Studies on acute toxicity, pharmacological and biological activities of *Aconitum napellus* Linn.

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Abstract

The *crude* extract of *Aconitum napellus* Linn. (Ranunculaceae) was obtained to evaluate their acute toxicity, pharmacological and biological effects. The pharmacological activities (acute toxicity study, neuropharmacological, analgesic and anti-inflammatory activities) were performed on mice at different doses whereas biological study was carried by immunomodulatory, antimicrobial, antimicrobial, phytotoxcity and antioxidant assays. The results of acute toxicity study revealed nontoxic behavior at 1-10 mg/kg dose while at 300mg/kg toxic symptoms appeared. *A. napellus* decreased the motor activity and exploratory behaviour and increased traction time at the dose of 10 mg/kg. The results of analgesic and anti-inflammatory activities were significantly reduced the number of writhes and edema at $p<0.05$ at 10 mg/kg which. At 15.60 mg/ml, the results of immunomodulatory assay showed maximum phagocytic index. Among antibacterial results the best activity was achieved against *Acineto bacterbaumanii* (21 mm zone of inhibition). FTIR, HPLC analysis and antioxidant studies were also carried out. The highest anti-oxidant values achieved with *A. napellus* were 98%, 92% and 78% at the concentrations of 0.5mg/ml, 1mg/ml and 5mg/ml respectively. FTIR and HPLC were also performed to standardize the extract. These findings may help to establish the useful medicinal and therapeutic application of *A. napellus*.

Keywords: *A. napellus*, toxicity study, pharmacological activity, Antioxidant, biological assays

Introduction

The use of some medicinal plants attributed to deaths and poisonings for example comfrey and chaparral. Most of the literature of Poisonous plant provides insufficient Knowledge about the safe use of herbal medicines (Kingsbury, 1979). Therefore, toxicological studies of medicines can be help ful for its utilization in therapy. It gives an added therapeutic value where other medicines failed. As we know that all medicines including herbal medicines can be harmful they are taking improperly. In this study we performed the experiments of toxicology and Pharmacology to determine the safety of compound or medicine as well as its therapeutic margin. This study also gives some helpful informations about the utilization of drug.

The literature survey of *Aconitum napellus* indicates the negligence of this herb for its medicinal and pharmacological action, therefore, different pharmacological experiments were carried out to explore its hidden medicinal value beside its poisonous category. Upon chemical investigation it reveals that *A. napellus* contains alkaloids such as jesaconitine, mesaconitine, aconitine, hypaconitine and diterpenoid alkaloids. Further it also consist of napelline, aconine and picraconitine, succinic acid, resins starch (Roberts and Michael, 1998).

Material and Methods

The material was purchased from local market of Karachi, Pakistan. The material was placed in a petri dish (weight of the empty petri dish was measured first) and the left the material to evaporate at room temperature. Then once again petri dish along with dry material was measured and the weight of plant material was calculated. This material was (5.5 g) used for further experiment.
Albino mice of either sex were used to evaluate the pharmacological and toxicological studies. These animals were obtained from the animal house of Dow University of Health Sciences, Karachi, Pakistan. The weights of mice were in between 25-30 gram and they were kept in home cages following (five animals in each group) the experimental conditions with access to food and water (Ahmad et al., 2012).

Determination of LD_{50} (Lethal Dose) and ED_{50} (Effective Dose)

For toxiclogical analysis slight modified method of Miller and Tainter (1944) and Litchfield & Wilcoxon (1949) was used. Albino mice of both sexes were divided into groups of six mice each and were given different doses according to body weight (1000, 500, 300, 200, 100, 10, mg/kg body weight). The signs of toxicity and death in mice were observed for a period of three days (Lorke, 1983). 0.5 ml saline solution is given to the control group.

Pharmacological effects

Pharmacological activities were performed on extract of *A. napellus* at 300, 100 and 10 mg/kg doses. These activities included gross behaviour, open field, head dip, cage cross,

light and dark test, forced swimming test ((Debprasad 2003, Florence 2000, Irwan 1968 and 1964, Kasture 2002, Kennett 1985, Sakina 1990, Sanchez-Mateo 2002, Ahmad et al., 2012).
The anti-inflammatory and analgesic activities were carried out by using the modified method of Ahmad *et al*., 2011 a and b and Hunskaar and Hole 1987. The doses of 300 and 100 and 10 mg/kg were tested. Edema was produced by 20 ul of 2% of formalin in the right hind paw of mice and the diameter of paw is measured by vernier caliper at 1, 2 and 3 hours. The activity was compared with 300 mg/kg aspirin orally. These analgesic test were performed according to the modified method of Koster *et al.*, 1959 and Turner 1965.

Antioxidant activity by DPPH radical scavenging activity and Total antioxidant activity by Phosphomolybdate assay

The DPPH radical scavenging activity and total antioxidant activity were carried out according to the methods described by Faten 2012 and Komal 2011. The absorbance were measured at 517 nm and 695 nm respectively for antioxidant and total antioxidant activity. The percentage was calculted at different concentration of drugs (0.5, 1, 5, 10 and 15 mg/2ml).

