Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 4, 2011

Research Article

ANIMAL STUDIES ON UNANI DRUG FORMULATION FOR ITS ANALGESIC, ANTIPYRETIC, ANTI-INFLAMMATORY AND NON-TOXIC EFFECT

WAHEED M.A[1], FARHA DEEBA[2], RABBANI SYED*[3], YOUSUF ALI M [1], ZEEYAUDDIN K.[4] ALI A. ALSHATWI[5]

[1.]Department of Ilmul Advia (Unani Pharmacology) Govt. Nizamia Tibbi College, Charminar Hyderabad, [2.]Jawaharlal Nehru Institute of Advance Studies, Secunderabad, [3.]Mahavir Hospital and Research Centre, Hyderabad, [4.]Deccan School of Pharmacy, Hyderabad. [5.]Molecular Cancer Biology Research Lab(MCBRL,) Dept. of Food Science and Nutrition. College of Food and Agriculture Sciences, King Saud University. Email: rabbanisyd @gmail.com

Received: 11 April 2011, Revised and Accepted: 8 May 2011

ABSTRACT

Background: 80% of the world's population presently use herbal medicines for some aspects of their primary health care. The practice of using herbal drugs for treating various diseases are well documented in the Unani classics as there are several herbs with known anti pyretic, analgesic and anti inflammatory properties that have been found safe, effective, dependable, affordable, and have negligible or no side effects compared to that of costly synthetic drugs, many of which have side /adverse effects. We evaluated analgesic, antipyretic and anti inflammatory activities of crude aqueous extract and crude ethanol extract of Unani Compound Drug (UCD), In-Vitro and In-Vivo.

Methods: Acute toxicity study is performed in mice after administration of Unani compound drug orally in graded doses. Analgesic activity of UCD was studied using hot plate and tail clip methods in rats. Antipyretic activity of UCD was evaluated in rats by using Brewer's Yeast induced pyrexia. Anti inflammatory activity of UCD was evaluated in rats by using formaldehyde induced arthritis. Estimation of SGOT and SGPT is performed by using Reitmann and Frankel Method. Histopathological examination of organs of rats was done by Clayeden Method.

Results: In acute toxicity study, no mortality was observed up to a dose of 2 gm/kg in aqueous extract and up to 5 gm/kg in ethanol extract of UCD. Both the extract of UCD showed significant central analgesic effect by increasing the reaction time in hot plate and tail clip tests. Both extract of UCD have shown significant and equal antipyretic activity compare to paracetamol in Brewer's Yeast Induced pyrexia in rats. Aqueous extract of UCD also showed In vitro anti oxidant activity in super oxide dismutase assay. SGOT and SGPT remains under normal level. Histopathological examination of six organs of rats reveals no pathological changes even after administration of UCD for 10 days.

Conclusion: The present study demonstrate the potential analgesic, antipyretic and anti inflammatory properties of Unani compound drug and concept of compounding medicine further supporting the claims of Unani medicine as safe and highly effective.

Keywords: Unani Compound Drug, Analgesic, Anti Pyretic, Anti Inflammatory

INTRODUCTION

In the long struggle to achieve mastery over the powerful sources of nature, man has always turned to plants for food, shelter, clothing, weapons, healing and even for relief from the hardship of life. Currently approximately 25% of all prescription drugs are derived from herbs, shrubs and trees. The WHO estimates that 4 billion people i.e., 80% of the world's population presently use herbal medicines for some aspects of their primary health care. The practice of using herbal drugs for treating various diseases are well documented in the Unani classics as there are several herbs with known anti pyretic, analgesic and anti inflammatory properties that have been found safe, effective, dependable, affordable, and have negligible or no side effects compared to that of costly synthetic drugs, many of which have side /adverse effects. Unani system of medicine is the oldest, natural, traditional system of medicine known to mankind famously called Greeco-Arab Medicine originated from Greece.

Inflammation has been known to humankind for thousands of years, ever since ancient peoples described its hallmark symptoms — redness, pain and swelling. Centuries later, researchers have discovered several of the cellular and molecular events that underlie these symptoms, but much more remains to be understood. Inflammation is an important feature of great number of diseases. It is a response of the tissue to an injury, infection, irritation or foreign substance. It is a part of host defense, but when response is too high it may be far worse than the disease itself and in extreme conditions, it may be fatal. Anti inflammatory drugs are considered important because of their wide therapeutic potential and their utility in a number of diseases. Now a days, the synthetic drugs are although dominating the market but the element of toxicity that these drugs entail, cannot be ruled out as they have many side effects such as nausea, vomiting, gastric disorders, rashes, nervous disorders, emotional disturbance, metabolic disorders and many more even in cancers and death [1]. Unani medicine claims to poses many effective and safe anti inflammatory agents, that are used widely used [2, 3, 4].

