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Comparative analysis of antioxidant, antidiabetic and analgesic activity of *Callestemon viminalis* L. and *Alcea rosea* L. leaves extracts

Khwaja Zafar Ahmed[1], Sadaf Naeem[2], Yousra Shafique[2], Saira Saeed Khan[3], Nausheen Alam[4], Saira Shahnaz[1] and Anosh Tahir[3]

[1]Department of Pharmacology, Faculty of Pharmacy, Nazeer Hussain University, Karachi, Pakistan
[2]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
[3]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[4]Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University, Karachi, Pakistan

Abstract: High levels of reactive oxygen species (ROS) in the body and diabetes are key factors for the development of hypercholesteremia and related neuropathic pains. Current study aimed to compare the antioxidant, antidiabetic and analgesic activities of aqueous methanolic extracts of *C. viminalis L.* and *A. rosea L*. leaves. HPLC method was used for phenolic content evaluation. Antioxidant capacity was determined by DPPH and analgesic activity was performed via acetic acid induced writhing reflex test. Whereas the antidiabetic activity was performed on Alloxan induced diabetes model. HPLC analysis indicated the presence of phenols in both extracts. Based on DPPH radical scavenging activity, *C. viminalis* and *A.rosea L*. both leaves extracts showed strong scavenging activity (IC50, 11.96±0.64lg/mL) and (IC50, 10.11±0.74lg/mL) respectively. Antidiabetic effect of *C. viminalis L* and *A. rosea L.* were also significant ($p<0.05$). Further biochemical analysis showed both leaves extracts significantly ($P<0.05$) reduces glucose, Low density lipid (LDL), triglycerides (TG), total cholesterol (TC) and urea while high density lipid (HDL) were improved. In writhing reflex test both extracts exhibited significant ($P<0.01$) analgesic activity which was comparable to Aspirin. In conclusion both *C. viminalis L.* and *A. rosea L.* leaves extracts displayed significant antioxidant, analgesic and antidiabetic activity.

Keywords: *C. viminalis* L., *A. rosea* L., poly phenols, antioxidant, analgesic activity, alloxan, antidiabetic activity,

INTRODUCTION

It is progressively being understood that a significant number of currently emerging diseases are because of the oxidative stress (OS) which occurs mostly due to the disproportion between pro oxidants formation and neutralization leading to compromised immune response (Tan & Suda, 2018). It's a need of time to find innovative approaches in order to boost the antioxidant defense mechanism (Nita & Grzybowski, 2016). Oxidative stress exposure leads to accumulation of oxygen reactive species (ROS) which is a hall mark causing damage of cells in the form lipid, protein and DNA oxidants (Tan & Suda, 2018). If the ROS (reactive oxygen species) level is not controlled, it will cause cell death as the basal cellular protective mechanism will be disturbed. In short, candidates that reduce or can control oxidative stress will be helpful in the treatment of different disorders and will be considered as potential candidates for treating ROS generated health issues.

Moreover, several evidences are showing that the primary source of oxidative stress induction is by hyperglycemia and it is interlinked with a vital procedures in the onset and disease progression of diabetic impediments (Amarowicz and Pegg, 2017); however, the exact mechanisms due to which the process of oxidative stress rushes the progress of complications associated with diabetes are solitary fairly known (Bashkin *et al*, 2021). The production of inflammatory mediators and their stimulation by oxidative stress which is actually induced by hyperglycemia, embark on to the creation of free radical species (Oguntibeju *et al*, 2019). Free radicals which are not steady species of molecules pair up with free electrons by attacking healthy cells, as a result forfeiture the structure of cell ultimately its function also (Phaniendra, *et al*, 2015). These damaged cells are responsible greatly for the degenerative diseases that include pain, inflammation, weakening of immune system, liver associated diseases, hyperglycemia and dysfunctioning of brain, cardiovascular disorders and others (Tan *et al*, 2018). Researchers suggested hyperglycemia and hyperinsulinemia accelerated lipolysis following insulin resistance increases the availability of free fatty acids, while hyperglycemia triggered triglyceride synthesis in the liver and decreased HDL. HDL dysfunction enhanced atherosclerosis in hyperglycemia (Phaniendra *et al*, 2015).

Plant antioxidants belong to the class of free radical-scavenging agents that are bound to control the damaging effect of these not so stable species on the human body (Haider, 2020) and functions as a treatment for few diabetic complications. Edible and non- edible herbs, fruits, oils, vegetables and some other plant parts are well known for their phenolic content existence (Alara *et al*, 2021). Plant metabolites that are secondary in nature like

*Corresponding author: e-mail: sadaf.naeem@jsmu.edu.pk

alkaloids, flavonoids, steroids, terpenoids, glycosides and saponins have acquired significance because of their different pharmacological functions as hypolipidemic, anti-inflammatory, analgesic, antidiabetic and so on (Faujdar *et al*, 2016).

