



Comparative evaluation of neuroprotective effect of three varieties of *Allium cepa* in chronic constriction injury induced neuropathic pain

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ABSTRACT

Allium cepa has been used extensively for culinary purposes as well as in traditional system of medicine. A number of varieties of *A. cepa* are available commercially with varietal differences in phytoconstituents and biological activities. However their effect on neuropathic pain has not been explored. The present study was designed to compare the effectiveness of three varieties of *A. cepa* in neuropathic pain. Methanol extracts and flavonoid-rich fractions (FRF) of outer scales of three varieties of *A. cepa* viz. Agrifound Dark Red (ADR), Agrifound White (AW) and NHRDF-Red (L28) were prepared. These were standardized with reference to their total phenol and flavonoid content. The marker- quercetin was determined by High Performance Thin Layer Chromatography (HPTLC). The effect of the prepared extracts was studied on neuropathic pain (NP) induced by chronic constriction injury (CCI) in rats. Behavioral indices of sensory dysfunction due to NP were evaluated by studying various behavioral tests viz. pin prick test, Eddy's hot plate test and Randall-Selitto test for hyperalgesia, and acetone drop test for allodynia. Markers of oxidative stress were studied by determining Thio-barbituric Acid Reactive Species (TBARS) and reduced glutathione (GSH) levels. FRF of variety L28 produced the most significant reduction of thermal and mechanical hyperalgesia and amelioration of cold allodynia. This also attenuated the CCI-induced increase in TBARS and decrease in GSH levels. L28 was found to contain highest amount of flavonoids and quercetin as compared to other varieties. These results indicate that FRF of *A. cepa* variety L28 may be a potential candidate for the management of NP.

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1. Introduction

Neuropathic pain (NP) has been defined as pain "caused by a lesion or disease of the somatosensory nervous system" [1] Rather than being a single entity, it is a heterogeneous group of conditions that differ not only in etiology but also in location [2]. NP can be

classified as peripheral or central and can originate from nerve injury following a wide array of conditions/ events, e.g. direct trauma to nerves, inflammation/ neuritis/nerve compression, diabetes, infections (herpes zoster, human immunodeficiency virus), tumors, toxins and primary neurological diseases [3]. It is a type of chronic pain, which unlike acute pain, is not self-

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limiting and serves no protective biological responses [4] NP is characterized by symptoms like spontaneous pain allodynia (characteristically burning or shooting in nature and is stimulus independent) and hyperalgesia (stimulus dependent) [5]. Conventional therapies for the management of NP are limited by numerous side effects [6]. Hence researchers are exploring alternative remedies for neuropathic pain. Natural products like flavonoids have demonstrated antioxidant and neuroprotective effects [7,8]. These are also useful for the management of NP [9-11]. Quercetin, a ubiquitous flavonoid, is a potent free-radical scavenger [12], neuroprotective and has demonstrated ameliorative effect on alcohol-induced NP [13,14]. Plants containing quercetin would, thus, have increased potential for neuropathology prevention [15].

One of the richest sources of dietary flavonoids is *A. cepa* Linn. or onion (family Alliaceae) [16]. The major flavonoids reported from onions include quercetin, kaempferol and myricetin [17]. A number of varieties of onion viz. red, white and yellow are available commercially [18]. The amount of phenolic compounds and flavonoids differ in these varieties [18-20]. Onions are common food plant reported to have numerous health benefits both in the traditional as well as modern systems of medicine [21,22]. Onions are used in the treatment and prevention of a number of diseases including cancer [23], coronary heart disease [24], obesity, hypercholesterolemia [25,26], diabetes type [27,28], cataract [29], microbial infections [30] and disturbances of the gastrointestinal tract. Most of these activities are related to the flavonoids, thiosulfonates and sterols. The anti-oxidant activity is attributed to the flavonoids- quercetin, kaempferol, myricetin and catechin [31] and anthocyanins found in red onions [18]. Onions have neuroprotective activity [32] due to flavonoids specially quercetin [13].

It has been reported that there are varietal differences in composition and concentration of phytoconstituents as well as in their biological activities [33]. The present study was designed to compare the total phenolic content, total flavonoid content and the amount of the marker - quercetin in three commercial varieties of *A. cepa* found in India, and to study their possible ameliorative effect on chronic constriction induced neuropathic pain in rats.

2. Materials and Methods

2.1 Plant Material

The bulbs of onion, *A. cepa* L. var. Agrifound Dark Red (ADR), Agrifound white (AW), NHRDF-Red (L28), family Alliaceae were procured from a cultivated source, National Horticulture Research and Development Foundation (NHRDF), Karnal (Haryana), India. These varieties were identified and authenticated by Dr. L.R. Verma, Deputy Director NHRDF, Regional Research Station, Karnal (Haryana) India) (vide receipt no. 937 and 966) in the month of November, 2012.

Animals

Wistar rats of either sex weighing 150-250 g were

used which are obtained from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. They were maintained on standard environmental conditions and fed with standard rodent diet (Ashirwad Feed, Chandigarh) and tap water ad libitum. They were housed in departmental animal house and were exposed to natural photoperiod. The experimental protocol was duly approved by Institutional Animal ethical Committee (IAEC) and care of animal was carried out according to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environmental and Forest, Government of India (Reg. No. 107/99/CPCSE A-2012-34).

2.2 Preparation of Various Extracts of *A. cepa*

2.2.1 Preparation of Methanol Extracts of *A. cepa* Outer Scales

Methanol extract of shade dried skin (outer scales) of onion bulbs of all three varieties was prepared separately. Dried skin (100 gm) was ground with 90% methanol and kept in an ultrasonic bath for 30 min and then allowed to stand for 24 h at room temperature. After 24 h, supernatant was collected and filtered. The solvent was removed under reduced pressure using rotatory vacuum evaporator with bath temperature around 35-40°C. The weight of the extract was calculated on the dry weight basis [34].