The aim of the study is to know the action of the compound Unani drugs with the help of modern parameters and techniques. According to modern pharmacologists most of the analgesics and anti inflammatory drugs are nephrotoxic, hepatotoxic, produce gastric ulcer and produce hindrance in the healing but in this study we give evidence that when the crude Unani drugs are used they are healing and have no side effect as those of allopathic medicine.

MATERIALS AND METHODS

All tests were performed in accordance with the guidance of CPCSEA, after approval and permission of institutional animal ethical committee (IAEC) Deccan College of Medical Sciences and Research Centre.

Extraction procedure

In the present work, continuous extraction is used for preparation of extract. The plant materials were cleaned and freed from foreign impurities dried under shed and were powdered by a mechanical grinder. The Unani Compound Drug was Qurs Shifa. About 150 gms of the pulverized plant materials was extracted with 250 ml of ethanol and distilled water using a soxhlet apparatus for 24 hours to get the extracts. Each time before extracting with the next solvent, the powdered material is dried in hot air oven with less than the 50^0C temperature; each extract is concentrated by distilling of the solvent and then evaporating to dryness on water bath. Semi solid mass was obtained (10.5% and 12.5%) w/w with respect to the powdered material and stored in refrigerator.

Acute toxicity procedure

Experimental design to find out the lethal dose for aqueous extract of UCD (Unani compound drug): Acute toxicity was carried out by

using various doses like 1000, 2000, 3000 mg/kg wt. Group of 3 female albino mice weighing between 20-25 gm were selected and is kept for overnight fasting with free access to water for the toxicological studies as per CPCSEA rules by using up and down method. Starting dose 2000 mg / kg according to body weight of animals of aqueous extract of UCD dissolved in distilled water was selected and administered via oral route with the help of oral catheter. Subsequent to administration of drug extracts the animals were observed closely for the first three hours for any toxic manifestations like increased motor activity, salivation, chronic convulsion, coma and death. The animals were subsequently observed at regular intervals for 24 hours and further for 1 week. Two mice died. The procedure was repeated again and another mouse was observed to have died. Then a dose of 300 mg / kg is given according to the body weight of the mice and not a single mouse died. It infers that the dose ranges from 300 – 2000 mg / kg. So the maximum non-lethal dose was found to be 2000 mg/kg body weight. [5].

Mechanical stimulus

Adult albino rats weighing 130-170 gm / kg were selected for the experiment. Four groups of 5 animals in each, Group I serve as a control receive rice bran oil 2ml/kg. Group II serves as a standard receive aspirin 400 mg / kg and group III receive aqueous extract of UCD 1000 mg / kg where as group IV receive ethanol extract of UCD 2500 mg / kg per orally through oral catheter. Rice bran oil was kept as vehicle and solvent in all the dosage of test, standard and control groups. Animals were fasted over night and marked for identification. After observing initial response of pain by applying tail-clip, animals those have not given the response within 5 seconds were discarded. The bull dog clips, with both arms protected at the tip with rubber tubing was used. It was applied with a constant pressure on the tail 1.5 cm distal to the root and the latency time (it takes the animal to bite the source of noxious stimulus i.e. the hemostat) was record. The doses of test drug, standard drug and control diluted in rice bran oil were given to the animals as per their growth and weight; latencies were measured at 30, 60, 90 and 120 minutes after administration of drugs. [6]

Anti pyretic activity

Experimental method for screening anti pyretic activity: In the present research study, anti pyretic activity is confirmed by using the Brewer's yeast induced pyrexia model. Brewer's yeast induced pyrexia [7]

Anti inflammatory activity

In the present study the following method is use to screen the anti inflammatory activity. Formaldehyde – induced arthritis: [8,9,10]. Male Wister rats weighing 130-170 gms were selected and divided into 4 groups each having 6 animals. Arthritis was induced by injecting 0.1 ml of a 2% formaldehyde solution into the plantar aponeurosis on the first and third day of experiment in all rats.

Group – I served as control received rice bran oil orally 2ml/kg P.O.

Group – II served as standard received aspirin 20 mg / kg through intraperitonial route (I.P.)

Group – III served as test group received aqueous extract of UCD 1000 mg / kg P.O.

Group – IV served as test group received ethanol extract of UCD 2500 mg/kg P.O. for 10 consecutive days and the mean increase in the paw diameter of every animal of each group is measured over a period of 10 days below the ankle joint using a micrometer screw gauge. The animals were sacrificed on 10th day after administration of drugs under anesthesia using chloroform and blood is withdrawn from the animal for the estimation of SGOT and SGPT levels by using Reitmann and Frankel method.