C. viminalis L. usually known as bottle brush is a flowering plant of family Myrtaceae. In herbal medicine it is used as an herbal tonic, tea and health food (Tiwari *et al*, 2013). Significant activities (antimicrobial, antioxidant, anti-inflammatory and hypolipidemic) are reported from various parts of this plant because of its terpinoidal, phenolic and flavonoidal constituents such as 1, 8-cineole and α-pinene (Zubair *et al*, 2013).

For local population of Pakistan, it has likewise been utilized to set up a hot beverage privately alluded to as "tea" for the treatment of loose bowels, gastroenteritis and diseases related to skin (Salem *et al*, 2017). However, there is no previous report on its analgesic and hypolipidemic effects with aqueous: Methanolic *C. viminalis L.* leaves extract. Petroleum ether extract of *C. viminalis L.* plant have been used in only one previous research to explore its hypoglycemic effect (Nazreen *et al*, 2011).

Alcea rosea L. (A. rosea) is well known as Hollyhock. It is an ornamental herb belonging to the family Mallow (Malvaceae) having huge attractive blooms of diverse shades. It is already reported in scientific literature for its multiple uses as anti-microbial (Mert *et al*., 2010), immuno-modulatory (Ghaoui *et al*., 2008) anti-inflammatory, analgesic (Mert *et al*., 2010) and hypoglycemic activity in diabetic mice models. Roots of this plant additionally been used for treatment of severe cough, bronchitis, inflammation and diarrhea (Dar, *et al*, 2017). Most commonly its extract were used to treat infections related to kidney, bladder, intestine. In folk medicine it is also reported to treat jaundice, snake bite, rheumatism along with diarrhea and vomiting (Akhi, 2020). Currently only Dar *et al*, (2017) has been reported the antidiabetic role of *A. Rosea* plant. We didn't found any other study and comparison among these two plants. Thus the purpose of current study is to explore the comparative antioxidant, analgesic and antidiabetic activity of *C. viminalis L.* and *A. rosea L.* leaves' extracts in different animal models.

MATERIALS AND METHODS

Methodology

Plant material identification and extraction

Fresh leaves from the branches of the plant *A. rosea L.* and *C. viminalis L.* were collected from the botanical garden of the Department of Botany, University of Faisalabad, Pakistan, in the month of August 2017. The plant identity was confirmed by the taxonomist of Faisalabad University with voucher number 708-16-17. The leaves were washed and air-dried in order to achieve the moisture content of 10.12% at the laboratory conditions for two weeks and the leaves were powdered. The extract for powdered leaves were made by soaking method. The process involves soaking of leaves' powder for one week in 3L of methanol: Water (70: 30) solvent in a sealed closed container. Both the extracts were filtrated using filter paper (Whatman no. 1). Then the solvent was evaporated under reduced pressure using a rotary evaporator to concentrate the extracts. Both extracts were stored in refrigerator before chemical and bioassay analysis (Saleem *et al*, 2016).

Chemicals

Alloxan monohydrate, 2,2-diphenyl-1-picrylhydrazyl, Ascorbic acid, Acetic acid (Sigma Aldrich, Limited), Analytical grade methanol and n-hexane (Merck, Limited), Glibenclamide (Daonil) 5mg tablets (Sanofi Aventis, Pakistan, Limited), Aspirin (Aspro) 300mg tablets (Reckitt Benckiser Pakistan LTD), All the reference compounds were purchased from Sigma-Aldrich i.e. chlorogenic acid, quercetin, gallic acid, Caffeic acid, Benzoic acid, Ferulic acid, syringic acid and Vitamin C and DMSO.

Phytochemical testing

For the presence of bioactive constituents such as terpenoids, alkaloids, saponins, tannins and flavonoids in C. viminalis and A. rosea methanolic leaves extracts, the standard operating procedures were followed. (Ahmed *et al*, 2018).

Determination of phenolic contents

Phenolic compounds in *A. Rosea L.* and *C. Viminalis L.* Leaves extract were evaluated by reversed phase - high performance liquid chromatography LC 2010AHT coupled with UV detector with direct injection. Detection and quantification was carried out by a reverse phase Luna 5u C18 (2) 100A (5µm particle size, i.d. 250 x 4.6 mm) and a diode array detector with wavelengths set at 280nm. The flow rate was 0.65ml min-1, injection volume was 20µl and the column temperature was set at 28°C. Gradient elution of two solvents was used: Solvent A consisted of: Acetic- water (2:98 v/v), solvent B: acetonitrile. The data were integrated and analyzed using the Laboratory Automated Software system. The given extracts, standard solutions and mobile phases were filtered by a 0.45-µm pour size membrane filter. The amount of phenolic compounds in the extracts was identified using their retention time and the content was calculated by analyzing the area under the curve, obtained from previously produced standard chromatograms. (Quirantes Piné *et al*, 2013).