Immunomodulatory, Antimicrobial and Phytotoxicity Assay

Immunomodulatory assay was carried out by modified method of Ramesh and Padmavathi, 2010 and Bhandari et al., 2017. crude extract of *A. napellus* different dilutions were used to observe process of phagocytosed *Candida albicans* cell to calculate the phagocyte index (PI). (Ramesh and Padmavathi, 2010 and Bhandari et al., 2017). Percentage of immunostimulation (%) was calculated as per blow formula;

$$\text{Stimulation (\%)} = PI_{(test)} - PI_{(control)} \times 100 / PI_{(control)}$$

The antibacterial and antifungal activity of *A. napellus* against different bacteria and fungi were performed by using agar-well method. Gentamicin antibiotic was used as a control (Vaghasiya *et al*, 2009). Stock solution of 10mg/ml of standard and sample extract was prepared and each well contains 10µl of this solution. Minimum inhibitory Concentration (MIC) of *A. napellus* was found out by Micro broth dilution method using 96-well microtitre plate (Sherwani *et al*., 201). Phytotoxicity assay was performed by method prescribed method of Ximenez *etal*., 2019.

HPLC and FTIR analysis

Basic chromatographic analysis was carried out on extract of *A. napellus* by the method described by Ahmad et al., 2011 and Muhammad 2006).

Statistics

The method described by (Alcaraz and Jimenez, 1989) was used for statistical analyses

(Mean ± S.E.M; N = 5; Significance with respect to control (* = Significant results, ** = highly significant results).

Results

For the evaluation of toxicity study 03 mice/group were treated with different doses (1000, 500, 300, 100 and 10 mg/k) of *A. napellus* orally. The animals of control group recieced 0.5 ml normal saline orally. All the animals were observed individually for a period of 24 hours. The results of these observation were recorded (Table 1). ED_{50} and LD_{50} value is also calculated (Table 2). For this purpose Albino mice of both sexes were divided into nine groups of six mice each and were given. After 24 hours number of dead animals were noted. The results showed non toxic and non lethal effect below the dose of 10m/kg (Table 1 and 2).

Table 1: Toxicity study of *Aconitum napellus*

Dose (mg/kg)	Onset of convulsion (min) Average	Death time (hr)
1000	3min	40 -80 min (03)
500	5 min	4 hours (3 die)
300	6 min moderate	12 hours recovered
100	15 moderate	recover
10	-	alive
0.5 ml normal saline	No	alive

Table 2: Percentage mortality in mice given the oral extract of *Aconitum napellus* at different doses

Group	Dose (mg/kg)	Dose difference (X)	No of dead	Mean mortality (Y)	X x Y
4	10	-	-	0	0
5	100	90	-	0	0
6	300	200	-	0	0
7	500	200	04	2	600
8	1000	500	06	5	3000

LD_{50}= 300, ED_{50} = 1-5mg/kg

The results of gross behavior study were compared with two reference drugs Histamine 10 mg/kg and 20mg/kg and Diazepam 2mg/kg. *A. napellus* at high dose showed similar effects like histamine which included shivering, fits, rashes, itching followed by loss of consciousness and highly relaxed muscles. At low dose (10mg/kg) *A. napellus* produces decrease in motor activity.

Table 3: Effect of *Aconitum napellus* on Traction test

Treatment	**Dose mg/kg orally**	**Traction Test (Mean no. of observations ± S.E.M)**
Control	0.5 ml saline	0.35 ±0.04
Crude extract *A. napellus*	10 mg/kg	2.8±0.58
	100	1.57 fall± 0.03*
	300mg/kg	1.06 fall ± 0.29**
Diazepam	2 mg/kg	1.63 fall ± 1.06

The behavior of animals (mice) was observed by method described by Irwan 1968. The drugs give positive signs of interaction at 10 mg/kg. Figure 1 exhibits the mean number of squares crossed by the mice were 100 ± 1.85 for control group, 65.8±1.28 for 10mg/kg, 46± 1.65 for 100mg/kg and 31 ± 2.01 for 300mg/kg which showed that *A. napellus* is CNS depressant drug. Figure 2 Showed that the *A. napellus* produced significant results with standard drug diazepam (Control group: 28 ± 0.04, 10 mg/kg: 13±0.03, 100 mg/kg: 06±0.51, 300mg/kg of *A. napellus*: 04 ± 1.34, Diazepam 2mg/kg: 05 ± 0.24). The results of cage cross activity was observed for the control group, test samples (crude extract of *A. napellus* at three dose i.e.10 mg/kg,100mg/kg and 300mg/kg) groups and for the standard drug i.e. Diazepam at 2mg/kg (Figure 3). The results showed that the *A. napellus* is sedative and depressant at the dose of 10mg/kg and 100mg/kg and 300 mg/kg but it showed some unwanted effect at 300 mg/kg as appear in gross behavior study. The results of control group, 10, 100, 300 mg/kg doses of *A. napellus* and Diazepam 2 mg/kg respectively were 25 ± 0.05, 14±0.61, 08± 0.85, 04 ± 1.13 and 06 ± 1.07. Figure 4 shows the results of light and dark activity. In this activity the time spent in light or dark side of box was recorded. The drug *A. napellus* showed depressant effect because the mice spent more time in the dark side of the box. The results of control (5.1 ± 0.07), 10 mg/kg (6.89±0.03), 100 mg/kg (7.47±0.20) and 300 mg/kg doses of *A. napellus* were highly significant and comparable with Diazepam 2 mg/kg (9.46 ± 0.06).