Histopathological studies

To study the histopathological damages of organs. Six organs such as heart, liver, kidney, stomach, spleen and lungs were dissected and examined histopathologically employing haemotoxylin and Eosin stain.

Microscopic evaluations were made in respect of the following parameters:

Architecture of organs parenchyma, sign of regeneration / degenerative changes, fatty changes, swelling of organs cells, inflammatory cell infiltration, focal necrosis, cell hyperplasia, number of normal cells.

Antioxidant activity of crude aqueous extract of Unani compound drug: the method employed was of Winterbourn [11] and is based on the ability of superoxide dismutase to inhibit the reduction of Nitro-blue Tetrazolium (NBT) by superoxide.

Statistical analysis of data by Social Packages for Statistical Sciences (SPSS) Data was statistically analyzed by using one way Anova followed by t-test and a probability level lower than 0.05 was considered as statistically significant. The increased pain threshold i.e., increase in normal reaction time by 10 seconds, indicates positive analgesic activity of drug. Values are expressed as mean ± SEM.

RESULTS

The acute toxicity study done by using up and down method, as per CPCSEA guidelines, shown that aqueous extract of UCD up to a dose of 2 g/kg body weight and ethanol extract of UCD up to a dose of 5 g/kg body weight both P.O were found to be safe and no mortality was observed in mice.

Analgesic effect of UCD

In hot plate test, the aqueous extract of UCD at a dose of 1 g/kg body weight and ethanol extract of UCD at a dose of 2.5 g/kg body weight per oral has shown highly significant ($P<0.001$)analgesic activity (Table 1). In tail clip test both extracts have shown highly significant ($P<0.001$) analgesic activity (Table 2). The response was much better and significant than the analgesic activity of aspirin (400 mg/kg P.O), a standard analgesic drug. The analgesic activity of both extracts of UCD started as early as ½ an hour and effect was maintained and increased during the whole observation compared to potent standard drug aspirin. The results of hot plate and tail clip tests indicate a significant increase in the pain threshold throughout the experiment.

Antipyretic activity of UCD: The antipyretic activity of UCD was evaluated by using Brewer's yeast induced pyrexia in rats. The experimental rats showed a mean increase of about 0.910 C in rectal temperature, 18 hour after Brewer's Yeast injection. The aqueous and ethanol extracts of UCD at a dose of 1 gm and 2.5 gm/kg body weight respectively have shown response comparable to that of anti pyretic activity of paracetamol (300 mg/kg P.O.), a standard antipyretic drug. The test drugs UCD started antipyretic activity as early as one hour and the effect was maintained and observed throughout the experiment up to 4 hour after its administration (Table 3).

In formaldehyde induced arthritis, the aqueous extract of UCD at a dose of 1 g/kg body weight and ethanol extract of UCD at a dose of 2.5 g/kg body weight have shown highly significant ($P<0.0001$) anti inflammatory activity by reducing the inflammation and swelling in the diameter of hind paw of experimental rats measured over a period of 10 days below the ankle joint using micrometer screw gauge when compare to potent anti inflammatory standard drug aspirin (20 mg/kg I.P). The anti inflammatory activity of UCD extracts started as early as first day and maintained for 10 days (Table 4). SGOT and SGPT levels estimated were slightly raised above normal level in test drugs. These enzymes level were very higher in experimental rats blood samples of standard aspirin compare to text drug UCD. This indicates that both extracts of UCD are safe even used for a longer duration (Table 5).

Since most of the analgesic, anti inflammatory and antipyretic drugs cause gastric irritation, ulcerations, hepato-nephro toxicity etc., so histopathological examinations of six organs that of liver, kidney, stomach, lungs, heart and spleen were done. No damages, necrosis or toxicity were noticed in the experimental rats of those received test drug (UCD) than the standard drugs (aspirin 20 mg/kg I.P) even

after 10 days of continuous administration of drug in such a large dosage. Whereas the standard drug aspirin has shown some damage to liver, kidneys and lungs. Based on the present study, standard drug aspirin in small dosage through I.P route was selected because P.O administration of it in 400 mg/kg and 300 mg/kg dosage resulted in 100% mortality in experimental rats within three days apart from producing pathology in organs.

Antioxidant activity: 50% maximum inhibition without drug and normal SOD activity was observed is 250 units / mg in 0.1 ml of the blood where as the 50 mg/ml of UCD is required to produce 333.3 units/ mg and 100 mg/ml to get 500 mg/units of SOD activity. So an increase in SOD activity in the presence of aqueous extract of UCD indicate the highly significant anti oxidant property.

An increase in SOD activity in the presence of the drug aqueous extract of UCD, which is an experimental proof in-Vitro of its antioxidant property. The results obtained in this model indicate that, activity of UCD is almost equal to that of the standard drug (paracetamol) and P values of the results obtained have confirmed statistical significance.