2.2.2 Preparation of Flavonoid Rich Fraction

Flavonoid rich fraction was prepared from the methanol extracts of outer scales of all three varieties of onion bulbs. Dried methanol extract was suspended uniformly in water and placed in three-necked round bottom flask and fractionated with ethyl acetate for 30 minutes at 500 C with continuous stirring, this procedure was repeated for 5 to 7 times and then all the ethyl acetate fractions were pooled and dried under reduced pressure to give flavonoid rich fraction (FRF) [35].

2.3 Standardization of Extracts

2.3.1 Total phenol content analysis

Total phenolic compounds of extracts were determined by Folin-Ciocalteu procedure [36,37]. The amount of total polyphenols was calculated as Gallic acid equivalent from the calibration curve of standard Gallic acid (Sigma Aldrich) solution and expressed as mg of Gallic acid/g dry plant extract. All measurements were done in triplicate and the mean percent phenolic content was calculated according to the formula:

Total Phenolic Content (%w/w) = GAE x V x D x 10⁻⁶ x 100/W

GAE - Gallic Acid Equivalent (% w/w)

V - Total Volume of Sample (ml)

D - Dilution Factor

W - Sample Weight (g)

2.3.2 Total flavonoid content analysis

Flavonoid content was determined [38] The flavonoid content was determined as quercetin equivalent from the calibration curve of quercetin standard solution and expressed as mg of quercetin/g dry plant extract. All measurements were done in triplicate and mean percent flavonoid content was calculated according to the formula: Flavonoid Content (%w/w) = $QE \times V \times D \times 10^{-6} \times 100/W$

QE - Quercetin Equivalent ($\mu\text{g/ml}$)

V - Total Volume of Sample (ml)

D - Dilution Factor

W - Sample Weight (g)

2.4 TLC-Densitometric Studies

2.4.1 Standard solution of marker

A stock solution of 1 mg/ml concentration of the marker (Quercetin, Sigma Aldrich) was prepared in methanol for TLC studies.

2.4.2 Test solutions

The test solutions of *A. cepa* ADR, L-28 and AW varieties was prepared by extracting accurately weighed 2 g of coarsely powdered plant material of each sample with 50 ml of methanol (90%) using Soxhlet apparatus in triplicate. Each extract was filtered, concentrated under reduced pressure and the volume was adjusted to 50 ml with methanol (90%). Similarly, the flavonoid rich fraction of each variety was prepared after fractionating the methanol (90%) extract of each variety with ethyl acetate. The methanol (90%) extract and flavonoid rich fraction of each variety were subjected for chemical standardization using TLC densitometric analysis.

2.5 Analytical Studies

2.5.1 Preparation of standard plot

A standard solution of quercetin was prepared by dissolving accurately weighed 5 mg of quercetin in 5 ml of methanol. The stock solution was diluted with methanol to get six dilutions of different concentrations (100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng). A volume of 10 μl from each dilution was applied in triplicate on pre-coated TLC plate. The plate was developed in solvent system Toluene: Ethyl acetate: Formic Acid (8.5:5.5:1) in a chamber saturated for 10 min, to a distance of 8 cm. The developed plate was dried in a current of hot air, and then scanned in TLC scanner at 254 nm. The AUC of the peak corresponding to quercetin was noted in each track.

2.5.2 Preparation of test sample

Test samples of *A. cepa* (methanol and flavonoid-rich fractions) were prepared as described above. These test solutions (10 μl) were applied separately in

triplicate on pre-coated TLC plate (20 \times 10 cm) and the plate was developed and scanned following the same procedure used for the preparation of standard plot and chromatogram was recorded. The average AUC of the peak corresponding to quercetin was noted at 254 nm in the test sample and its concentration was calculated from the standard plot.

2.6 Peripheral Neuropathic Pain Induction By Chronic Constriction Injury

Peripheral neuropathic pain was induced by chronic constriction injury [39] with slight modification using silk 4-0 suture instead of chromic gut suture as it has been documented that chromic gut suture initiates the inflammatory reactions in sciatic nerve [2,40]. Rats were deeply anesthetized with chloral hydrate (400 mg/kg i.p.). The hairs of rat's lower back and thighs were shaved, and skin was sterilized with 0.5% chlorhexidine. The skin of its lateral surface of left/right thigh was incised and cut made directly through the biceps femoris muscle to expose the sciatic nerve. Once exposed the sciatic nerve was ligated with silk 4-0 thread at 4 sites with 1 mm gap. The ligatures were loosely tied until a short flick of the ipsilateral hind limb was observed. The muscles and skin were closed in two layers with the use of thread and topical antibiotic was applied. All surgical procedures were carried out under normal sterile conditions.

2.7 Experimental Protocol

Seven groups, each comprising of six Wistar rats, were employed in present study. The behavioral tests were employed on 1st, 7th and 14th day. Thereafter on 14th day, animals were sacrificed and biochemical estimations were done.

Group 1: Normal Control

Rats were not subjected to any treatment and surgical procedure and were kept for 14 days.

Group 2: Sham Control

Rats were exposed surgical procedure to expose left sciatic nerve on day 1 without any nerve ligation.

Group 3: CCI Control

Rats were subjected to surgical procedure to expose and ligate the left sciatic nerve on day 1 as described earlier.