Figure 5 shows that the mean activity time in water bath of animals with *A. napellus* (control group: 4.28 ± 0.06, 10mg/kg: 2.89±0.58, 100mg/kg: 2.53± 0.71, 300 mg/kg: 1.45

± 1.51, Diazepam 2mg/kg 1.8 ± 1.12 minutes of mobility time). This indicates that *A. napellus* is depressant at lowest dose while stimulant at the high doses.

Table 3 shows that the time took to travel iron rod was significantly increased at the dose of 10, 100 and 300 mg/kg. For control group it took 0.35 ±0.04 min., where as 10, 100 and 300 mg/kg took 2.8±0.58, 1.57 (fall) ± 0.03, 1.06 (fall) ± 0.29 min. respectively. Diazepam 2 mg/kg used as a reference drug, takes 1.63 (fall) ± 1.06 min.

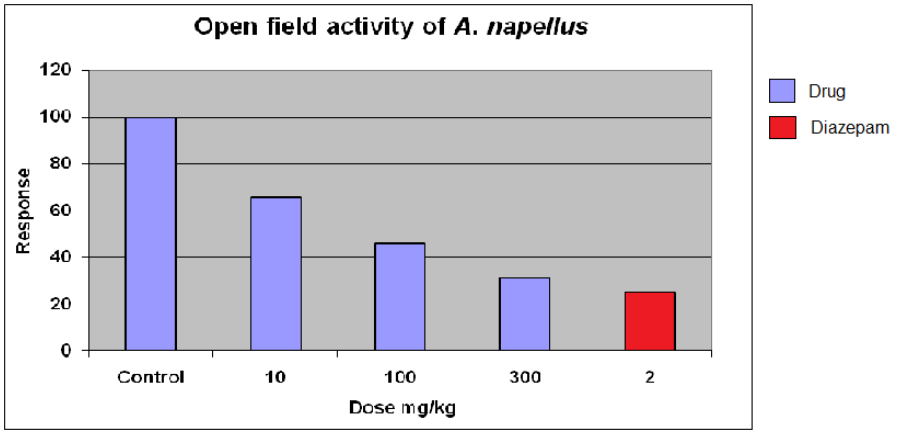


Figure 1: Open field activity of *A. napellus*

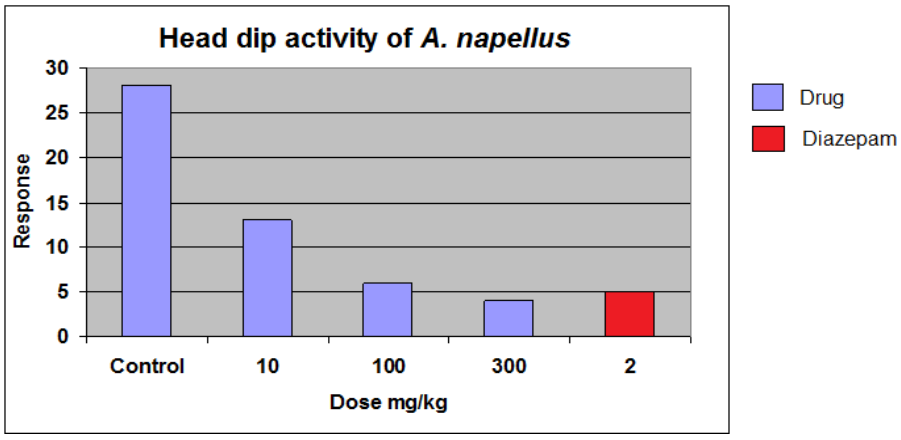


Figure 2: Head dip activity of *A. napellus*

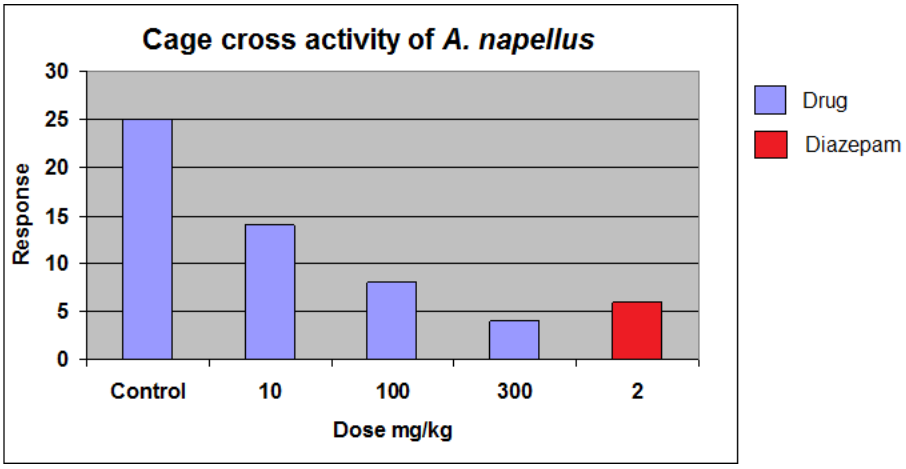


Figure 3: Cage cross activity of *A. napellus*

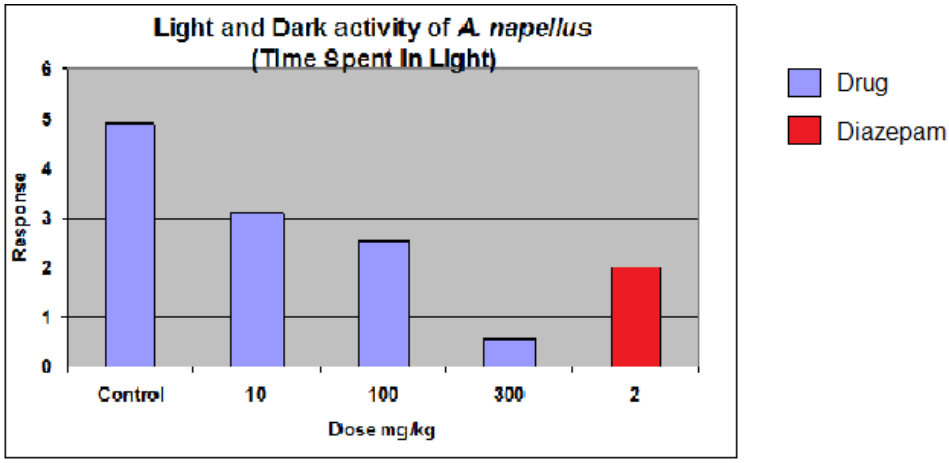


Figure 4: Light and Dark activity of *A. napellus*

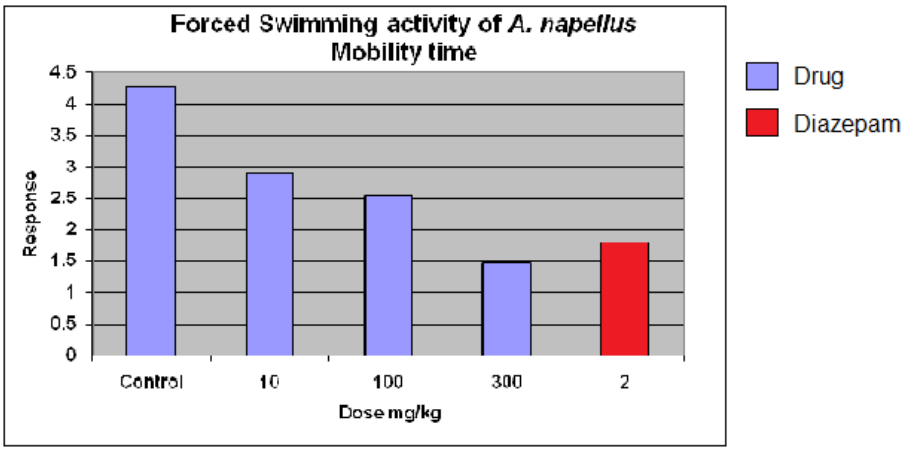


Figure 5: Forced Swimming activity of *A. napellus*

The extract of *A. napellus* at 10, 100 and 300 mg/kg reduced the intensity of edema in the 1st, 2nd and 3rd hr of anti-inflammatory activity. Maximum response is observed at the dose of 10 and 100 mg/kg respectively at 3rd hr with 18.5% and 19.11%, $p<0.05$ inhibition of edema. Aspirin showed 14.11% inhibition of edema at 3rd hour which is less than *A. napellus* (Table 4).

During analgesic activities control group produced licking and biting response for 1st and 2nd phase respectively is 2.58± 0.31and 2.35± 4.67 min; $p<0.05$ (Table 5). The crude extract of *A. napellus* showed highest % of inhibition in second phase that is 82.1%, 96.59%, 89.78% respectively for 10, 100 and 300 mg/kg doses. Aspirin showed 45.95% of inhibition in the second phase. These results indicates that *A. napellus* has more significant analgesic effect at non toxic dose (10mg/kg). In case of writhing test, the control group produced 68± 5.67 and 39 ± 4.67 number of writhes in 1st and 2nd phase respectively after the administration of 0.6% acetic acid according to body weight of mice. The percentage of inhibition of 1st and 2nd phase respectively at the dose of 10, 100 and 300 mg/kg were 47.05 and 38.46; 51.47 and 51.28; 58.82 and 64.10; 39.70 and 48.72. The results were highly significant in comparison with Aspirin treated group at $p<0.05$ (Table 6). HPLC and FTIR finger prints are presented in figure 6 and 7 where as figure 8 and 9 shows the results of antioxidant and total antioxidant activities. The results of immunomodulatory activity, antibacterial, antifungal and phytotoxic effects were presented in table7-12 respectively.