Histopathological studies: Aspirin 20 mg/kg intraperitoneally

Organs	Histo pathology
Liver	PPICC.
Heart	NAD.
Kidney	OCCFCC.
Lungs	MHPLA.
Spleen	NAD.
Stomach	NAD.

PPICC = Periportal Inflammatory Cell Collection
NAD = No Abnormality Detected
OCCFCC = Occasional Focal Collection of Inflammatory Cells
MHPLA = Medial Hypertrophy & Peribronchial Lymphoid Aggregates

Histopathological evaluation to verify the protective effect of Aspirin in the dose of 20 mg/kg through I.P route showed pathological changes in the liver, kidneys and lungs. Based on the present study, standard drug aspirin in small dosage through I.P route was selected because P.O administration of it in 400 mg/kg and 300 mg/kg dosage resulted in 100% mortality in experimental rats within three days apart from producing pathology in organs (Fig 1).

Aqueous extract of UCD: 1000 mg/kg per oral

Organs	Histopathology
Liver	NAD.
Heart	NAD.
Kidney	NAD.
Lungs	NAD.
Spleen	NAD.
Stomach	NAD.

Histopathological examination of six organs of experimental rats treated and tested with crude aqueous extract of UCD in the dose of 1 gm/kg P.O for 10 days in formaldehyde induced arthritis. This study reveals that the test drug is highly protective and safe in larger doses even for a longer duration compared to the standard drug aspirin (Fig 2).

Ethanol extract of UCD: 2500 mg/kg per oral

Organs	Histo pathology
Liver	NAD.
Heart	NAD.
Kidney	SFA – RCC
Lungs	NAD.
Spleen	NAD.
Stomach	NAD.

SFA – RCC = Single Focal Area of Round Cell Collection

Histopathological examination of six organs of experimental rats treated and tested with crude ethanol extract of UCD in the dose of 2.5 gm/kg P.O for 10 days in formaldehyde induced arthritis. This study reveals that the test drug is highly protective and safe in larger doses even for a longer duration compared to the standard drug aspirin (Fig 3).

Table 1: Analgesic effects of UCD on Thermic Induced pain (Hot plate method) in rats

Drug	Dose (mg/kg)	Reaction time in minutes				
		0	30	60	90	120
Control	Saline	4.92±0.16	4.5±0.01	5.75±0.11	4.92±0.09	4±0.13
Aspirin	400 mg/kg PO	7±0.58	7.25±0.74	7.33±0.61	7.5±0.66	7.5±0.58
Aqueous	1 gm/kg PO	10±1.51	8.66±1.38	9±1.67	8.41±1.49	8.25±1.78
Ethanol	2.5 g/kg PO	10.66±2.0	9.41±1.39	10.25±1.74	9.25±1.11	9±1.07

n: five animals in each group; Values are mean ± SEM, P<0.001 when compared to control

Table No.2 Analgesic effects of UCD on Tail clip method in rats

Drug	Dose (mg/kg)	Reaction time in minutes				
		0	30	60	90	120
Control	Saline	3.25±0.11	4±0.13	4.92±0.16	5±0.13	5.92±0.16
Aspirin	400 mg/kg PO	7.83±1.39	8.16±0.95	8.66±1.06	8.08±1.25	8.41±1.19
Aqueous	1 gm/kg PO	9.66±1.23	9.16±1.33	9.58±1.38	9.41±1.40	8.58±1.32
Ethanol	2.5 g/kg PO	9.83±1.48	8.58±1.44	10.16±1.47	9.5±1.20	8.66±1.20

n: five animals in each group; Values are mean ± SEM, P<0.001 when compared to control

Table.3 Antipyretic activity of UCD on Brewer's Yeast Induced pyrexia in rats

Drug	Dose (mg/kg)	Rectal temperature in °C at time (h)					
		0	18	1	2	3	4
Control	Saline	35.15±0.10	36.7±0.14	36.7±0.14	36.6±0.16	36.6±0.14	36.5±0.11
Paracetamol	300 mg/kg PO	35.2±0.13	36.8±0.12	35.18±0.04	35.1±0.9	35.8±0.08	35.1±0.11
Aqueous	1 gm/kg PO	35.2±0.06	36.5±0.09	36.08±0.15	35.8±0.15	35.5±0.12	35.3±0.11
Ethanol	2.5 g/kg PO	35.1±0.1	36.57±0.14	36.43±0.10	36.42±0.08	36.40±0.06	36.37±0.08

n: six animals in each group; Values are mean ± SEM, P<0.001 when compared to control

0 : Temperature just before yeast injection

Table.4 Anti inflammatory activity of UCD on Formaldehyde Induced Arthritis in rats