Group 4: Treated (Methanol Extract of ADR outer scale) Methanol extract of outer scales was administered at a dose of 100 mg/kg p.o. once a day in chronic constriction injury induced rats for 14 days, from day 1 (30 min prior to anesthesia for surgery) to day 14.

Group 5: Treated (Methanol Extract of L-28 outer scales) Methanol extract of outer scale was administered at a dose of 100 mg/kg p.o. once a day in chronic constriction injury induced rats for 14 days, from day 1 (30 min prior to anesthesia for surgery) to day 14.

Group 6: Treated (Methanol Extract of white onion outer scales)

Methanol extract of outer scales was administered at a dose of 100mg/kg p.o. once a day in chronic constriction injury induced rats for 14 days, from day 1 (30 min prior to anesthesia for surgery) today 14.

Group 7: Treated (Flavonoid Rich Fraction of L-28 onion outer scales)

FRF of outer scales was administered at a dose of 50 mg/kg p.o. once a day in chronic constriction injury induced rats for 14 days, from day 1 (30 min prior to anesthesia for surgery) today 14.

2.8 Behavioral Examination

2.8.1 Cold-Allodynia (Acetone Drop Test)

The cold allodynia was assessed by spraying 100 μ l of acetone onto the surface of paw of rat (placed over a wire mesh), without touching the skin. The response of rat to acetone was noted for 20s [41]. Acetone was applied thrice to hind paw with a gap of 5 min between each application and the individual scores noted in 20s interval were added to obtain a single score over a cumulative period of 60s.

2.8.2 Mechanical Hyperalgesia (Pin Prick Test)

The mechanical hyperalgesia was assessed by the pin prick test [42]. The surface of injured hind paw was touched with the point of the bent gauge needle (at 900 to the syringe) at intensity sufficient to produce a reflex withdrawal response. The paw withdrawal duration was recorded in seconds and the normal quick reflex withdrawal response was given the value of 0.5s Heat-hyperalgesia (Hot Plate Test)

The thermal nociceptive threshold, as an index of thermal hyperalgesia, was assessed by Eddy's Hot Plate, maintained at a temperature of 52.5 ± 1.0 °C. The rat was placed on the hot plate and withdrawal latency, with respect to licking of hind paw was recorded in seconds. The cut-off time of 20s was maintained [43].

2.8.3 Static Mechanical Hyperalgesia Test (Randall-Selitto Test)

Mechanical (static) nociceptive threshold as an index of mechano-hyperalgesia were assessed by pressure stimulation method [44]. Briefly, nociceptive threshold, expressed in grams, as measured by applying increasing pressure to the left hind paw. Withdrawal of left hind paw or vocalization response was noted to assess the static mechanical nociceptive threshold. The cut-off pressure of 450 g was maintained.

2.8.4 Biochemical Estimations

All the animals were sacrificed by high dose of anesthesia on 14th day of surgery. Sciatic nerve and tissue beneath the sciatic nerve was isolated immediately. Freshly excised sciatic nerve homogenate (10 %w/v) was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The tubes with homogenate were kept in ice cold water for 30 min. and then centrifuged at 40 C (2000g, 10 minutes). The supernatant of homogenate was separated, and employed to estimate total protein content, TBARS and

GSH.

2.8.5 Estimation of Total Protein Content

The nerve total protein was determined by Lowry's method with slight modifications [45] using total protein modified biuret, end point assay test kit (Span diagnostics Ltd, Surat, India). Absorbance was noted spectrophotometrically (DU 640 B spectrophotometer, Beckman coulter inc., CA, USA) at 750 nm.

2.8.6 Estimation of Thio-barbituric Acid Reactive Substances (TBARS)

The quantitative measurement of TBARS, an index of lipid peroxidation in sciatic nerve was performed as described in method by Ohkawa et al., (1979) [46]. 0.2 ml of supernatant of 10% homogenate was pipetted out in a test tube, followed by addition of 0.2 ml of 8.1% sodium dodecyl Sulphate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of 0.8% of thio-barbituric acid and the volume was made up to 4 ml with distilled water. The test tube was incubated at 950 C for 1 hr, then cooled and added 1 ml of distilled water followed by addition of 5 ml of n-butanol: pyridine mixture (15:1 v/v). The tube was centrifuged at 4000g for 10 min. The absorbance of developed pink color was measured spectrophotometrically at 532 nm. A standard calibration curve was prepared by using 1–10 nM of 1, 1, 3, 3-tetramethoxy propane. The TBARS value was expressed as nM per mg of tissue.

2.8.7 Estimation of Reduced Glutathione (GSH)

The reduced glutathione (GSH), content in sciatic nerve was estimated [47]. Supernatant of homogenate (0.5 ml) was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were then centrifuged at 1000 g for 10 min. at 40C. The supernatant obtained (0.5 ml) was mixed with 2 ml of 0.3 M Disodium hydrogen phosphate. Then 0.25 ml of 0.001 M freshly prepared DTNB [5, 5'-dithiobis (2-nitrobenzoic acid) dissolved in 1 %w/v citric acid] was added and absorbance was noted spectrophotometrically at 412.

2.9 Statistical Analysis

The data of behavioral results was analyzed by two way ANOVA method followed by Tukey's post-hoc test using Sigma Stats 3.5 software and expressed as mean \pm S.E.M. The data of biochemical results was statistically analyzed by one way ANOVA and using Tukey's post-hoc. The p-value < 0.05 was considered to be statistically significant.

3. Results

The extracts and fractions prepared from the outer scales of three varieties of onion were standardized with respect to total phenolic content (Fig.1) and total flavonoid content (Fig. 2) by UV method. Quercetin content of prepared extracts was quantified by HPTLC.