Acute toxicity testing

Acute oral toxicity study of the leave extracts was analyzed as per the methods described by Lorke (1983). After the oral administration of *C. viminalis L.* and *A.*

rosea L. leaves extracts' in the dose of 2000mg/kg, Wistar rats (150-200g, n=6) were observed individually for the initial 30 min, providing special attention during the first 4h and then regularly monitored for 14 days for toxicity determination with comparison to control.

Antioxidant assay

The DPPH assay was performed as described by Fernandes *et al*. (2016). Each concentration (0.02-4mg/ml) of *C.viminalis* and *A. rosea* extract sample (10µL) in methanol: Water was added to 190µL of 150µM DPPH in methanol. The mixture after vortex mixing was incubated for 10 minutes at room temperature and 517nm was set as the wavelength to measure the absorbance values. 20 minutes were given to keep the sample in dark, and the absorbance were taken as the reading reached plateau and from this values corresponding percentage of inhibition were calculated. Ascorbic acid was taken as standard at the concentration value of 5mg/ml. IC50 value was determined from % inhibition v.s. log concentration graph.

The formula used to calculate % inhibition ratio was as follows:

% Inhibition = Absorbance of blank-Absorbance of sample/ Absorbance of blank × 100

Antidiabetic activity

Animals and housing

Wistar rats of either sex weighing 150 to 200g were purchased from the animal house of the University of Agriculture, Faisalabad, Pakistan. The animals were housed in polypropylene cages at room temperature and fed with standard feed and water *ad libitum*. Fourteen hours prior to the experiment, the animals were kept in the experimenting lab in order to familiarize with the laboratory environment and fed only with standard diet and water *ad libitum*. The experiments were performed following the rules of Helsinki Resolution 1964. The protocol was approved by Ethical Review Committee, GC University, Faisalabad, Pakistan.

Induction of diabetes

The Wistar rats were fasted overnight and their normal blood glucose levels were checked using Accu-Check strips of Accu-Check Glucometer from Roche, (Switzerland) before induction of diabetes. Intraperitoneal Alloxan (150mg/kg) was used to induce experimental diabetes. To control the drug-induced hypoglycemia five percent glucose (5%) was given to all experimental rodents. Control rodents were given distilled water only. After induction of diabetes we only selected those rodents for study that blood glucose values reaches above 250 mg/dl on the third day. The treatment with *C. viminalis* and *A. Rosea* leaves extracts started after Alloxan administration from 4th day and continued up to thirty days (Akpan *et al*, 2012).

Experimental design

All Experimental Wistar rats (n=54) were allocated into nine groups each contain n=6: Group I: Control receiving distilled water, Group II: Negative control, Group III: 100 mg/kg/day of the aqueous: Methanolic extract of the *C. viminalis L.*, Group IV: 200mg/kg/day of the aqueous: methanolic extract of the *C. viminalis L.*, Group V: 400 mg/kg/day of the aqueous: methanolic extract of the *C. viminalis L.*, Group VI: 100mg/kg/day of the aqueous: methanolic extract of the *A. rosea L.*, Group VII: 200 mg/kg/day of the aqueous: Methanolic extract of the *A. rosea L.*, Group VIII: 400mg/kg/day of the aqueous: methanolic extract of the *A. Rosea L.,* Group IX: (Standard): 0.5mg/kg/day Glibenclamide.

All the drugs and extracts used in the experiment were administered in aqueous suspension, orally by gauge technique. After thirty days of treatment, all the animals were sacrificed and analyzed for alteration in blood glucose levels and biochemical tests.

