"Evaluation of Unani preparations by *in vivo* analgesic activity test and *in vitro* antioxidant activity test."

This Thesis Paper is submitted to The Department of Pharmacy, East West University in Conformity with the Requirements for the Degree of Bachelor of Pharmacy

Submitted by:

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EAST WEST UNIVERSITY

This thesis paper is dedicated
to my beloved parents and sister

CERTIFICATE

This research paper, submitted to the Department of pharmacy, East West University in conformity with the requirements for the degree of Bachelor of pharmacy (B.pharm) was carried out by Md. Shamiul Islam (2008-1-70-057)

…….

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Associate Professor and Chairperson

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CERTIFICATE

This is to certify that the thesis on "Evaluation of Unani preparation by *in vivo* analgesic activity test and *in vitro* antioxidant activity test" submitted to the Department of Pharmacy, East West University, Aftabnagar Dhaka. In partial fulfillment of the requirement for the degree of Bachelor of pharmacy (B.pharm) it was carried out by Md. Shamiul Islam (2008-1-70-057) under my supervision and that no part of the thesis has been submitted for any other degree. I further certify that all the sources of information in this connection are duly acknowledged.

……

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ABSTRACT

Since centuries unani system of medicine is practiced in this continent. A comparison of unani medicine with the allopathic could give reliable clinical evaluation. For the experiment male Swiss albino mice of 1-2 Weeks of age, Weighing between 20-25gm, were collected from international center for diarrheal disease and research, Bangladesh (ICDDRB).Sorobin has analgesic activity. Its effect in writhing inhibition (Sorobin 3.4±2.8) was more than (Diclofenac Na 7.2±3.7). Balarista has antioxidant activity which was found by free radical scavenging activity using DPPH with IC_{50} value of 7.596.

Keywords: Unani medicines, *In-vivo*, *In-vitro*, Sorobin, Balarista, Diclofenac, Writhing test.

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Chapter 1: Introduction

The Unani system of the medicine is one of the oldest forms of alternatives, traditional and complementary medicine. It incorporates medicinal sources from the plant kingdom, the animal kingdom and natural in its raw form. The current practice of this complementary medicine is preserved in its original form by the unani scholars and physicians. Because it is seen to effective and due to its simplicity and affordability is becoming popular and is being introduced and practiced in many parts of the world.

1.1 Unani Medicine

Unani system is a science which deals with the preventive and primitive aspects of human being and health problems occurred by the Ecological and Environmental factors, this may vitiate humors i.e. Blood, phlegm, Yellow bile, and the fluids circulating in the body vessels. It teaches to maintain the health and treat if effected by disease by bringing back the balance in imbalance humors .Humors are one of the basic principles and concepts conceptualized by the Father of medicine, Hippocrates.(national Institution of Unani Medicine,2006)

1.2 Basic Principles and concepts

In basic principles and concepts the human body is considered to be made up of the following components:

→ Arkan(Element)

→ Mizaj(Temperament),

→ Akhlat(Humours)

→ Aza(Organs)

→ Arwah(Spirit)

→ Quwa(powers)

→ Afal(Function)

1.2.1 Element (Arkan)

These are four i.e. Air, Water, Earth and Fire. Actually these four elements represent several other elements found in the modern science. There are four states denoting the temperaments of these four elements

>Air, >Hot and Moist, >Fire, >Hot and Dry, >Earth, >Cold and Dry, > Water, > cold and Moist.

1.2.2 Temperaments (Mizaj)

The interaction between the chemical combinations of four elements, Produces various states which determine the temperament of an individual human being, plants, and minerals. Temperament (Mizaj) is simply defined as having the following states and their combinations:

>Hot Single……..compound hot and Dry,

>Dry Hot and Moist,

>Cold and Dry,

>Moist cold and Moist.

1.2.3 Humors (Akhlat)

The humors are actually the body fluids which are classified broadly in four. The fluid of the body contains various hormones, enzymes and humours etc. They are responsible for nutrition to the whole of body.

These fluids are (a) Primary (b) secondary.

