**
Control- 10 Days	4.1±0.98	1.83±0.16
Diabetic -10 Days	3.3±0.33	0.83±0.75
DFU-10 Days	1.50±0.84	0.42±0.36
MP1 + DFU-10 days	2.4±0.45	1.00±.084
MP1 + MP2 + DFU-10 Days	3.6 ±0.96	1.4±0.36
Control- 20 Days	6.6±0.61	4.3±1.23
Diabetic -20 Days	5.6±0.33	1.23±0.35
DFU- 20 Days	4.50±0.84	0.82±0.36
MP1 + DFU-20 days	5.6±0.44	1.00±.084
MP1 + MP2 + DFU-20 Days	5.5±0.98	3.38±0.66

Fig.5. Influence of Marketed product treatment on number of entries & time spent in EAin elevated plus maze.



Influence of treatment of Marketed product containing Aloe Vera on the number of entries & time spend in enclosed arm for the time intervals, for the elevated plus maze test. Each value represents mean ± S.E.M. of 6 observations ƐP<0.0001 vs. Diabetic group; *P<0.0001 vs. DFU test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); Alloxan (150mg/kg) ;MP1; oral marketed product (300mg/kgp.o): MP1 + MP2 ; topical marketed product (30 mg/kg): treated

Fig.6. Influence of Marketed product treatment on number of entries & time spent in OA In elevated plus maze.

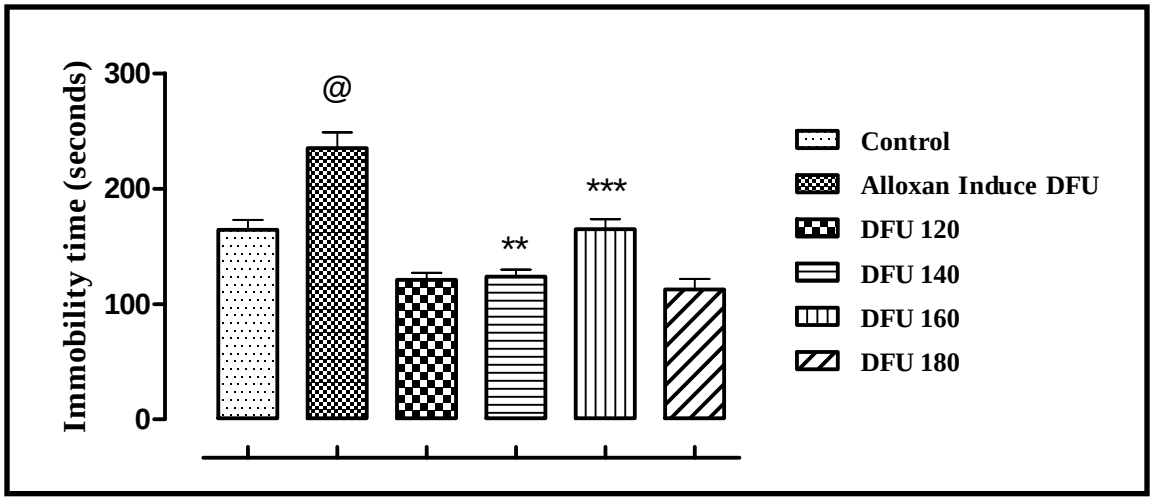


Influence of treatment of Marketed product containing Aloe Vera on the number of entries & time spend in open arm for the time intervals, for the elevated plus maze test. Each value represents mean ± S.E.M. of 6 observations ƐP<0.0001 vs. Diabetic group; *P<0.0001 vs. DFU test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); Alloxan (150mg/kg) ;MP1; oral marketed product (300mg/kgp.o): MP1 + MP2 ; topical marketed product (30 mg/kg): treated

Table 4**.** Effect of acute treatment with Marketed Product on DFU Depression and Anxiety.