The standard plot of quercetin was prepared (Fig. 3). Figure 4 shows the HPTLC chromatograms of the marker (quercetin) and the test extracts. The yields and results of standardization are reported in Table 1.

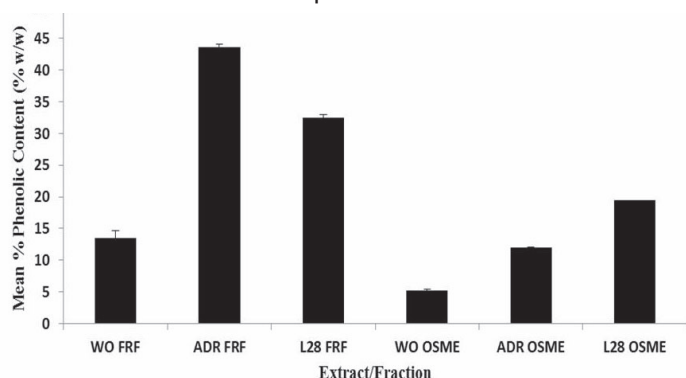


Figure 1 Total phenolic content of various extracts/fractions of *A. cepa*.

WOFRF - Agrifound white flavonoid rich fraction, **ADR FRF** - Agrifound dark red flavonoid rich fraction, **L28 FRF** - NHRDF - Red flavonoid rich fraction, **WO OSME** - Agrifound white outer scale methanol extract, **ADR OSME** - Agrifound dark red outer scale methanol extract, **L28 - OSME** - NHRDF - Red outer scale methanol extract

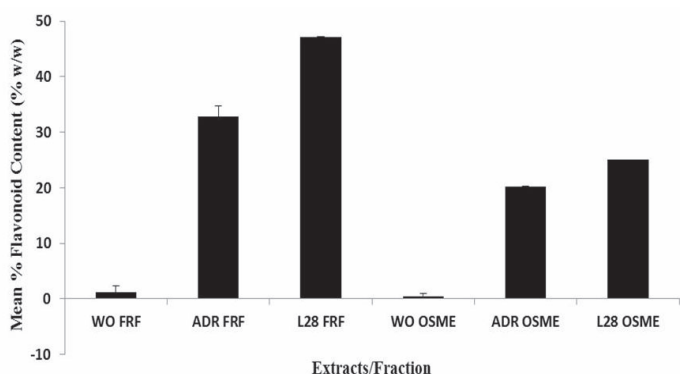


Figure 2 Total flavonoid content of various extracts/fractions of *A. cepa*.

WOFRF-Agrifound white flavonoid rich fraction, **ADR FRF** - Agrifound dark red flavonoid rich fraction, **L28 FRF** - NHRDF - Red flavonoid rich fraction, **WO OSME** - Agrifound white outer scale methanol extract, **ADR OSME** - Agrifound dark red outer scale methanol extract, **L28 - OSME** - NHRDF - Red outer scale methanol extract

3.1 Effect of various extracts of outer scales of *A. cepa* on chronic constriction induced cold allodynia

Chronic constriction injury (CCI) resulted in significant development of cold allodynia, demonstrated by increase in hind paw lifting duration, after the day of surgery as compared to sham control ($P < 0.05$). The allodynia was more pronounced on 7th and 14th day after surgery. Out of three varieties methanol extracts of ADR and

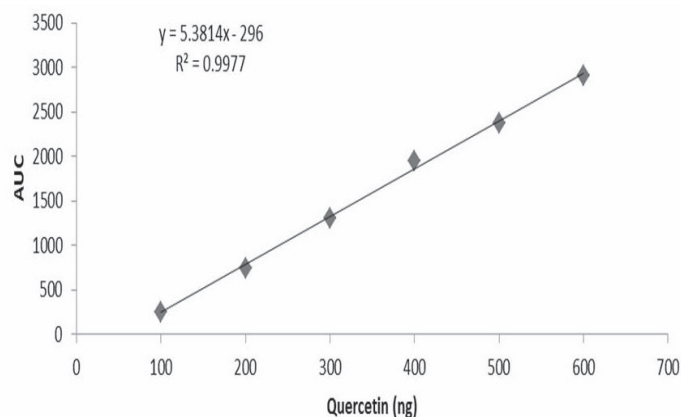


Figure 3 Standard plot of quercetin in TLC densitometric analysis.

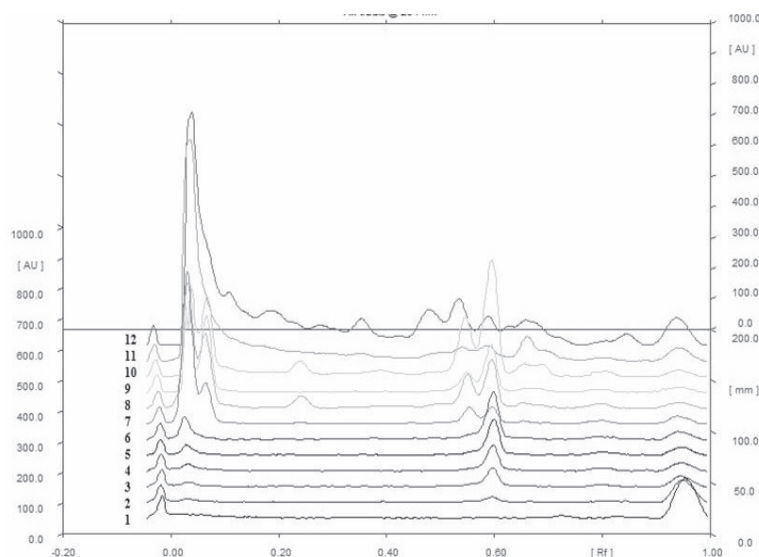


Figure 4 TLC densitometric chromatogram of marker with test solutions of all three *A. cepa* varieties.