Primary fluids are four humor i. e. blood, phlegm, yellow bile and black bile. Secondary fluids are hormones, enzymes, and plasma etc. (National Institution of Unani Medicine, 2006)

1.2.4 Organs (Aaza)

Organs are composed of cell, tissues, nerves and blood vessels. Various organs of the body and health in disease condition of each individual affect state of health. (National Institution of unani Medicine, 2006)

Pneuma-Gaseous material (Arwah)

The pneuim is a life force which carries of different powers, without which human body is dead. This is a source of life and vitality. (National Institution of Unani Medicine, 2006)

1.2.5 Pneuma – Gaseous material (Arwah)

The pneuima is life force which carries of different powers, without which human body is dead. This is a source of life and vitality. (National Insitution of Unani Medicine, 2006

1.2.6 Faculties (Qawa)

Faculties/Powers are of three kinds.

>Natural powers,

>Psychic Powers,

>Vital powers.(National Institution of Unani Medicine, 2006)

1.2.7 Functions (Afaal)

Afaal includes movements of various organs. It is necessary to ensure that various organs should be in proper shape and condition to perform proper functions. (National Institution of Unani Medicine, 2006)

1.3 History of Unani Medicine

The Origin of Unani system of medicine is from Greece. The term UNANI is derived from the word UNAN or YUNAN which means Greece in Arabic It's also known as Greco-Arab medicine. The treatment of Unani is based on teachings of Hippocrates. It was the work of the greek philosopher-physician Hippocrates (buqrat in arabic) (406-377 B.C.), who freed medicine from the realm of superstition and magic and gave it the status of science.

He considered illness to be natural rather than a supernatural phenomenon, and he felt that medicine should be administered without ritual ceremonies or magic. By his method of careful study and comparison of symptoms, he laid the foundation for clinical medicine (history of unani Medicine, 1940).

After Hippocrates Many scholars enriched the system of Unani Medicine. Of them Galen (jalinus in Arabic) (131-200 A.D.) stands out as the one who established its foundation on which Arab physicians like Rhazes (Al-Razi in Arabic) (850-932 A.D.) and Avicenna (Ibn-Sena in Arabic) (980-1037A.D.) constructed an imposing edifice. Galen introduced and practiced the Unani system of medicine in pre-Islamic Egypt, researched, experimented and developed hundreds of new medicines and cures for almost all types of diseases (History of Unani Medicine, 1940)

Unani medicine was the first to establish that disease was a natural process and that symptoms were the reactions of the body to the disease. It believes in the humeral theory which presupposes the presence of the four humors- Dam (blood), Balgham (phlegm), Safra (yellow bile) and Sauda (black bile).

Each humor has its own temperament – blood is hot and moist, phlegm cold and moist, yellow bile hot and dry and black bile cold and dry. Every person attains a temperament according to the preponderance in them of the humors which represent the person's healthy state, which are expressed as sanguine, phlegmatic, choleric and melancholic.

It was further enriched by imbibing the best of contemporary system of medicine in the Middle Eastern and far eastern countries like Egypt, Syria, Iraq, Persia, India, China, and other Middle East and Far East countries enriched the Unani system.

That is why this system is known, in different parts of world, with different names such as Greco-Arab Medicine, Ionian Medicine, Arab Medicine, Islamic Medicine, Traditional Medicine, Oriental Medicine, etc.

A Unani physician does not prescribe the strongest drug at the beginning of the treatment. He selects the drug according to the degree of variation from the normal healthy condition and observes the effect produced by the treatment. At the same time, he instructs the patient to observe some restrictions in diet and lifestyle."

These techniques include: mushily (purging), taareeq (sweating), hammam (bath therapy), munzij (ripening), mahajim (cupping), and riyazat (exercise) (History of Unani Medicine, 1940)

1.4 Product Information

1.4.1 SUROBIN®

Herbal anti-inflammatory & analgesic

Description:

Surobin is a unique combination of *Colchicum luteum, Aloe barbadensis, Solanum nigrum, Terminalia chebula*, which is highly effective in all kinds of rheumatism such as chornic rheumatoid arthritis osteoarthritis, sciatica, lumbago, gout, joint pain etc. (SUROBIN, 1906)

Figure 1: Hamdard Product Surobin

Composition:

Each tablet contains:

Aloe barbadensis	149.93 mg
Colchicum luteum	149.93 mg
Terminalia chebula	149.93 mg
Solanum nigrum	149.93 mg

(SUROBIN, 1906)

Indications:

Hepatitis, jaundice, ascites, pleurisy, alveolitis, uterine inflammation & constipation. (SUROBIN, 1906)

Dosage:

One to two tablets twice daily after meal or as directed by the physician. (SUROBIN, 1906)

Contraindication:

There is no known contraindication. (SUROBIN, 1906)