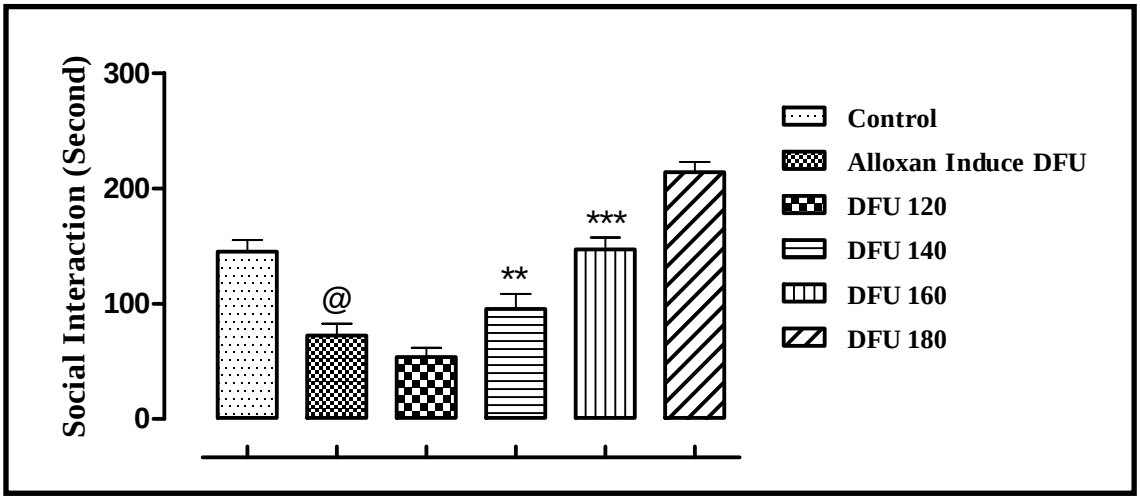
Duration	Control	Alloxan Induce DFU	Marketed product in DFU			
			Alloxan Doses (Mg / Kg)			
			120	140	160	180
Immobility Time (In Seconds)	180	230	120	124	190	135
	175	240	135	109	150	109
	178	238	106	139	163	116
	185	242	125	123	156	90
Social Interaction Time (In Seconds)	70	45	57	90	177	227
	75	48	75.	132	144	209
	70	40	45	138	157	240
	68	42	38	112	190	250

Fig.7. Influence of Marketed Preparation treatment on immobility time in forced swim test.



Influence of treatment of Marketed product containing Aloe Vera on Immobility time spends in Forced Swim Test. Each value represents mean ± S.E.M. of 6 observations
ƐP<0.0001 vs. Diabetic group; *P<0.0001 vs. DFU test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); Alloxan (150mg/kg) ; Alloxan (120 mg/kg); alloxan (140mg/kg) ;alloxan (160mg/kg); alloxan (180mg/kg)AGE; oral extracted aloe vera (300mg/kgp.o): + AG; topical aloe vera (30 mg/kg): treated

Fig. 8.Influence of Marketed Product treatment on Interaction time in social interaction test.



Influence of treatment of marketed product containing Aloe Vera on social interaction time spends in social interaction test in anxiety protocol. Each value represents mean ± S.E.M. of 6 observations
ƐP<0.0001 vs. Diabetic group; *P<0.0001 vs. DFU test group (One-way ANOVA followed by Tukey's post hoc test). Control: Control(saline solution); Alloxan (150mg/kg) ; Alloxan (120 mg/kg); alloxan (140mg/kg) ;alloxan (160mg/kg); alloxan (180mg/kg)AGE; oral extracted aloe vera (300mg/kgp.o): + AG; topical aloe vera (30 mg/kg): treated

Extracted Drug (120,140,160,180 Mg/kg I.P.) or saline (10 ml/ kg.I.P.) was administered 30 min. prior to the peak hour (24 h) of DFU to rat. After these administration individual rat was subjected to the either social interaction test and time spend in social interaction and immobility time in forced swim test was recorded as describe above. Separate group of rat (n=6) was employed for each treatment dose and test

Discussion:-

Performance in elevated plus maze:-

The anxiety protocol observed that the diabetic rats made fewer entries and spent less time in to open arm (OA) and also the conditioned get worsened with the DFU induced group. The animals in diabetic and DFU groups showed no significant differences compared with the control in number of entries in to enclosed arm (EA). Significant decrease was observed in the number of entries in to OA and also the time spent on OA in diabetic and DFU groups compared to that of control (Fig.1,2). Furthermore the treatments groups 1 (AGE, AGE+AG) indicated that the increased entries and the time spent in to OA as near to control group. The condition were observed to be more improved with the combination therapy (AGE+AG).significant rise was observed in the number of entries into OA & EA and also the time spent on OA as compared to diabetic and DFU groups but no significant difference observed in comparison with control rats (Table 1). The treatment group 2 (MP1, MP1+MP2) showed that the increase entries but less as compared to that of treatment groups 1 and not closed to normal group (Table 3,Fig.3,4). Furthermore no significance differences were observed in other behavioral (ethological) parameters, such as rearing, grooming & number of excreted boli in both control and diabetic. Significant differences were observed in control and DFU. The condition gets normalized after the treatment groups 1 & 2.