Analgesic activity

The analgesic activity of both plant leaves extracts were carried out using the Acetic acid-induced writhing test (Ali *et al*., 2021). Total of 54 Albino mice were divided into 9 groups (n=6 per group) and were treated 30 min prior to acetic acid injection as: Group A, B and C were orally treated with *C. viminalis L.* in the dose of 100, 200 and 400mg/kg/day respectively; Group D, E and F were orally treated with 100, 200 and 400mg/kg/day of *A. rosea L.* respectively all by gastric gavage; Group G was orally treated with aspirin in the dose of 150mg/kg and considered as positive control. Group H was given normal saline and considered as control group. Group I was given acetic acid and labelled as negative control group. After one hour administration of drug and extracts, 0.7% glacial acetic acid (10ml/kg) was given intraperitoneally (i.p) to all the mice to induce pain characterized by abdominal constrictions or writhes. The number of writhes observed in each mouse was counted at 15min of time interval over a period of 5 min and they were expressed as % of constriction inhibition and this percentage of reduction of writhes in 15 minutes was calculated as follows:

% age reduction = $\bar{x}$ control group - $\bar{x}$ treated group/$\bar{x}$ of control group × 100

Estimation of biochemical parameters

The serum lipid profile that includes Total Cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), and triglycerides (TG) were measured using commercially available kits. The serum creatinine and urea levels were also analyzed through commercial kits specific for the tests. All above biochemical parameters were estimated using Elisa Kit.

STATISTICAL ANALYSIS

The obtained data were analyzed on SPSS (version 21) software using One-way ANOVA followed by Tukey's post hoc test. The data were presented as Mean ± Standard Deviation (SD), percentage and $P<0.05$ indicates statistical significance.

RESULTS

Acute toxicity study

No signs of toxicity were produced after oral administration of both extracts in experimental rats at 2000mg/kg body weight dose and it was also observed that no rat died till day 14.Such findings reflects that *C. viminalis L.* and *A. rosea L.* found to be safe. Keeping in mind this safety report, further investigations on antidiabetic, analgesic and hypoglycemic effects of selected plant extracts at variable doses (100, 200, 400 mg/kg) were performed.

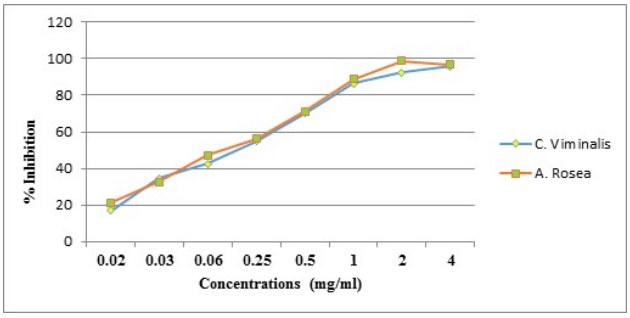

Fig. 1: % age inhibition of *C. viminalis L.* and *A. rosea L.* leaves extracts on DPPH radical scavenging activity

Phytochemical analysis

Secondary metabolites are important for diverse functions in plants. Through phytochemical testing we find out some important constituents. Our analysis revealed the presence of flavonoids, glycosides, steroids and tannins at various concentrations in both leaves extracts. Phytochemical constituents present in the leaves extracts' of both plants are shown in table 1.

Determination of phenolic contents

Table 2 and 3 represents the total flavonoids from fresh leaves of both tested plants. *C. viminalis L.* leaves extract showed presence of chlorogenic acid (162.3) followed by quercetin (22.4), gallic acid (21.3), syringic acid (14.15), and ferulic acid (13.8). Moreover *A. rosea L.* also contain flavonoids (table 3) i,e chlorogenic acid (761.5),gallic acid (120.09), quercetin (52.6), Caffeic acid (57.6),Vitamin C (19.5) , Benzoic acid (498.6).

Antioxidant assay

DPPH assay was used to investigate the antioxidant capability of both selected plant extracts. Dose dependent anti -oxidant effect was observed in both extracts shown in fig. 1. *A.Rosea L.* found to be potent antioxidant (98.81±0.76) with IC_{50} value of (10.11±0.74lg/mL) as compared to *C.viminalis L.* (95.26±1.55) with IC_{50} value of (11.96±0.64 lg/mL) in presence of ascorbic acid that showed (97.18±0.40) with IC_{50} value (7.11±0.54 lg/mL) selected as standard for this assay at the dose of 4mg/ml.

Analgesic activity

In acetic acid induced writhing reflex test we observed significant reduction in writhes count after treatment with given extracts comparative to both control and negative group at given doses of 100mg/kg, 200mg/kg and 400mg/kg (12.6%,48.07%,55.96% with p value of $p<0.05$,<0.01,<0.01) respectively for *C. viminalis L.* while for *A. rosea L.* extract also dose dependent activity observed (46.37%,50%,71.2% with $p<0.05$, $p<0.01$, $p<0.001$) at 100mg/kg,200mg/kg and 400mg/kg respectively as compared to negative control (table 4).

Standard drug showed significant (79.2%, $p<0.01$) decrease in the writhes count just like both extracts. Post hoc analysis exposed that animal treated with *C. viminalis L.* and *A. rosea L.* at the dose of (200ml and 400ml/kg/day) via oral route showed significant inhibition ($p<0.05$) of the abdominal writhes in dose dependent compared to control group and negative control group.