Side effect:

No significant side effect has been observed in proper dosage. (SUROBIN, 1906)

Precaution:

Keep out of reach of the children. (SUROBIN, 1906)

Storage:

Store at cool and dry place, protect from light. (SUROBIN, 1906)

Presentation:

Box containing 10x10 tablets in strip. (SUROBIN, 1906)

One remedy for ten ailments

DIRECTIONS FOR USE

- Headache, Toothache and Burns:

  Apply Surobin

- Earache: Instill Surobin mixed with 2 drops of sesame oil into the ears,2-3 times daily.
- Cough, cold and catarrh : mixed with 2 drops of sesame oil into the nostrils, twice a day. Massage the oil gently on the neck and chest.
- Itching and Scabies : Apply Surobin mixed with Lemon juice and sesame oil on the affected part.
- Nose Bleeding: Surobin mixed with 2 drops of sesame oil into nostrils twice a day.
- Pneumonia and Lumbago: Melt a little beeswax in sesame oil Allow to cool and mix Surobin to it. Massage the oil gently on the affected part.
- Stomach Troubles: In case of Indigestion, Flatulence, Loud Eructating, Loose Motion, Vomiting, Nausea and Dysentery-take 3-4 times daily. (SUROBIN, 1906)

PROPHYLACTIC USE

- Cholera: Take Surobin with cold water for 2-3 times a day.
- Plague and Other epidemics: 3-4 drops of Surobin with cold water should be taken by whole family 2-3 times daily.

  (SUROBIN, 1906)

1.4.2 BALARISTA®

General tonic

Description:

Hamdard Balarista is effective in malnutrition, general debility, loss of weight, anorexia, rheumatism. **(**Balarista, 2009)

Composition:

Each 5 ml syrup contains

Sida cordifolia 1.35 g,

Withania somnifera 1.35 g,

Fritillaria roylci 28.15 mg,

Ricinus communis 28.15 mg,

Vanda roxburghii 14.08 mg,

Amomum subulatum 14.08 mg,

Paederia aromaticum 14.08 mg,

Syzygium ingredients 14.08 mg

& other ingredients Q.S

Figure 2: Hamdard Product Balarista

(Balarista, 2009)

Direction:

Adults: 1-2 teaspoonfuls twice daily.

Children: *VI* teaspoon twice daily or as directed by the physician. **(**Balarista, 2009)

Dosage:

Adults: 2-4 teaspoonful 2-3 times daily or as directed by the physician.

Contraindication:

There is no known contraindication. (Balarista, 2009)

Side effect:

No significant side effect has been observed in proper dosage. (Balarista, 2009)

Precaution:

Keep out of reach of the children. (Balarista, 2009)

Storage:

Protect from light. Keep in cool & dry place. Shake well before use. (Balarista, 2009)

Presentation:

Amber glass bottle containing 450 ml syrup. (Balarista, 2009)

Diclofenac

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in certain conditions. The name is derived from its chemical name: 2-(2, 6-dichloranilino) phenyl acetic acid. (Diclofenac, 2012)

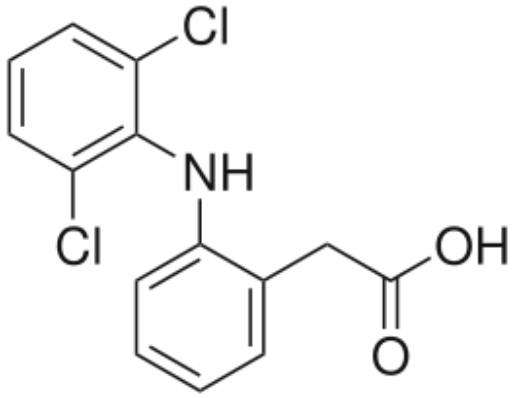


Fig 3: Structure of Diclofenac

Medical uses

Diclofenac is used to treat pain, inflammatory disorders, and dysmenorrhea.Diclofenac is used commonly to treat mild to moderate post operative or post-traumatic painAs long-term use of diclofenac and similar NSAIDs predisposes for peptic ulcer,eye-drops are sold to treat acute and chronic non-bacterial inflammations of the anterior part of the eyes (e.g., postoperative states). (Diclofenac, 2012)

Trade names

Diclofle

Diclogem

Dolex

Ortofen

Seradic

Voltaren

Votrex

Zipsor

(Diclofenac, 2012)

Mechanism of action

The exact mechanism of action is not entirely known, but it is thought that the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis.