Peak 1 to 6 is of different concentrations of standard (100 to 600 ng); 7 is of ADR OSME; 8 is of ADR FRF; 9 is of L28 OSME; 10 is of L28 FRF; 11 is of WO OSME; 12 is of WO FRF

L28 of *A. cepa* significantly attenuated CCI-induced cold allodynia when compared to CCI-control, on different day intervals. Maximum attenuation was seen with FRF of L28 (Fig. 5).

3.2 Effect of various extracts of outer scales of *A. cepa* on chronic constriction induced mechanical hyperalgesia

Chronic constriction injury (CCI) resulted in significant development of mechanical hyperalgesia, demonstrated by increase in hind paw lifting duration, after the day of surgery as compared to sham control ($P < 0.05$). The hyperalgesia was more pronounced on 7th and 14th day after surgery. Out of three varieties methanol extracts of ADR and L28 and flavonoid rich fraction of L28 of *A. cepa* significantly attenuated CCI-induced cold allodynia when compared to CCI-control, on different day intervals. Most significant attenuation was seen with FRF of L28 (Fig. 6).

Table 1 Standardization of Extracts/Fractions by UV Method and HPTLC.

Sample No.	Variety of <i>Allium cepa</i>	Extract/ Fraction	Yields (% dry weight)	UV Method		HPTLC Method	
				TPC % w/w (Mean ± SD)	TFC % w/w (Mean ± SD)	Quercetin in plant % w/w (Mean ± SD)	Quercetin in extracts % w/w (Mean ± SD)
1	Agrifound Dark Red (ADR)	OSME	15.10	11.94 ± 0.2	20.22 ± 1.07	0.0252 ± 0.0062	1.52 ± 0.020
		FRF	45.45	43.69 ± 1.13	32.83 ± 0.48	0.0633 ± 0.0025	8.25 ± 0.040
2	NHRDF-Red (L-28)	OSME	13.6	19.46 ± 0.08	23.14 ± 1.83	0.1676 ± 0.0045	9.21 ± 0.030
		FRF	46.82	32.53 ± 0.44	47.19 ± 0.01	0.2065 ± 0.0052	25.30 ± 0.080
3	Agrifound White (AW)	OSME	5.44	5.27 ± 0.43	0.48 ± 0.01	0.0006 ± 0.0012	0.01 ± 0.001
		FRF	3.54	13.46 ± 0.80	1.2 ± 0.7	0.0100 ± 0.0034	0.33 ± 0.04

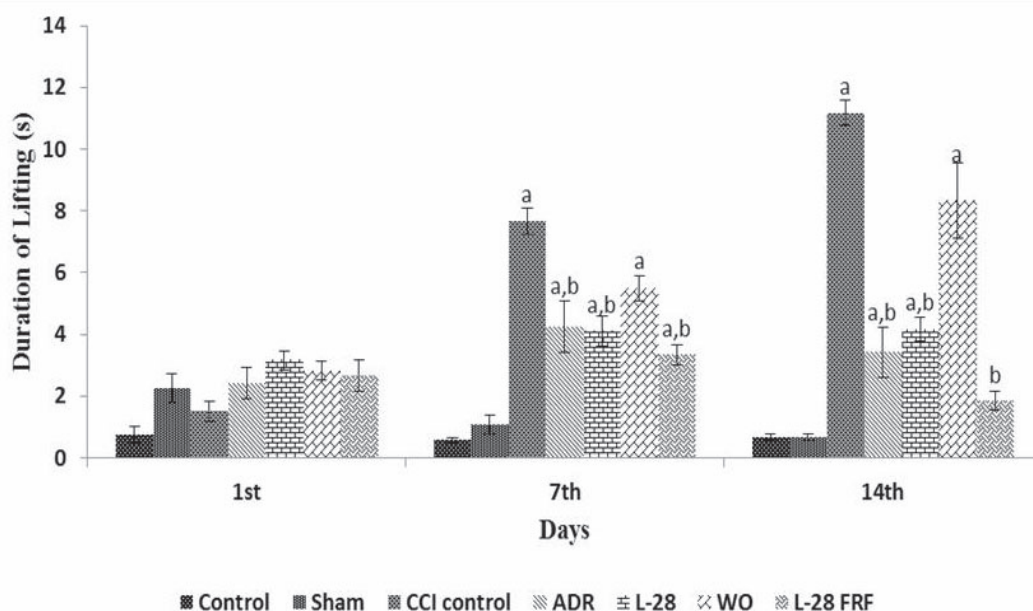


Figure 5 Effect of various extracts of outer scales of *A. cepa* on chronic constriction induced cold allodynia assessed by acetone drop test.

ADR - Agrifound dark red outer scale methanol extract, **L28 NHRDF-Red** outer scale methanol extract,

WO - Agrifound white outer scale methanol extract,

L28 - FRF - NHRDF-Red flavonoid rich fraction, . a p < 0.05 as compare to Sham group, b p < 0.05 as compared to CCI Control.