Antidiabetic activity

Current study presented that Alloxan injection at the dose of 150 mg/kg produce significant hyperglycemia ($P<0.05$) in tested animal model. Daily treatment till one month with aqueous methanolic leaves extract of *C. viminalis L.* and *A. rosea L.* at selected doses of 100, 200 and 400mg/kg/day cause a dose-dependent reduction in blood sugar levels (90.20±1.74; 83.60±1.33; 77.80±1.43) and *A. rosea L.* (142.0±3.33; 129.8±4.18; 108±3.49) respectively comparable to the Glibenclamide (standard drug-99.1±1.59) responsible for reduction in blood glucose levels after 30 days of study (table 5).

Both extracts after treatment in given time interval of thirty days at (100, 200mg/kg and 400mg/kg) selected doses exhibited highly significant ($p<0.01$) hypoglycemic effects compared to diabetic control group. It was found that *A. rosea L.* worked as a more powerful hypoglycemic agent with ($p<0.01$) compared to *C. viminalis L.* at the dose of 400mg/kg/day.

Effect of C. viminalis L and A. rosea L. on Biochemical analysis

In Alloxan-induced diabetic rats model the levels of total cholesterol, triglyceride, LDL, were augmented and HDL levels were reduced significantly ($P<0.05$) when compared to normal control rats. Significant decrease ($P<0.05$) in levels of TC, TG and LDL was noticed on administration of both extracts at the given doses of 100, 200 and 400mg/kg. On the other hand, a significant ($P<0.05$) increase in HDL level was seen in diabetic rats treated with fore mentioned experimental drugs and Glibenclamide compared to diabetic control rats (table 6).

Table 1: Phytoactive Constituents of Aqueous: Methanolic Extract of *C.viminalis L.* and *A. rosea L.* Leaves

Groups	Name of Phytochemical Test	Aqueous: Methanolic Extract of *C. viminalis L.*	Aqueous: Methanolic Extract of *A. rosea L.*
Alkaloids	Mayers & Dragendorff's	+	+
Glycosides	Keller Kilani test	-	+
Saponins	Foam test	++	-
Tannins	Ferric chloride test, potassium dichromate	++	++
Flavonoids	Shinoda's and Zn-HCl test	++	++

(+) means presence in a single method test, (++) means presence experimented in two methods and (-) = absence.

Table 2: HPLC Analysis of *C. ciminalis L.* Aqueous Methanolic Leaves Extract

C. viminalis L.			
Compound name	Retention time	Area %	Quantity (ppm)
Chlorogenic acid	2.653	6.1	162.3
Quercitin	2.987	5.9	22.4
Gallic acid	4.707	9.1	21.3
Syringic acid	16.380	8.7	14.15
Feurulic acid	32.827	3.0	13.8

The presence of functional constituents (ppm) with different retention time

Table 3: HPLC Analysis of Aqueous: Methanolic (30:70) *Alcea. rosea L.* Leaves

A. rosea L.			
Compound name	Retention time	Area %	Quantity (ppm)
Chlorogenic acid	2.64	10.4	761.5
Quercitin	3.493	5.6	52.6
Gallic acid	4.800	18.8	120.09
Caffeic acid	12.440	7.1	57.6
4 hydroxy 3-methoxy benzoic acid	14.560	26.5	498.6
Vitamin C	23.74	5.5	19.5
Sinapic acid	26.980	2.3	5.3