Inhibition of COX also decreases prostaglandins in the epithelium of the stomach, making it more sensitive to corrosion by gastric acid. This is also the main side-effect of diclofenac. Diclofenac has a low to moderate preference to block the COX2-isoenzyme (approximately 10-fold) and is said to have, therefore, a somewhat lower incidence of gastrointestinal complaints than noted with indomethacin and aspirin.

The action of one single dose is much longer (6 to 8 hours) than the very short half-life that the drug indicates. This could be partly because it persists for over 11 hours in synovial fluids.

There are marked differences among NSAIDs in their selective inhibition of the two subtypes of cyclo-oxygenase, COX-1 and COX-2. Much pharmaceutical drug design has attempted to focus on selective COX-2 inhibition as a way to minimize the gastrointestinal side-effects of NSAIDs like aspirin.

Besides the well-known and often-cited COX-inhibition, a number of other molecular targets of diclofenac that could contribute to its pain-relieving actions have recently been identified. These include:

- Blockage of voltage-dependent sodium channels (after activation of the channel, diclofenac inhibits its reactivation also known as phase inhibition)
- Blockage of acid-sensing ion channels (ASICs)
- Positive allosteric modulation of KCNQ- and BK-potassium channels diclofenac opens these channels, leading to hyperpolarization of the cell membrane. (Diclofenac, 2012)

Contraindications

- Hypersensitivity against diclofenac
- History of allergic reactions (bronchospasm, shock, rhinitis, urticaria) following the use of aspirin or another NSAID
- Third-trimester pregnancy
- Active stomach and/or duodenal ulceration or gastrointestinal bleeding. (Diclofenac, 2012)