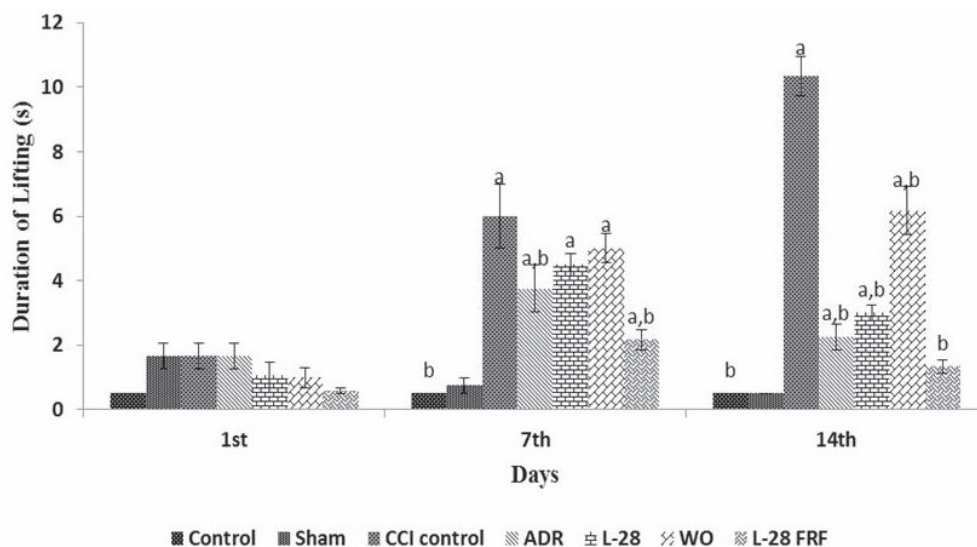


Figure 6 Effect of various extracts of outer scales of *A. cepa* on chronic constriction induced mechanical hyperalgesia assessed by pin prick test.

ADR - Agrifound dark red outer scale methanol extract, **L28 NHRDF-Red** outer scale methanol extract, **WO** - Agrifound white outer scale methanol extract,

L28 - FRF - NHRDF-Red flavonoid rich fraction, . a $p < 0.05$ as compare to Sham group, b $p < 0.05$ as compared to CCI Control.

3.3 Effect of various extracts of outer scales of *A. cepa* on chronic constriction induced heat hyperalgesia

Chronic constriction injury (CCI) resulted in significant development of heat hyperalgesia, demonstrated by increase in hind paw withdrawal latency, after the day of surgery as compared to sham control ($P < 0.05$). The hyperalgesia was more pronounced on 7th and 14th day after surgery. Out of three varieties methanol extracts of ADR and L28 and flavonoid rich fraction of L28 of *A. cepa* significantly attenuated CCI-induced cold allodynia when compared to CCI-control, on different day intervals. Maximum attenuation was seen with FRF of L28 (Fig. 7).

3.4 Effect of various extracts of outer scales of *A. cepa* on chronic constriction induced static mechanical hyperalgesia

Chronic constriction injury (CCI) resulted in significant development of static mechanical hyperalgesia, demonstrated by decrease in hind paw withdrawal threshold, after the day of surgery as compared to sham control ($P < 0.05$). The hyperalgesia was more pronounced on 7th and 14th day after surgery. Out of three varieties methanol extracts of ADR and L28 and flavonoid rich fraction of L28 of *A. cepa* significantly attenuated CCI-induced cold allodynia when compared to CCI-control, on different day intervals. Maximum attenuation was seen with FRF of L28 (Fig. 8).

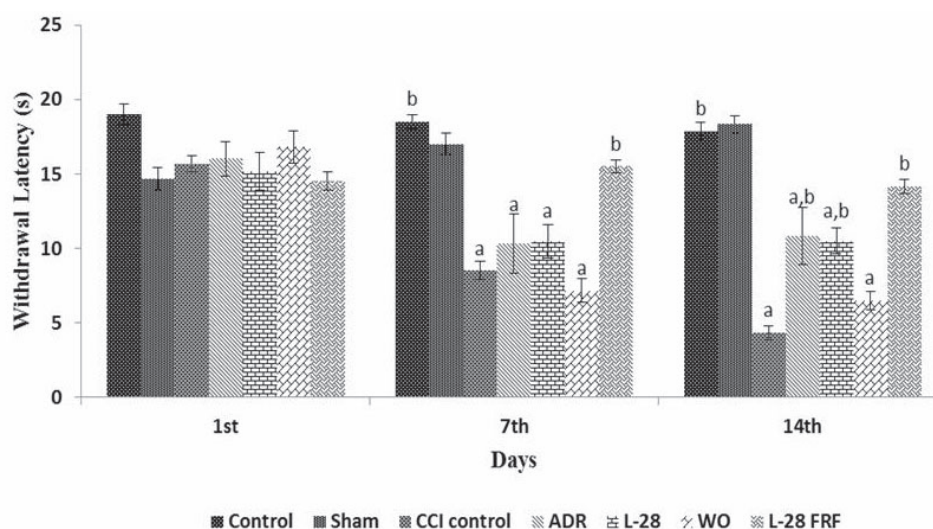


Figure 7 Effect of various extracts of outer scales of *A. cepa* on chronic constriction induced hyperalgesia.

ADR - Agrifound dark red outer scale methanol extract, **L28 - NHRDF-Red** outer scale methanol extract, **WO** - Agrifound white outer scale methanol extract, **L28 FRF - NHRDF-Red** flavonoid rich fraction, a $p < 0.05$ as compare to Sham group, b $p < 0.05$ as compared to CCI Control.