Treatment	**Dose mg/kg orally**	**Paw diameter in mm ±S.E.M**			**Inhibition (%)**		
		1hr	2 hour	2 hr	1hr	2hr	3hr
Formalin	20µl	3.85± 0.67	3.65± 0.25	3.4± 0.37	-	-	-
A. napellus Crude extract	10mg/kg	3.43±1.23	3.03±1.14	2.77± 1.12	10.90	16.98	18.5
	100mg/kg	3.03±1.42	3.01±1.24	2.75± 0.14	21.29	17.53	19.11
	300mg/kg	3.25± 1.94	3.15± 0.23	3.05± 0.22	15.58	13.69	10.29
Aspirin	300mg/kg	3.11± 1.35	3.12± 0.45	2.92± 0.14	19.22	14.52	14.11

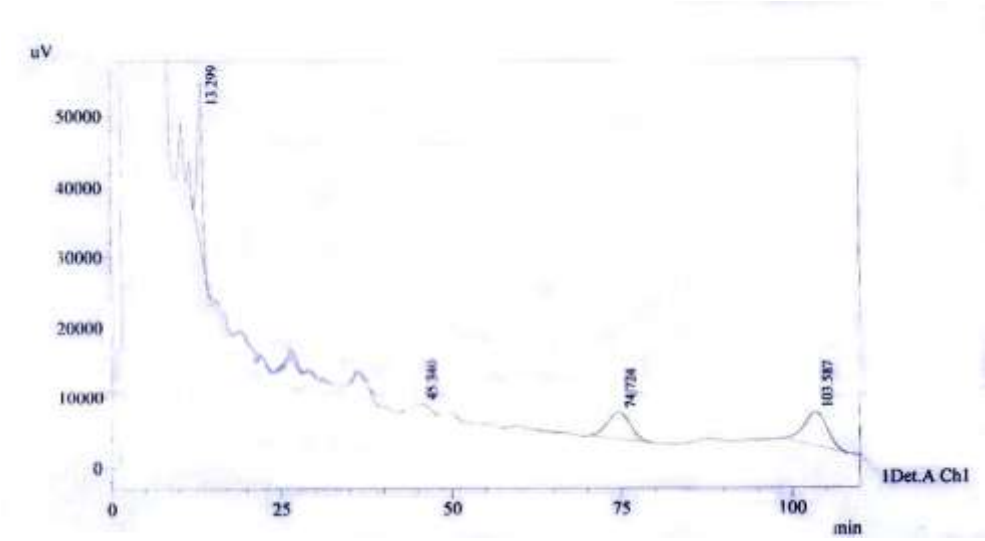
Table 4: Ant-inflammatory effect of *A. napellus* by formalin

Treatment	Dose mg/kg orally	Mean No. of Licking and biting ±S.E.M Time in minutes		Inhibition (%)	
		1st phase	2nd phase	1st phase	2nd phase
Control	0.5 ml Saline	2.58± 0.31	2.35± 4.67	-	-
Crude extract *A. napellus*	10 mg/kg	1.49±0.04	0.42±1.10	42.24	82.1
	100mg/kg	1.45± 0.06	0.08 ± 0.05	43.79	96.59
	300mg/kg	1.25±0.07	0.24±0.91	51.55	89.78
Aspirin	300mg/kg	1.29± 0.10	1.27± 0.03	50	45.95

Table 5: Analgesic effect of *A. napellus* by formalin induced licking and Biting

Treatment	Dose mg/kg orally	Mean No. of Writhes ±S.E.M		Inhibition (%)	
		1st phase	2nd phase	1st phase	2nd phase
Control	0.5 ml Saline	68± 5.67	39 ± 4.67	-	-
Crude extract *A. napellus*	10mg/kg	36±2.10	24±1.33	47.05	38.46
	100mg/kg	33±2.31	19±1.72	51.47	51.28
	300mg/kg	28±2.11	14±1.32	58.82	64.10
Aspirin	300mg/kg	41.5± 0.15	20± 0.45	39.70	48.72

Table 6: Analgesic effect of *Aconitum napellus* by acetic acid Induced writhing test



Peak	Ret. Time	Area	Height	Area %	Height %
1	13.299	1023823	22891	31.253	69.665
2	45.34	227792	1354	6.953	4.122
3	74.724	872211	3792	26.625	11.54
4	103.587	1152128	4821	35.169	14.673
total		3275954	32858	100	100

Figure 7: Represent the HPLC analysis of A. napellus (Injection Volume 80 ul, Detection Wavelength 275 nm)