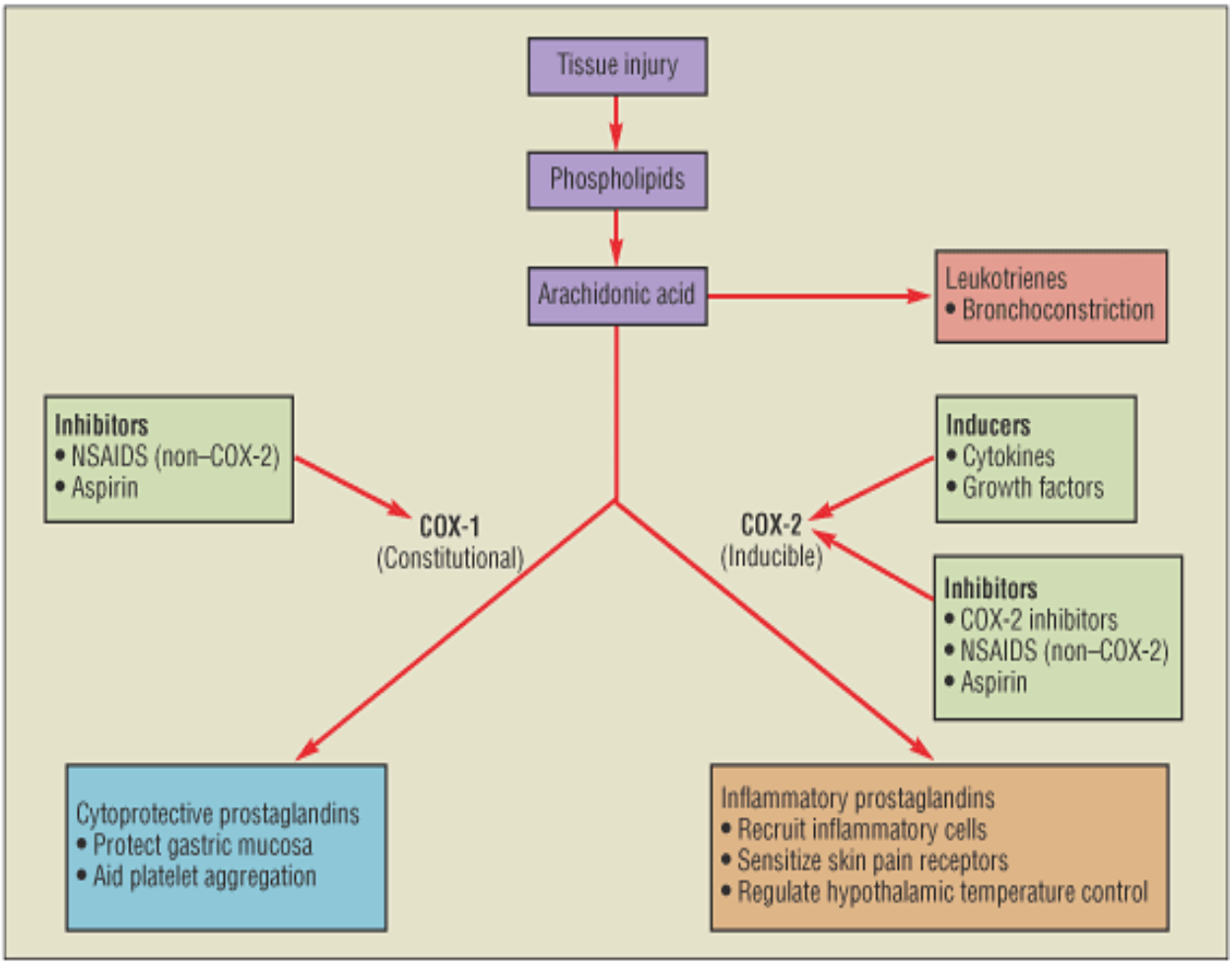


FIGURE 1. Algorithm of the biochemical pathway shows that the formation of prostaglandins occurs via both cyclooxygenase enzymes (COX-1 and COX-2).

(Stovitz, S. D. 2003)

SIDE EFFECTS

- Upset stomach,
- Nausea,
- Heartburn,
- Diarrhea,
- Constipation,
- Headache,

- Tiredness,
- Drowsiness,
- Dizziness,
- Swelling of the hands or feet (edema),
- Sudden or unexplained weight gain,
- Hearing changes (such as ringing in the ears),
- Mental/mood changes,
- Difficult/painful swallowing,
- Change in the amount of urine,
- Unexplained stiff neck,
- Allergic reaction, including: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing, should be consulted with doctor. (Diclofenac sodium Oral,2011)

Chapter 2: Literature review

2.1 Pharmacological Studies of Sorobin

1. The antipyretic activity: Puspak Jyoti Singh et al., designed to investigate the analgesic and antipyretic activities of ethanolic and aqueous extracts of *Terminalia bellirica* (family: Combrataceae) fruits (200 mg/kg, p.o.) in acetic acid-induced writhing, Eddy's hot plate method and brewer's yeast-induced fever models in mice and rats. Both extracts showed a significant decrease in the number of the writhes in acetic acid-induced writhing and increase in paw licking time to heat stimuli in the hot plate method. Both extracts showed a significant inhibition of elevated body temperature when compared to corresponding control. The results suggested that the ethanolic and aqueous extracts possessed significant analgesic and antipyretic activities. (Uma Shankar Sharma et al., 2010)

2. The Analgesic activity: Anilkumar Patra et al., evaluated the analgesic activity of aqueous leaf extract of *solanum nigrum* using hot plate, tail flick & acetic acid induced writhing method. Aqueous extract of *solanum nigrum* show significant analgesic effect ($P<0.05$) as compared to control at a dose of 50mg/kg and at 100mg/kg oral dose it shows analgesic effect equally significant with standard (i.e. diclofenac 100mg/kg IP). Hence finally it is concluded that *solanum nigrum* may possess opioid analgesic activity. (K. Bhavani et al., 2010)

3. The Anti-inflammatory activity: K. M. Y. Amin et al., studied with an increased incidence of drug toxicity and resistance to allopathic drugs, natural products from plants could be Interesting alternatives. Some plant extracts and phytochemicals are known to have anti-analgesic properties, and can be of great significance in treatment of pain related disorders. These considerations require the

scientific evaluation of the most important and commonly used traditional herbal formulations. A study has been done to find anti-analgesic activity of Unani formulation derive from medicinally important plants like *Zingiber officinal* (Ginger), *Colchicum luteum* (Colchicum), and *Aloe barbadensis* (Aloe). In this proposed work, modified powder of different mentioned plants into its solid state (tablet) by using gum acacia extracts showed a significant decrease in the number of the writhes in acetic acid-induced writhing. Its 50% alcoholic extract and aqueous extract were used to determine its anti-analgesic activity. Efficacy of Unani formulation was compared with a standard referent drug, Diclofenac sodium. Results clearly indicate that, modified form of the test formulation possesses significant anti-analgesic activity in both acute and sub-acute phase. (Aziz ur Rahman et al., 2011)