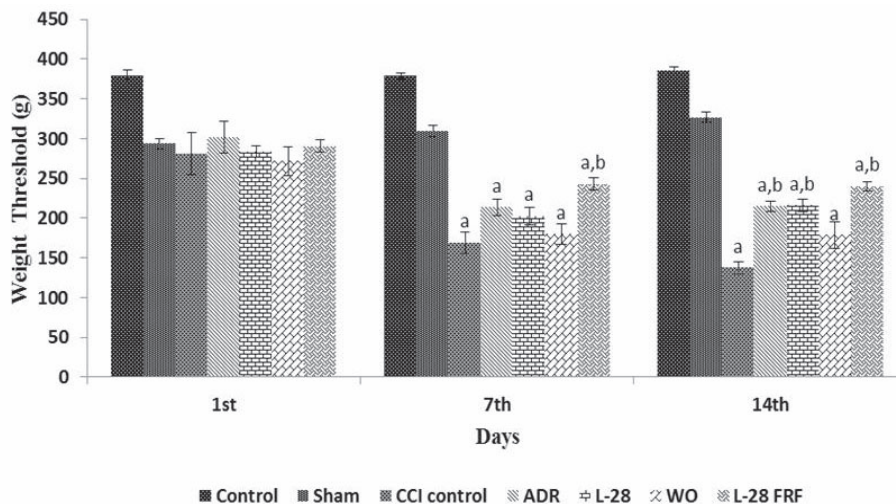


Figure 8 Effect of various extracts of outer scales of *A. cepa* on chronic constriction induced static mechanical hyperalgesia.

ADR - Agrifound dark red outer scale methanol extract, **L28** - **NHRDF-Red** outer scale methanol extract,

WO - Agrifound white outer scale methanol extract,

L28 FRF - **NHRDF-Red** flavonoid rich fraction, . a p < 0.05 as compare to Sham group, b p < 0.05 as compared to CCI Control.

3.5 Effect of various extracts of outer scales of *A. cepa* on thiobarbituric acid reactive species and reduced glutathione (GSH)

Chronic constriction injury (CCI) resulted in significant rise in TBARS and decrease in the levels of reduced glutathione, noted after 14th day of surgery as compared to sham (P < 0.05). However, total protein levels were not

affected significantly as a result of CCI. Administration of the methanol extracts of ADR and L28 and FRF of L28 of *A. cepa* attenuated CCI-induced rise in sciatic nerve malondialdehyde (Fig. 9) and decrease in reduced glutathione levels (Fig. 10). FRF of L28 variety of *A. cepa* resulted in normalization of CCI-induced biochemical abnormalities in a significant manner.

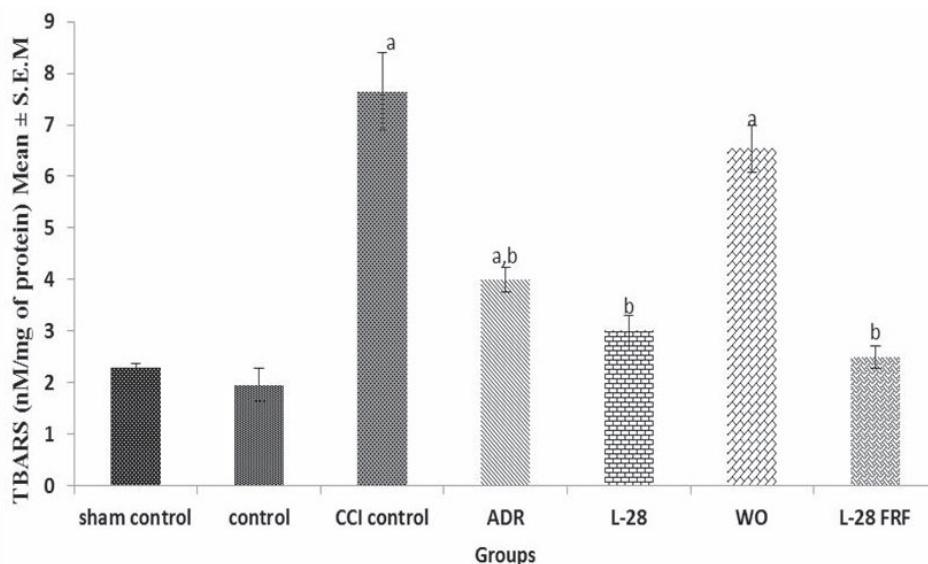


Figure 9 Effect of various extracts of outer scales of *A. cepa* on TBARS level.

ADR OSME - Agrifound dark red outer scale methanol extract, **L28 OSME** - **NHRDF-Red** outer scale methanol extract, **WO OSME** - Agrifound white outer scale methanol extract,

L28 FRF - **NHRDF-Red** flavonoid rich fraction, . a p < 0.05 as compare to Sham group, b p < 0.05 as compared to CCI Control.