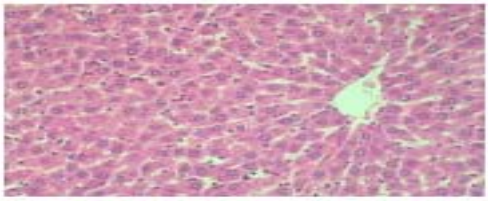
Drug	Dose (mg/kg)	Reaction time in minutes					
		1	2	3	4	5	6
Control	Saline	11.75±2.78	10.50±2.53	11.25±2.62	11±2.59	11.08±2.66	9.25±2.27
Aspirin	20 mg/kg IP	11.83±2.73	11.08±2.62	11±2.63	10.33±2.47	9.75±2.30	9.33±2.23
Aqueous	1 gm/kg PO	9.17±2.12	8.17±2.05	7.5±1.8	6.17±1.49	5.5±1.37	13.33±5.7
Ethanol	2.5 g/kg PO	20.67±4.41	18.33±4.33	16.42±3.95	15.08±3.70	14.25±3.54	12.5±3.02

n: six animals in each group; Values are mean ± SEM

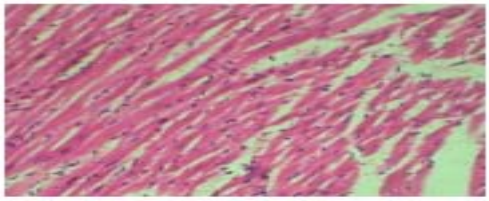
Table.5 Effects of test drugs and standard drug on different bio medical parameters in formaldehyde induced arthritis (mean + SEM).

Treatment	No. of days	No. of animals	SGPT Iu/L	SGOT Iu/L	P - value
Aspirin	10	3	84.67 + 8.12	230 + 11.56	
Aqueous	10	3	27 + 2.65	73.67 + 4.98	<.001
Ethanol	10	3	26 + 2.52	72.33 + 6.50	<.001

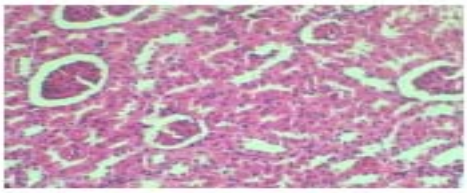
The result was analyzed statistically using student t-test where applicable.



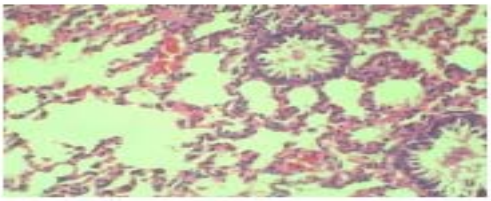
Liver section from aspirin treated shows PPICI



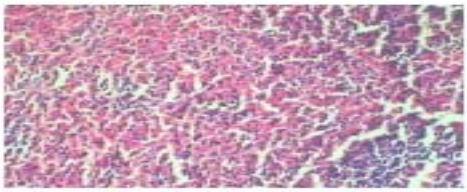
Heart section from aspirin shows no abnormalities



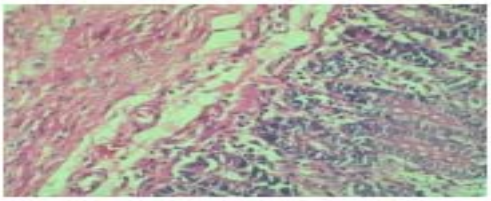
Kidney section of aspirin treated shows OCCFCC



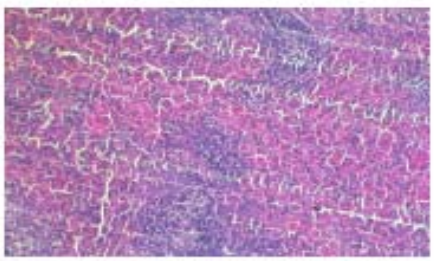
Lungs section of aspirin treated shows MHPLA



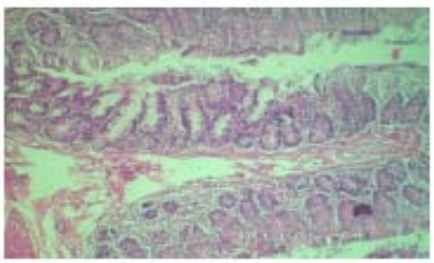
Kidney section shows SFA-RCC



Lung section shows no abnormality



Spleen section shows no abnormality



Stomach section shows no abnormality treated orally with 2.5 gm / kg of UCD for 10 days.