2.2 Activity Studies of Balarista

1. antioxidant activity: Bhattacharya SK et al., studied with active principles of *Withania somnifera,* consisting of equimolar concentrations of sitoindosides VII-X and withaferin A, was investigated for their effects on rat brain frontal cortical and striatal concentrations of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) for antioxidant activity. Results were compared with effects induced by deprenyl, an agent with well documented antioxidant activity. Active glycowithanolides of *W. somnifera* (WSG) (10 and 20 mg/kg, i.p.), administered once daily for 21 days, induced a dose-related increase in SOD, CAT and GPX activity in frontal cortex and striatum, which was statistically significant on days 14 and 21, except with the lower dose of WSG on GPX activity, where the effect was evident only on day 21. The data were comparable to those induced by deprenyl (2 mg/kg/day, i.p.) with respect to SOD, CAT and GPX activities, which were evident by day 14. These findings are consistent with the therapeutic use of *W. somnifera* as an Ayurvedic rasayana and medhyarasayana. Antioxidant effect of active principles of *W. somnifera* may explain, at least in part, the reported antistress, immunomodulatory, cognition-facilitating, anti-inflammatory and anti-aging effects produced by them in experimental animals, and in clinical situations. (Satyan KS et al., 2008)

2. The genotoxicity test: Erasmus E et al., said that the antioxidant properties of the fruit of the *Rosa roxburghii* (RR) plant have been associated with several putative health promoting effects. The possible cytotoxic, mutagenic/antimutagenic and genotoxic effects of RR fruit extract were investigated. The effect on antioxidant status and protection against induced oxidative stress were also investigated using primary rat hepatocytes. A RR fruit extract containing 45 g/L total ascorbic acid and 65 g/L total polyphenols was used in this study. Dilutions up to 0.08% (v/v) increased significantly the antioxidant status in primary rat hepatocytes. The glutathione redox state was decreased with RR treatment but was increased in Chang liver cells and MT-2 lymphoblast. No cyto- or genotoxicity were observed at levels of up to 5% (v/v) of the fruit extract. In addition, a significant protection against t-BHP induced oxidative stress was observed in primary rat hepatocytes. The Ames test revealed no mutagenic activity using the Salmonella typhimurium strains TA98, TA100 and TA102. A significant antimutagenic effect of the extract was observed against the metabolic activated mutagens 2-acetylaminofluorene and aflatoxin B1 and to a lesser extent against methyl methanesulfonate. It is concluded that these results support the associated health promoting potential of Rosa Roxburghii fruit and in particular against oxidative stress. (Van Der Westhuizen FH et al., 2008)

3. anti oxitoxic activity: Ghosal S et al., evaluated the antioxidant activity of *Withania somnifera* (WS) glycowithanolides was assessed in chronic footshock stress induced changes in rat brain frontal cortex and striatum. The stress procedure, given once daily for 21 days, induced an increase in superoxide dismutase (SOD) and lipid peroxidation (LPO) activity, with concomitant decrease in catalase (CAT) and glutathione peroxidase (GPX) activities in both the brain regions. WS glycowithanolides (WSG), administered orally 1 h prior to the stress procedure for 21 days, in the doses of 10, 20 and 50 mg/kg, induced a dose-related reversal of the stress effects. Thus, WSG tended to normalize the augmented SOD and LPO activities and enhanced the activities of CAT and GPX. The results indicate that, at least part of chronic stress-induced pathology may be due to oxidative stress, which is mitigated by WSG, lending support to the clinical use of the plant as an antistress adaptogen. (Bhattacharya SK et al., 2001)

Chapter 3: Statement of purpose

Unani medicines are the form of herbal products. In modern science allopathic drugs are more valuable than herbal. But from history we can say allopathic drug are modernized from of herbal.

My objective was to establish a value of herbal products to general people. For that evaluation is done for analgesic activity [Experimental (Sorobin)] through *in-vivo* test with Swiss albino mice procured from ICDDRB. Quantitative analysis using DPPH method for antioxidant activity [Experimental (Balarista)] was done as *in-vitro.*

Consider a wide spread use of Unani medicines one cannot emphasize enough the need for establishing the safety profiles of Unani medicines. Keeping on mind, the present scenario this research work on Unani preparation, explore a spectram of its pharmacological aspects utilizing experimental animals. The objective is to have a better understanding of the
Possible pharmacological profile of the drug under the study .This study was performed in an effort to evaluate the activity according to modern pharmacological parameters so as to fully fathom the activity of these preparations for alternative medical care.