The presence of functional constituents (ppm) with different retention time

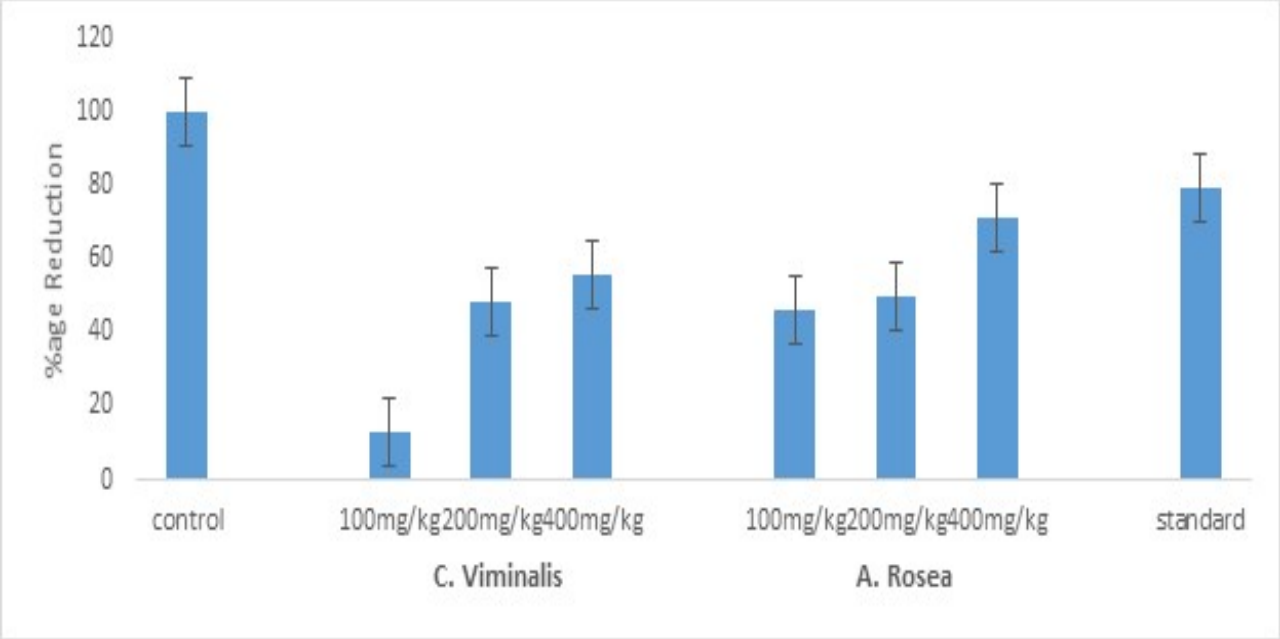

Fig. 2: %age reduction of writhes in 15 minutes

Table 4: Antinoceceptive Effect of *C. viminalis L.* and *A. rosea L.* on Acetic Acid Induced Mice

Groups	Average no of writhes	% inhibition
Control	2.11±0.11	100%
Negative Control	28.91±1.23*	_ _
C. viminalis L.		
100mg/kg	22.22± 1.18#	12.6%
200mg/kg	15.66±1.15##	48.07%
400mg/kg	12.32±0.75##	55.96%
A. rosea L.		
100mg/kg	18.52±1.37##	46.37%
200mg/kg	14.0±7.4##	50%
400mg/kg	9.0±4.29##	71%
Standard Aspirin (150mg/kg)	6.42±0.32##	79.2%

Values are $\bar{x} \pm SD$, *$p < 0.05$, **$p < 0.01$, as compared to control group. #$p < 0.05$, ##$p < 0.01$, as compared to negative control group

Table 5: Effect on Blood glucose in Alloxan Induced Diabetic Rats Treated with Aqueous Methanolic *C. viminalis L*. and *A. rosea L.* Leaves Extract (Mean ±SD)

Groups	Before treatment mg/dl	After treatment mg/dl	P-value
Control	99.60±1.57	102.6±1.96	<0.05
Diabetic control	247.0±13.6**	266.4±13.7**	>0.05
Standard (Glibenclamide)	224.3±5.62	99.1±1.59	>0.001
C. Viminalis L.			
100mg/kg	249.4±9.16**	90.20±1.74##	<0.001
200mg/kg	365.2±17.5**	83.60±1.33##	<0.001
400mg/kg	345.2±20**	77.80±1.43##	<0.001
Alcea. Rosea L.			
100mg/kg	272.0±11.0**	142.0±3.33##	<0.05
200mg/kg	281.6±10.6**	129.8±4.18##	<0.001
400mg/kg	273.2±19.3**	108±3.49##	<0.001

Values are $\bar{x} \pm SD$, *$p < 0.05$, **$p < 0.01$, as compared to control group. #$p < 0.05$, ##$p < 0.01$, as compared to negative control group

Table 6: Effect of *C. viminalis L.* and *A. roseae L.* Extract on Lipid Profile in Diabetic Rats

	Total cholesterol	Triglycerides	HDL	LDL
Control	133.20±2.31	145.00±1.84	36.26±0.23	57.50±1.24
Diabetic control	165.40±4.03*	191.20±3.02*	33.480±0.252*	93.76±3.58*
Standard (Glibenclamide)	131.81±2.28##	153.26±3.13#	27.53±1.71	68.37±3.56#
C.viminalis L.				
100mg/kg	144.80±1.88#	162.80±2.31#	36.68±0.343#	83.56±1.46
200mg/kg	137.0±1.64#	141.40±1.75#	37.760±0.232#	74.96±1.67#
400mg/kg	130.40±1.36##	135.20±2.01##	38.020±0.206##	69.30±1.68##
A.rosea L.				
100mg/kg	148.80±3.28#	188.20±1.53	35.82±0.215	75.26±3.39#
200mg/kg	140.00±2.30#	178.0±4.09#	36.40±0.16#	64.92±1.64#
400mg/kg	128.00±1.58##	159.20±1.07#	39.300±0.173##	58.86±1.66##