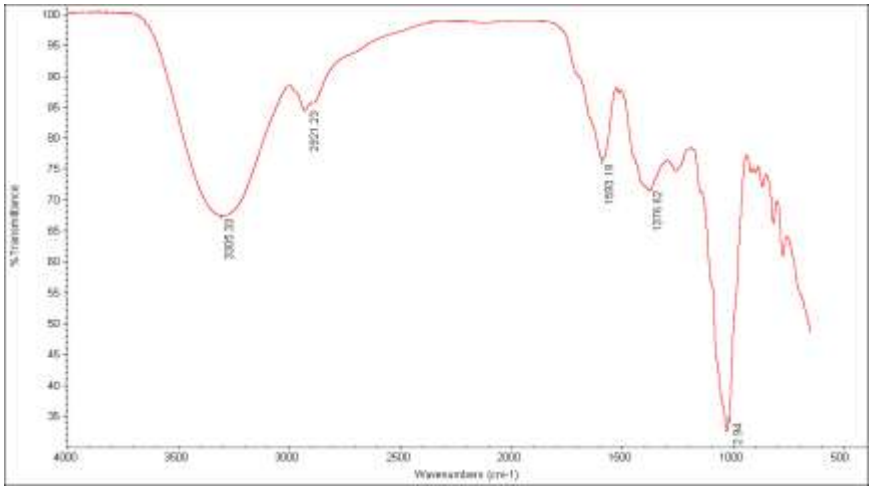


Figure 8: Represent the FTIR analysis of *A. napellus*

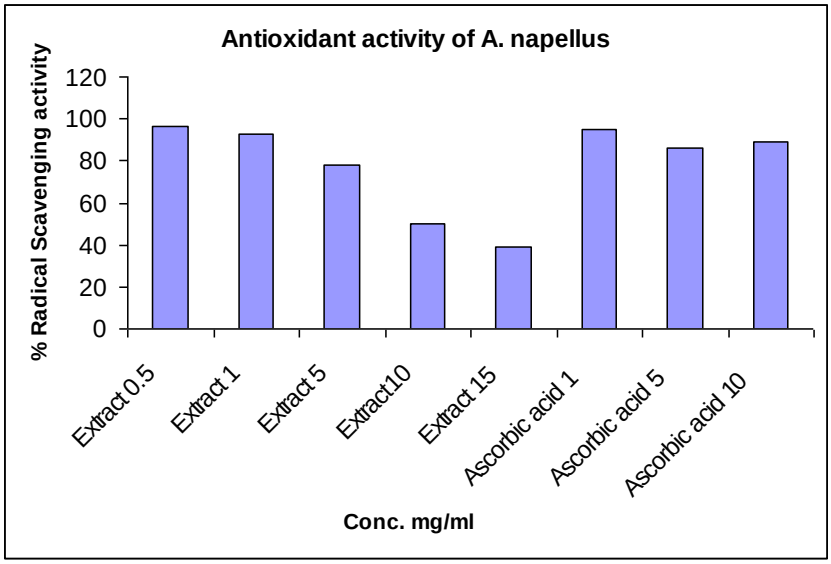


Figure 8: Represent the % of antioxidant activity by DPPH method

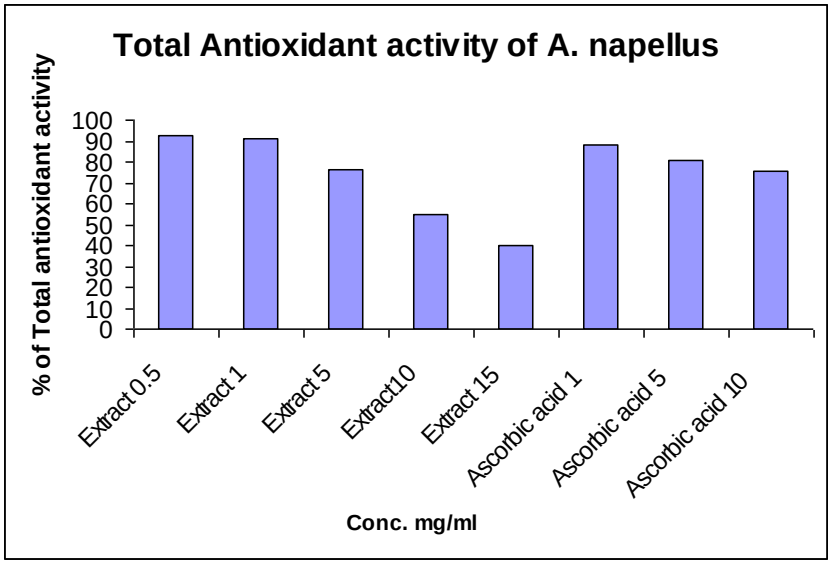


Figure 9: Represent the % of total antioxidant activity by phosphomolybdate method

Table 7: **Immunomodulatory effect of *A. napellus* extract**

Concentration of leaf extract (mg/ml)	Phagocytic index	% immunostimulation
500	48	37.64
250	57	35.68
125	52	23.80
62.5	78	85.70
31.25	70	66.69
15.60	79	88.14
7.80	60	42.82
3.90	51	21.44
1.95	56	33.33
0.98	57	35.76
0.48	58	38.15
0.24	30	-28.48
0.12	22	-47.64
0.06	19	-54.76