Fig. 1: Histopathological changes occurring with aspirin treated with experimental rats for 10 days in formaldehyde induced arthritis

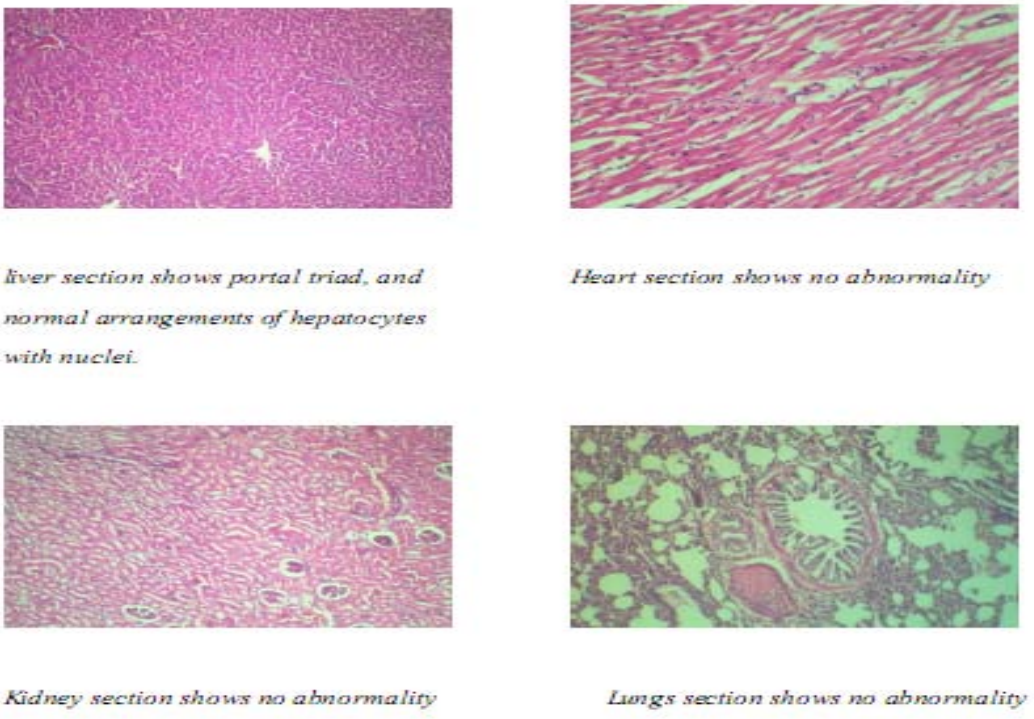


Fig. 2: Histopathological changes occurring with aqueous extract of UCD treated with experimental rats in formaldehyde induced arthritis for 10 days

DISCUSSION

Pain, fever and inflammation are the most common ailments with which every human being suffers and these are the features of various diseases. Conventional NSAIDs of modern medicine are confronted with common and serious unacceptable side effects as well as adverse effects notably gastro-intestinal bleeding, hepato-nephro toxicity [12,13,14]. Hence there has been an active search for "better" NSAIDs and this inspired the scientists and researchers to find out some other method of therapy or update the knowledge and the philosophical background of existing indigenous system of medicine [15].

The practice of using herbal medicines for treating various diseases is well documented in the Unani classics. The Unani system of medicine claims to possess number of single and compound drugs which act as analgesic, antipyretic and anti-inflammatory agents. These are free from hazardous side effects and adverse effects based on the observation, experience and clinical practice (Isteqra) of ancient Unani physicians. The purpose of compound formulation in Unani system of medicine is to increase the potency of the drugs by synergistic action of the other ingredients and for treatment of multiple symptoms of disease. In-Vitro and In-Vivo experimental evaluation of analgesic, antipyretic and anti-inflammatory effects of crude aqueous and ethanol extracts of Unani compound drug was conducted in the present study [16,17,18].

The results reported in the present study demonstrate that acute toxicity study on oral administration of crude aqueous and ethanol extracts of UCD did not produce any toxic manifestations like increase motor activity, salivation, chronic convulsion and coma in mice. The crude aqueous extract up to a dose level of 2 gm/kg and crude ethanol extract up to a dose level of 5 gm/kg are safe and did not produced any mortality in mice. This study shows that crude ethanol extract of test drug is better and safer, tolerable even in larger dose compared to another test drug aqueous extract of UCD.

Both crude aqueous and ethanol extract of UCD exhibit significant analgesic activity as evidenced by the increase reaction to the pain stimulus. The result were significant at $P<0.001$ for Hot Plate method as well as for tail clip method, suggesting its central (narcotic) analgesic activities compared to standard drug ($P<0.001$). The present study showed that both the test drugs cause significant effect against central analgesic activity in comparison to control group as well as standard drug. As far as, the test drugs are concerned crude ethanol extract of UCD produce much better analgesic effect than the crude aqueous extract. This may be due to the presence of more alcohol soluble chemical constituents (or) active principle than the water soluble in the UCD.