Performance in social interaction test and forced swim test:-

The social interaction test which is the measure of the alloxan induced DFU anxiety. The results indicated that the reduction in social interaction time induced anxiety in rat (Fig.7, 8). Further forced swimming test, the measure of the DFU induced depression study was carried out in animals using limited access protocol described earlier porsolt et al (1977) with minor changes and the result indicated that increase in the immobility time in forced swimming test during DFU induced depression in rat (Fig.5,6). The observations indicated that the improvisation in social interaction time made anxiolytic action in rats and the forced swimming test, increased duration of immobility time was observed after the application of extracted Aloe Vera and the marketed product containing Aloe Vera (Table 2, 4).

Conclusion:-

In the present study, the result showed alloxan induced diabetic foot ulcers was indicated that the behavioural dysfunction such as anxiety and depression. The use of Aloe Vera extract (oral & topical application) shows excellent activity against diabetic and diabetic foot ulcer induced anxiety and depression by stepwise wound healing process in comparison with marketed product containing Aloe Vera. The present observation were indicated that the extracted herbalsample shows equivalent or in somewhere better indirect activity against diabetic foot ulcer generated anxiety and depression as compared to marketed product.

Acknowledgment:-

The deep expression of gratitude to my research guide, Head, Department of pharmaceutical sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur; for providing me valuable guidance, continuous support and necessary facilities.

References:-

1. **Allan V. Kalueff (2007)et al.**Hindawi Publishing CorporationNeural PlasticityVolume, Article ID 52087,7pages doi:10.1155/2007/5208.
2. **Das AK (1994).**Diabetic Neuropathic Foot. Int. J. Diab. Dev. Countries; 14: 85-90.
3. **Dunning T (2003).** Care of people with diabetes: a manual of nursing practice, ed 2.Oxford Blackwell, 1-21.
4. **Goodson WH, Hunt TK (1979).** Wound healing and diabetic patient. Surgery, Gynecology and Obstetrics; 149, 600-608.
5. **Lavery L, Armstrong D, Wunderlich R, et al (2003)**. Diabetic foot Syndrome: evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanicwhites from diabetes disease management cohort. Diabetes care 26: 1435.
6. **Levin E (1995).**Preventing amputation in the patient with diabetes.Diabetes Care; 18:1383- 1394.
7. **Moral Y, Fujita S, Goto T, Kubo K (2004).**Clinical evaluation of allogeneic cultured dermal substitutes for intractable skin ulcers after tumor resection .Eur J Dermatol; 14: 172–6.
8. **Moulik PK, Mtonga R, Gill GV (2003).**amputation and mortality in new onset diabetes foot ulcers stratified by etiology. Diabetes Care; 26:491-494.
9. **Raghow R (1994**).The role of extracellular matrix in post inflammatory wound healing and fibrosis. Federation of American Society for Experimental Biology Journal; 8; 823-831.
10. **Ramsey S, Newton K, Blough D (1999) et al.** Incidence, outcomes, and cost of foot ulcers in patients with diabetes. Diabetes Care; 22: 382.
11. **Ravishankarrajashree (2011)et al**. Malaysian J.Med.Scioct-Dec 2011; 18(4): 26-31.
12. **Reiber G (1996).**The epidemiology of diabetic foot problems. Diabetes Med; 13:S6.
13. **Spaheimer G (1988).**Decreased collagen production in diabetic rats. Diabetes; 37; 371-376.
14. **Trautner C, Haastert B, Giani G, (1996) et al.**Incidence of lower limb amputation and
15. diabetes. Diabetes Care: 19; 1006.
16. **International Diabetic Federation, IDF** Diabetes Atlas I Sixth edition.
17. **Pan American Health Organization (Nov 2005).**the newsletter of the Pan American Health Organization, available at : http://www.paho.org/english/DD/PIN/ptoday20_nov05.htm
18. **WHO, (2002).** World health organization: Diabetes: the cost of diabetes. Fact sheet no.236.Geneva.