Table 8: Antibacterial activity of *A. napellus* extract

Gram positive bacteria	**Zone of inhibition in mm (mean± S.D)**	**Gram negative bacteria**	**Zone of inhibition in mm (mean± S.D)**
B. s cereus	13±3	*E. aerogenes*	-
B. subtilis	15±0	*E. coli ATCC 8739*	-
B. thruingiensis	16±1	*E. coli*	-
C. diptheriae	-	*E. coli multi drug resistance*	-
C. hofmanii	-	*K. pneumoniae*	-
C. xerosis	-	*S. typhi*	-
S. epidermidis	19±0	*S. paratyphi A*	-
S. saprophyticus	19±1	*S. paratyphi B*	-
M. smegmatis	-	*S.dysenteriae*	-
S. fecalis	-	*S. marcesens*	-
S. pyogenes	-	*A. baumanii*	21+1
		C. jejuni	13+1
		C. coli	17+2
		H. pylori	-
		H. influenzae	-
		V. cholerae	-
		A. hydrophila	13+1

Table 9: Minimum Inhibititory Concentration (MIC) *of A. napellus* extract in µg/ ml against different bacteria

Bacteria	MIC µg/ ml of *A. napellus* extract
Gram positive bacteria	
B. cereus	140
B. subtilis	160
S. epidermidis	200
S. saprophyticus	220
B. thruingiensis	160
Gram negative bacteria	
A. baumanii	260
C. jejuni	120
C. coli	140
A. hydrophila	200

Table 10: Antifungal activity of *A. napellus* extract

Yeast	Zone of inhibition mm	Dermatophytes	Zone of inhibtion mm	Saprophytes	Zone of inhibition mm
C. albicans	12±2	*M. canis*	-	*A. flavus*	8±1
C. albicans ATCC 0383	8±1	*M. gypseum*	-	*A. niger*	10±1
S. cerevisiae	-	*T. rubrum*	-	*Fusarium specie*	-
C. galbrata	-	*T. mentagrophytes*	-	*Penicillium sp*	-
C. tropicalis	-	*T. tonsurans*	-	*Rhizopus*	-
C. kruzei	6±2			*Helminthosporum*	-
				Neurospora	-

Table 11: Minimum Inhibititory Concentration (MIC) *of A. napellus* extract in µg/ ml against different fungi

Yeast	MIC µg/ml	Dermatophytes	MIC µg/ml	Saprophytes	MIC µg/ml
Candida albicans	320	*Microsporum canis*	-	*A. flavus*	240
Candida albicans ATCC 0383	360	*Microsporum gypseum*	-	*A. niger*	440
Saccharomyces cerevisiae	-	*Trichophyton rubrum*	-	*Fusarium specie*	-
Candida galbrata	-	*Trichophyton mentagrophytes*	-	*Penicillium sp*	-
Candida tropicalis	-	*Trichophyton tonsurans*	-	*Rhizopus*	-
Candida kruzei	400			*Helminthosporum*	-
				Neurospora	-

Table 12: Phytotoxic activity of *A. napellus*

Name of plant	Concentration of sample (µg/ml)	Number of fronds survived		Concentration of standard drug (µg/ml)
Lemna minor		*A.napellus*	control	
	1000	10	15	0.015
	100	12	15	0.15
	10	14	15	0.15