Values are $\bar{x} \pm SD$, *$p < 0.05$, **$p < 0.01$, as compared to control group. #$p < 0.05$, ##$p < 0.01$, as compared to negative control group

Table 7: Effect of *C.viminalis L.* and *A.rosea L.* Extracts on Urea and Creatinine

Groups	Urea	Creatinine
Control	58.2±0.44	1.02±0.004
Diabetic Control	70.55±15.3*	1.39±0.04*
Standard (Glibenclamide)	59.64±11.71#	1.11±0.03#
C.viminalis L.		
100mg/kg	67.4±5.3	1.25±0.02
200mg/kg	60.0±6.7#	1.22±0.03#
400mg/kg	55.2±11.6#	1.01±0.1#
A.rosea L.		
100mg/kg	68.52±14.71	1.22±0.03#
200mg/kg	56.0±11.4#	1.21±0.04#
400mg/kg	54.0±14.9##	1.00±0.02##

Likewise, in diabetes induced rats, dose administration of both extracts at different doses considerably (P<0.05) reduced urea and creatinine levels as compared to diabetic control rats. Additionally we observed the dose dependent effect of both extracts *C. viminalis L.* and *A. rosea L.* at 400 mg/kg treatment showed significantly (P<0.01) higher reduction of urea and creatinine levels compared to 100 and 200mg/kg dose (table 7).

DISCUSSION

Oxidative stress characterizes imbalance state among production of free radical and the reaction of anti-oxidant defense system of host. Researchers have demonstrated that DM and its related complications are connected with over production of free radicals as a result reduction in antioxidant status. Hyperglycemia-induced upsurge in protein glycosylation brings about overproduction of free radicals that mediates neuropathy, hypercholestremia and other complications (Tan & Suda, 2018; Kondhare and Lade, 2017).

Phytochemicals especially polyphenols obtained from natural plant have been utilized as helpful agents in therapeutic management of different diseases since ancient time. They possess the ability to modify human metabolism and restore antioxidant defensive mechanism in a satisfactory manner for the inhibition and prevention of chronic and degenerative diseases (Mishra *et al*, 2013; Kumar *et al*, 2020). *C. Viminallis L* and *A. Rosea L* are those medicinal plants that have been studied for their antidiabetic, antioxidant and antibacterial properties however their comparative study has not been studied before. Thus, the investigation was performed to give an experimental evidence to their comparative therapeutic applications. We have found alkaloids, saponins, glycosides and flavonoids in both plant leaves extracts. Further poly phenolic contents were also identified, table 2 represents concentrations of poly phenols in *A. Rosea L.* leaves and *C. Viminalis L.* which indicated both leaves contains poly phenols. Poly Phenols are natural phytoconstituents known to possess numerous valuable effects, together with their antioxidant potential (Oyedeji *et al*, 2009). Phenolics and flavonoids having strong anti-oxidant potential probably because of free radical scavenging activity and these free radicals are basically involved in multiple disorders but mostly in diabetes and pain (Al-Ishaq *et al*, 2019; Ferraz *et al*, 2020).

In DPPH assay we found significant concentration-dependent radical scavenging ability by A. Rosea and *C. Viminalis* extracts which were similar to ascorbic acid. However A. Rosea extract showed maximum inhibition. The DPPH scavenging activity of *C. Viminalis* and *A. Rosea* leaves extracts can be correlated with the presence of different flavanoidal contents. Saleem *et al*, 2015 reported that methanolic extract of *C. viminalis* leaves exhibited potent antibacterial potential because of the occurrence of flavanoidal contents. In the same way abdal-salam *et al*, 2018 investigated that leaves extract of A. Rosea possess highly antioxidant, cytotoxic and immune stimulant activities because of the presence of its poly phenolic contents.

Oxidative stress induces DM via generating changes in enzymatic systems, impaired glutathione metabolism, lipid per oxidation and diminished levels of vitamin C (Ahmed *et al*, 2018). Alloxan produces diabetes by producing metabolite (dialuric acid), start a redox cycle along with the production of super oxide radicals which undergoes to dismutation and finally produce hydrogen peroxide induced oxidative stress. The resultant product of reactive species responsible for enormous rise in cytosolic calcium levels along with lipid per oxidation causing rapid destruction of the insulin-producing pancreatic β cell (Abd El Latif *et al*, 2014).