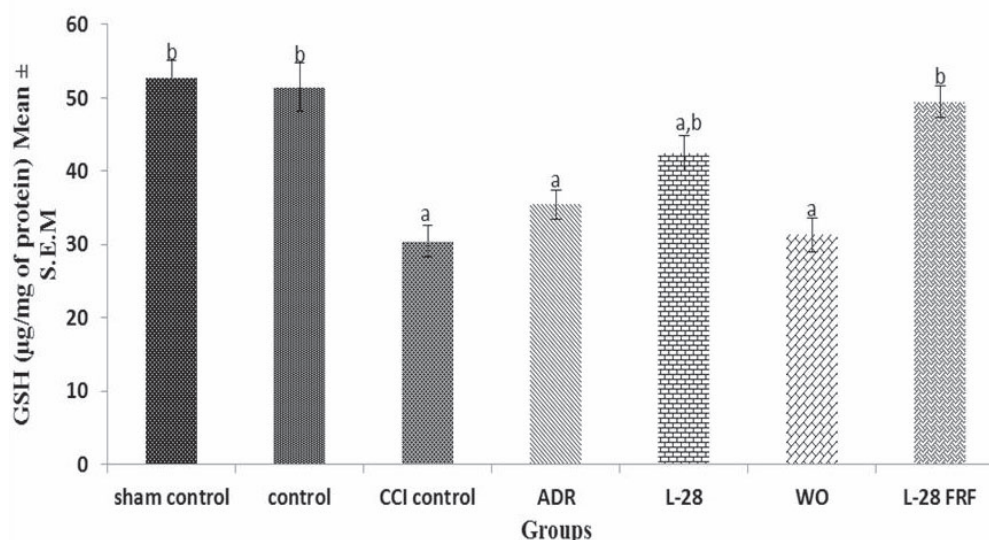


Figure 10 Effect of various extracts of outer scales of *A. cepa* on reduced glutathione (GSH) level. **ADR OSME** - Agrifound dark red outer scale methanol extract, **L28 OSME** - **NHRDF-Red** outer scale methanol extract, **WO OSME** - Agrifound white outer scale methanol extract, **L28 FRF** - **NHRDF-Red** flavonoid rich fraction., a $p < 0.05$ as compare to Sham group, b $p < 0.05$ as compared to CCI Control.

4. Discussion

A. cepa is a common but medicinally valuable plant. A number of varieties and hybrids are available commercially [19]. In the present study two red (ADR and NHRDF-Red (L28)) and one white (WO) varieties of onion developed in India were selected for phytochemical and biological studies. These were procured from a cultivated source that insured minimum variation.

The various medicinal properties of onions are attributed to the different constituents like organosulfur compounds, phenols and flavonoids present in them [48].

The present study investigated the phenolic and flavonoid content of the selected varieties. It is reported that the phenol and flavonoid content tend to vary in different coloured varieties of onion viz. red, white and yellow [49,50].

It has been reported that outer layers of onions have better nutritional value [51]; these also contain higher amount of flavonoids and quercetin [52,53]. Hence methanol extract and FRF were prepared from the outer scales of the selected varieties. The extracts and fractions were standardized in terms of total phenolic content (TPC) and total flavonoid content (TFC). The highest phenolic content was recorded in methanol extract of L28 and FRF of ADR. The highest flavonoid content was found in both methanol extract and FRF of L28. Earlier reports also show that red variety of onions contains higher amounts of phenolics [54], flavonoids [17] and quercetin [52].

Of the major flavonoids reported in the onion quercetin is the most abundant [55]. Hence this was used as the marker compound in the TLC densitometric studies. TLC densitometry is a simple, rapid and sensitive technique. This technique has been used for the comparison of quercetin content in the bioactive extracts/fraction of the

three varieties of onion studied. The result shows the highest percentage yield in L28 variety, that is 9.21 % w/w of dry methanol extract and 0.1676 % w/w of dry plant material. The percentage yield of quercetin in FRF of L28 methanol extract was found to be 25.30 % w/w of dry FRF. This content of quercetin is higher than what is theoretically expected from FRF of methanol extract. This may be due to 3-4 h continuous heating involved in the process of preparation of FRF may lead to break down of quercetin 3,4-O-diglucoside and quercetin-4-O-monoglucoside and quercetin 3-monoglucoside to aglycone form i.e. quercetin [56]. This method may be used for standardization of the extracts/ fractions in future.

Onions are reported to be neuroprotective in an experimental stroke model [32], thus it was thought to be worthwhile to study and compare neuroprotective effect of three selected varieties for the management of chronic constriction injury induced neuropathic pain. In this study peripheral neuropathic pain was induced by ligation of the sciatic nerve of left hind paw of rat with silk 4-0 sutures due to which there is intense pain in rat paw which increases and at peak on 14th day [57]. CCI resembles human neuropathy resulting from trauma of peripheral nerves, with some functional preservation of the innervation (nerve entrapment or compression). The model of CCI is among the most commonly used models because its reliability and reproducibility. The procedure results in intraneural edema, which strangulates the nerve, effectively axotomizing many but not all of the nerve axons this may be because the levels of mitochondrial reactive oxygen species increase in rat neuropathic dorsal horn neurons, superoxides produced by mitochondria may be one of important free radicals for neuropathic pain [58,59]. It is reported that CCI induces hyperalgesia and allodynia to mechanical and thermal stimulation on the lesion side [60].

Hence, the behavioural experiments carried out in this study measured the degree of hyperalgesia and allodynia i.e. pin-prick test for mechanical hyperalgesia, acetone drop test for cold allodynia, Eddy's hot plate test for thermal hyperalgesia and Randall-Selitto test static mechanical hyperalgesia. These tests confirmed the induction of peripheral neuropathic pain CCI control group that shows significant increase in pain with time from day 1st to 14th with respect to sham group. No significant difference in sham and normal control was observed. The methanol extract of L28 showed most significant improvement in CCI induced NP.

The biochemical estimations show that CCI causes oxidative stress and leads to increased lipid peroxidation which further leads to generation of reactive oxygen species. This point is well supported by the literature according to which ROS plays an important role in CCI neuropathic pain model and ROS scavengers temporarily reversed mechanical hyperalgesia [61,62].

The neuroprotective activities of onion have been attributed to flavonoids like quercetin [32]. A report states that quercetin attenuates diabetic neuropathic pain by opioidergic mechanism or due protein kinase C (PKC) inhibition. Another study reports PPAR- γ receptor agonistic activity of quercetin [63,64]; this receptor is present in dorsal root ganglion and contributes to neuropathic pain.65. This study shows that the fraction containing highest amount of total flavonoids and quercetin also shows the most significant antioxidant effect and improvement in neuropathic pain.

5. Conclusion

The results of the present investigation show that FRF of *A. cepa* variety L28 attenuated CCI-induced neuropathic pain in rats. The high total flavonoid and quercetin content may be responsible for the bioactivity. This fraction may be a potential candidate for the management of NP.

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