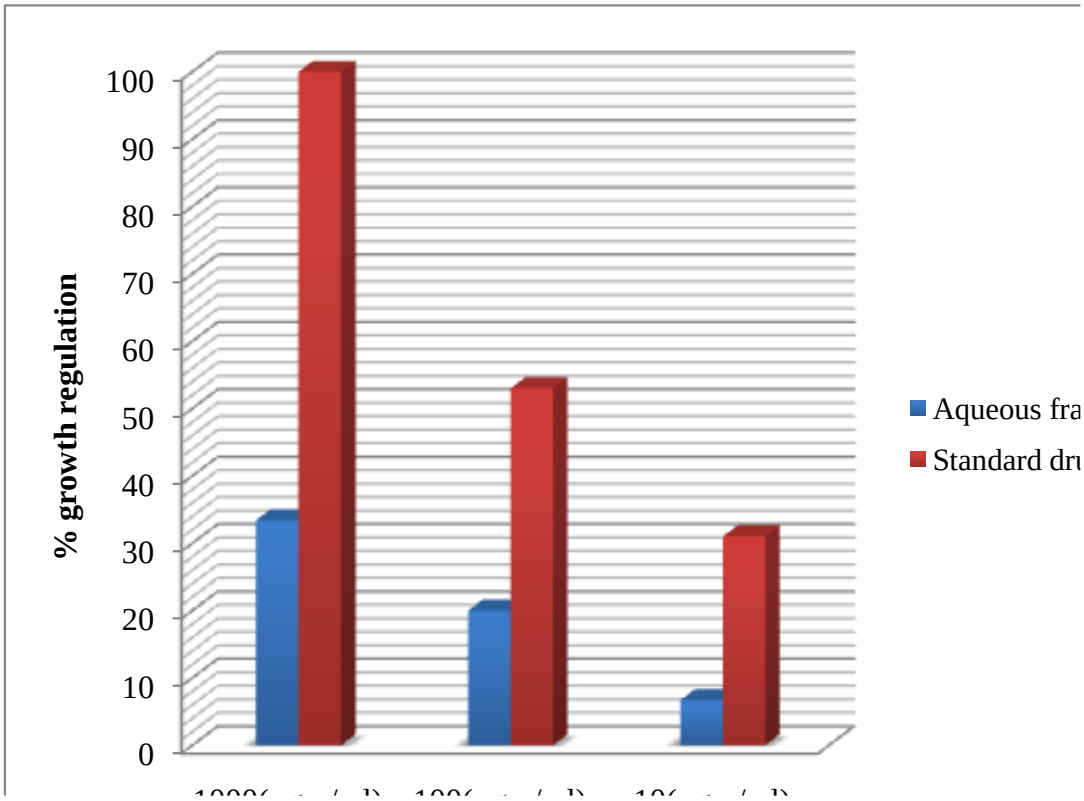


Figure 9. Phytotoxic activity of *A. napellus* (aqueous fraction)

Discussion

The main objective of this study is to provide the beneficial effect of *A. napellus*. Traditionally different species of Aconitum have been used and a number of researches have carried out on these species. However, only few data is available regarding *A. napellus* Linn. It is an important medicine in different system of medicine and used in many diseases (Day, 1993, ABC Homeopathy 2011). Literature also reports its toxic manifestation (Ratsch 1998 and Day, 1993). Therefore, pharmacological and toxicological investigations were performed, mainly related to its LD_{50}, ED_{50} value, Analgesic, anti-inflammatory and neurological effects.

The results obtained from toxicological findings suggested that it produces 100 % of mortality (death) at 1000 mg/kg dose and 66.6% mortality (death) at 500 mg/kg. The dose of 300 and 100 mg/kg showed non lethal effect but symptoms of toxicity is produces which included fits, rashes and loss of consciousness. At the dose of 10mg/kg mice showed non lethal and non toxic behaviour and remain alive. LD_{50} of *A. napellus* is then calculated and it was found 300m/kg. The ED50 value of the drug is calculated as below 10 mg/kg. These finding gives an idea to performed further pharmacological acitivites.

The results of gross behaviour activities suggested that *A. napellus* produces dose dependent decrease in the activity. At high dose 300 mg/kg it produces anxiety followed by sedative effect. At the dose of 10 mg/kg it produces therapeutic effects which show slight increase in diuresis, and number of faeces. These findings were compared with Diazepam 2mg/kg and Histamine 10 and 20 mg/kg. Interestingly the findings of toxic symptoms were more or less same like histamine. The dose of 10 mg/kg suggests that the drug showed depressive effect.

Other neuropharmacological activities were also performed on these doses. The results of Open field, head dip, cage cross, Light and Dark activity, traction, forced swimming test suggest that drug produces highly significant CNS depression. Mice showed decrease in motor acitivity, exploratory behaviour at the dose of 10 mg/kg. In traction test mice showed similar behaviour like Diazepam. At 10 mg/kg the traction time of mice were increased and produces significant muscle relaxant effect. In case of Forced induce swimming activity it also shows decreased in mobility time which is highly significant at 10 mg/kg.

In case of anti-inflammatory effect, the results showed that the drug (extract) produces reduction in the inflammation. This may be influence by the CNS depressive effect of drug which probably reduces the pain response. The dose of 10mg/kg showed 18.5% inhibition where as Aspirin showed 14.11% of inhibition at 3rd hour which is significant at $P<0.05$ when compare with the standard drug Aspirin.
In case of analgesic activity, the crude extract/drug *A. napellus* significant analgesic effect. It reduces the time of licking and biting as well as abdominal cramps induce by

acetic acid. The drug showed more significant response at the 2nd phase of formalin induce licking and biting (82.1% for 10mg/kg of *A. napellus* and 45.95% for Aspirin at P<0.05. HPLC and FTIR finger prints in this study are also helpful for the authentication of extract. The results of antioxidant activity of *A. napellus* extract also shows its potential to reduce the oxidation process. At 15.60 mg/ml, the results of immunomodulatory assay showed maximum phagocytic index that generated potent 88.14% immune stimulation. Among antibacterial results the best activity was achieved against *Acinetobacter baumanii* (21 mm zone of inhibition). MIC against best tested strains of bacteria and fungi were also given quite promising results. The extract of *A. napellus* also shows promising phytotoxic effect.

It has already been reported that *A. napellus* coantains alkaloid aconitine and aconitine acid. The highest concentrations of these alkaloids are found in the root, making that the most dangerous and toxic part of the plant (Bugatti *et al.,* 1992). In order to get therapeutic effects the dose should be adjusted, consequently that decreases the toxicity of *A. napellus*.

CONCLUSION

The crude extract of *A. napellus* showed potent analgesic, anti-inflammatory and CNS depression effect. The present study also exhibited that this toxic plant can be used for different diseases effectively after the determination of its LD_{50} and ED_{50} value.

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