Studies have recommended that antioxidants are able to recuperate insulin activity and can diminish the risk of diabetes mellitus (Asmat *et al*, 2016; Obafemi *et al*, 2017). Hussain *et al*., 2020 reported that flavonoids have antioxidant potential and may be because of this they put forth strong activity against diabetes by modulation of signaling network based on cellular targeting, hence, progressing glucose metabolism, α -glycosidase, and aldose reductase or glucose transport via carbohydrate metabolic pathway in β-cells of pancreas. Furthermore, polyphenols and flavonoids encounter diabetic related complications (hyperlipidemia and renal problems) because of their anti-diabetic properties (Al-Ishaq *et al*, 2019). We observed significant decrease (p<0.05) in levels of blood glucose after one month of treatment with extract of *C. viminalis* (leaves)with gradual increased in doses at 200 and 400mg/kg respectively. However, the hypoglycemic effect of *A.rosea* was slightly higher and dose dependent just similar to standard hypoglycemic drug Glibenclamide. Findings from current research is in accordance with previous studies that explained the presence of flavonoids, saponins, tannins and phenolic compounds in the leaves of *C. viminalis* and *A. rosea* that considered as bioactive constituents in diabetes management (Tiwari *et al*., 2014; Dar *et al*, 2017).

Hyperlipidemia induced by diabetes is in turn cause excess mobilization of fat from the adipose tissues because of the underutilization of glucose. Increased levels of fatty acids due to high levels of TGRs (triglycerides), LDL and low levels of HDL in turnarouse insulin resistance as well as dysfunctioning of β-cells (Hamzah, 2019). We have observed high levels of TC, LDL and TG's in negative control group although our *C.viminalis* and *A. rosea* 200, 400mg/kg/day animals showed significant reduction in TC, LDL, TG's and raised values of HDL. Our results are in accordance with previous researches that explained polyphenols rich

extracts exhibited hypolipidemic effect (Ramchoun *et al*, 2009).

Diabeties consequences are also faced by kidneys in which small vessels within the kidneys begin to bleed, as a results kidneys are incapable to filter blood and produce urine. Therefore, salt and water production is more than normal in the body leading to ankle swelling, weight gain and protein in the urine, alongside pooling of waste materials in the blood. Levels of urea and creatinine also raised in blood due to renal failure (Baxmann *et al*, 2008). Our negative control group animal showed high levels of urea and we observed slight change in creatinine levels also. However treatment with *C.viminalis* and *A. rosea* 200, 400mg/kg/day ameliorate rise in urea and creatinine levels in dose dependent manner, however more promising results were obtained by A. rosea because of their high polyphenolic contents. Previous researches (Dar *et al*., 2017) explained that this experimental extract has high phenolic content (412.23mg/g) which may be one of the reason for its tremendous effect in decreasing the creatinine and urea levels in tested animals although more research is required to explore underlying mechanism.

Painful diabetic neuropathy is related with signs and symptoms as tingling hyperalgesia and spontaneous pain (Khdour, 2020). That's why it's a high need of time to search a novel therapeutic agent for the satisfactory treatment of neuropathic pain associated with diabeties. Accumulated levels of free radicals are responsible for damage of membrane as a result anti-oxidant defense mechanism becomes weaker leading to cell and tissue damage (sen *et al*, 2010). In current study administration of *C. viminalis* and *A. rosea* leaves extract relieved pain in writhing reflex test compared to that of the standard group, aspirin. In practice painful diabetic neuropathic pains are controlled by antioxidant supplementation (Rajanandh *et al*, 2014). In context to these literature both plant extracts are helpful in reducing symptoms realated to diabetic neuropathy but in current study results of *A. rosea* are more promising as compared to *C. viminalis*. Results are also in accordance with Saha, 2019, who reported that methanol extract of the leaves of *A. rosea* have effective in-vitro anti-inflammatory activity. Current study suggested that the analgesic and antidiabetic capacity in *A. rosea* and *C Viminalis* extracts might derive from the phenolic compounds.

CONCLUSION

In the light of present research, it can be concluded that the experimental extracts from selected plants *C. viminalis* and *A. rosea* aqueous methanolic (70:30) possess strong antioxidant, hypoglycaemic and analgesic activity. In conclusion, our results showed that both *C. Viminalis* and *A. Rosea* leaves extracts reduce oxidative stress by free radical scavenging activity and protect against hyperglycemia, pain and also able to manage hyperlipidemia by decreasing serum level of glucose, cholesterol and triglycerides. Further scientific conformation should be done for characterization, identification and purification of phyto-constituents liable for effective analgesic and antidiabetic action of *A. rosea* and *C. viminalis* extracts and mechanism responsible for their effects.

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