Review of literature reveals the phyto-chemical analysis of single drug present in the test drug UCD extracts showed the presence of flavonoids, alkaloids, tannins, saponins, phytosterol, beta-sitosterol, triterpenoid, steroids, glycosides, ursolic acid, oleonoic, campesterol, triterpenes [19,20,21]. This analgesic activity observed can be attributed to the presence of flavonoids and tannins which have been reported to produce analgesic effect by inhibiting prostaglandin synthatase or presumption is that as analgesic drugs acts mainly by the inhibition of prostaglandin synthesis or cyclooxygenase and lipooxygenase inhibition, this test drug may also act in the same mechanism [22,23]. It is well known that analgesic and anti inflammatory drugs possesses antipyretic activity and vice versa. The present study has demonstrated that both the test drugs i.e., crude aqueous extract at a dose of 1 gm/kg and crude ethanol extract of UCD at a dose of 2.5 gm/kg exhibit significant antipyretic activity carried out in albino rats weighing 130-170 gm using Brewer's Yeast induced pyrexia. Both extracts shown significant fall in body temperature up to 4 hour following their administration. The antipyretic activity started as early as one hour and the effect was maintained for whole experiment. The response was comparable to that of antipyretic activity of paracetamol (300 mg/kg) a standard antipyretic drug. The result in this test indicate that, antipyretic activity of the UCD is almost equal to that of the standard drug and $P<0.001$ of the results obtained have confirmed statistical significant. The antipyretic activity of the test drug may be due to presence of flavonoids compounds or inhibition of prostaglandin synthesis could be possible mechanism of antipyretic action as that of paracetamol. Also, there are several other mediators of multi-processes underlining the pathogenesis of fever inhibition any of these mediators may bring about antipyresis or inhibition of cyclooxygenase or lipo-oxygenase or inhibition of prostaglandin

synthetase within the thalamus [24,25]. Anti-inflammatory activity was carried out by using formaldehyde induced arthritis. The crude aqueous extract in the dose of 1 gm/kg and crude ethanol extract in the dose of 2.5 gm/kg were used. Both extracts have shown highly significant results P<0.001 compared to aspirin 20 mg/kg I.P. a standard anti-inflammatory drug. As far as both extracts are concerned, the ethanol extract have shown much better result P<0.0001 than the aqueous extract of UCD P<0.001. The standard drug aspirin was first given in the dose of 400 mg/kg P.O. and then 300 mg/kg P.O. to the experimental rats but no animal survive for more than 3 days. So we have used aspirin in a dose of 20 mg/kg intra peritoneally. Hence, the test drugs even in the large doses for a longer duration is found to be safe and better tolerable than the standard drug aspirin. Both the extracts progressively reduced the edema of the rat hind paw induced by formaldehyde, this mechanism of action may be that of aspirin, a cyclo-oxygenase inhibitor or due to presence of flavonoids or ursolic acid or β-sitosterol or terpenoids which are reported to possess potent anti inflammatory activity and can inhibit human leukocyte elastase [26].

However participation of other compounds in the pharmacological actions observed should not be discarded. SGOT and SGPT levels were estimated in the blood samples of test drugs and standard drug after 10 days of experiment. The standard drug have shown highly significant rise in SGOT and SGPT levels (P<0.01) than the test drugs indicating the toxicity and damage to the liver and other organ. Both the extracts of Unani compound drugs are very safe and do not have any toxic effect on the liver and other organs in large dosage even for longer duration (Table 5). Based on the present study, standard drug aspirin in small dosage through I.P route was selected because P.O administration of it in 400 mg/kg and 300 mg/kg dosage resulted in 100% mortality in experimental rats within three days apart from producing pathology in organs. Whereas, UCD showed neither mortality nor any histopathologies. In-Vitro efficacy of the crude aqueous extract of the UCD as an antioxidant has shown highly significant results in Super Oxide Dismutase (SOD) assay. 50% maximum inhibition without drug and normal SOD activity was observed is 250 units / mg in 0.1 ml of the blood where as the 50 mg/ml of UCD is required to produce 333.3 units/ mg and 100 mg/ml to get 500 mg/units of SOD activity. So an increase in SOD activity in the presence of aqueous extract of UCD indicates the highly significant anti oxidant property of In-Vitro assay.

CONCLUSION

In the present scientific research study, the test drug i.e., crude aqueous and ethanol extracts of Unani Compound Formulae (UCD) showed very striking and highly significant analgesic, antipyretic and anti-inflammatory activities without any side effects and toxicity. This study reveals that UCD is safe and devoid of toxic effects even in large doses and also for a longer duration.

We invite the attention of scientific world for further study and recommend for the administration of the trial Unani Compound Drug (UCD)

REFERENCES

1. Dr. Sayeed Ahmad D. I. Hom. (London) , Allopathy, 2004
2. Guidelines on acute oral toxicity CPCSEA Rules, Annex.2d. OECD no.425, 2001.
3. Emmanuel B. Thompson, Drug Bio screening, Drug Evaluation Technique in pharmacology V.C.H. Publishers (UK) Ltd. 8 Wellington Court Cambridge CBI, 2HZ, U K, p.no.101-107 ,1999.
4. Ghosh M.N. Fundamental of Experimental Pharmacology, Scientific book agency Calcutta p.no. 175 ,2005.
5. Kulkarni SK, Handbook of Experimental pharmacology, New Delhi . p.no. 125 ,1987.
6. Bianchi, C. & Franceschini, J. . Experimental observations on Haffner's method for testing analgesic drugs. British Journal of Pharmacology and Chemo-therapy :1954, 9, 280-284.
7. Loux JJ, Depalma PD, Yankell SL. Antipyretic testing of aspirin in rats. Toxical.Appl.Pharmacol.:1972,22:672-675.
8. Aslam M, Singh S. Research methodology and biostatistics in pharmacology, academa publishers, New Delhi, p.no. 149 ,2006
9. Selye H , Br. Med. Journal : ,2:1129,1949.
10. Singh Sand Majumdar DK), Int.J.Pharmacogmnosy,: 1996, 34(3):218.
11. C C Winterbourn, B M McGrath, and R W Carrell ,Reactions involving superoxide and normaland unstable haemoglobins, Biochem J.;1976.155(3): 493–502. PMCID: PMC1172871.
12. Kantoory GH. Kamil-us-Sana, Vol.I, Lucknow (India): Matab Munshi Nawal Kishore; , p.no. 319, 403, 422 ,1889.
13. Goth's. Medical pharmacology 12thed. Galgotia publications, New Delhi, ,p.no. 368 ,1990.
14. Rang HP,Dale MM.Pharmacology 2nded.ELBS London.p.no. 720 ,1991
15. chopra, R.N., chopra. I. CL, Handa, K L and Kapur, L D. . Chopra's Indigenous drugs of India , e4, by Chopra et al., 1958, 2nd ed. pp. 325-327.
16. Archana, P. Tandan, S.K., Chandra, S., Lal, J., Antipyretic and Analgesic activities of C. Bonducella seed kernel extract. Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, India . Phytother. Res2005,. 19, 376–381.
17. Gupta, Mazumder, U.K. Kumar R.S., Thangavel S.K., Studies on Anti – inflammatory, Analgesic and Antipyretic properties of Methanol Extract of Caesalpinia bonducella leaves in Experimental Animal Models. Iranian J. Pharma & Therapeutics,:2003, Vol.2, No.2 .
18. Mahnaz K., Neda B, Hadi F.M. Effect of Matricaria Recutita on acute pain in the presence andabsence of sex hormones JRMS : 12 (4) , 190-197, .
19. Jimenez, MJ, Alcaraz MJ, Ferrandis ML, Viller A. Anti-inflammatory activity of a flavone from S.leucantha. planta medica :1986 52, 541.
20. Safayhi H, Sailer ER. Anti-inflammatory actions of pentacyclic triterpenes. Planta medica 1997: 63.487-493.
21. Aboutabl EA, Nassar MI, Elsakhawy FM, Maklad YA, Osman AF, El-khrisy EAM phytochemical and pharmacological studies on Sideritis taurica Stephen ex Wild. J of Ethnopharmacology:2002, 82, 177-184.
22. Mathew AG, Parpia HAB. Food browning as a polyphenol reaction. In:Chichester research, New York academic press ,p.no. 75-145.1971.
23. Hardman JG, Limbird LE, Gilman GA. Goodman and Gillman's The Pharmacological basis of therapeutics, 10th ed, Macgraw Hill medical publishing division, USA 2001.
24. Raj Narayana K, Reddy MS, Chaluvadi MR, Krishna DR. Bioflavanoids classification and antipyretic potential. Indian J. Pharmacol.:2001,32:2-16.
25. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Anti-inflammatory and antipyretic activity of Michelia champaca linn. (white variety) Ixora brachiata Roxb and Rhynchosia cana (willd). D.C.flower extract. Indian J.exp.Biol.35:1997,1310-1314,.
26. Gupta. M.B, Nath. R, Srivastava.N, Shankar. K, Kishore.K, Bhargava. K. P,. Anti-inflammatory and antipyretic activities of ß-sitosterol. International journal of immunopharmacology: 1996,.18, 693-700.