Chapter 4: Materials

ANALGESIC ACTIVITY

(Acetic Acid Induced Writhing Test)

4.1. ANIMAL

4.1.1. SWISS ALBINO MICE

For the experiment male Swiss albino mice of 1 – 2 weeks of age, weighingbetween 20 -25 gm, were collected from the animal research branch of the international center for diarrheal disease and research, Bangladesh (ICDDRB).Animals were maintained under standard environmental conditions temperature 23.0 ± 2.0 ^{o}C , relative humidity: 55 – 65% and 12 h light/12h dark cycle) and had free access to feed and water and water ad labium.The animals were acclimatized to laboratory condition for one week to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee [Hossain, M.M.2009].

Figure 5: Swiss albino Mice

Table 1: Materials used in analgesic activity method

Drugs	Sources	Chemicals	Instruments	Sources
*Sorobin tablet(experimenl) *Normal Saline *Diclofenac sodium 50mg/tablet (CLONAC®)	*Hamdard **Beximco pharma **Somatec Pharmaceutical Ltd.	*Tween 80 (polysorbate 80) *Acetic acid	*Injection (100 unit) *Needle *Syringe *Beaker *Weight Balance *Pump *Pipette *Volumetric flask	*EWU Lab (Chemicals and Instruments)

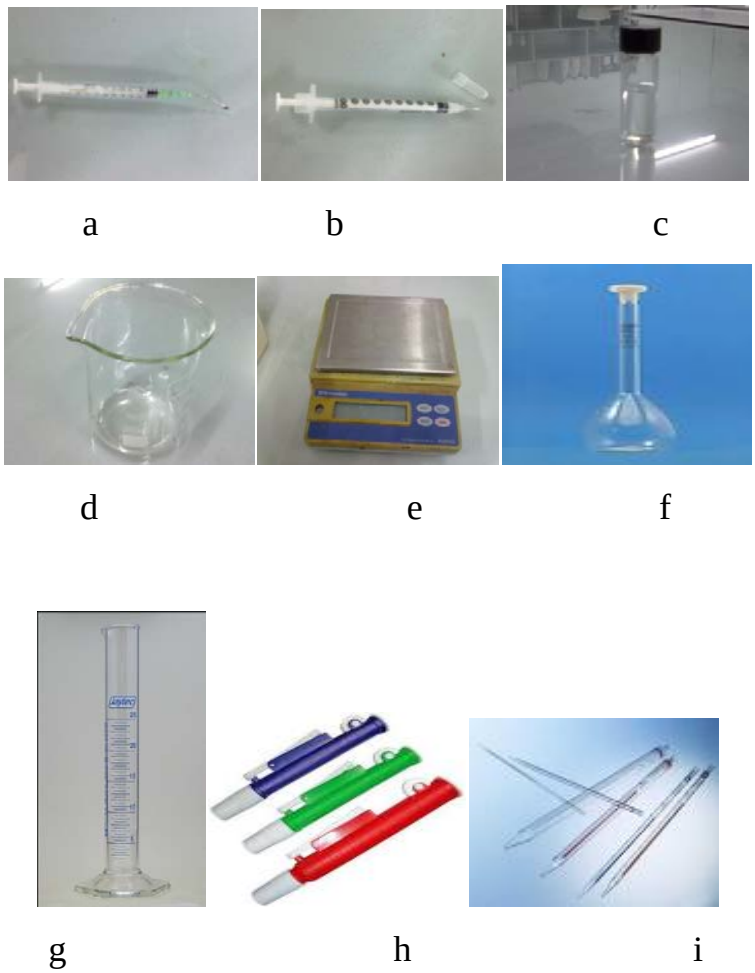


Fig.6 : a. Needle; **b.** Injection; **c.** Vial; **d.** Beaker; **e.** Weight balance; **f.** Volumetric flask; **g.**measuring cylinder; **h.** Pump; **i.** Pipette;

ANTIOXIDANT ACTIVITY

(Determination of DPPH radical scavenging assay)

Table 2: Materials used in antioxidant activity method

Drug	Source	Chemicals	Instrument	Sources
*Balarista syrup (experimental)	*Hamdard	*DPPH (2,2-dipheny1-1-picrylhydrazyl) *Methanol Distilled water	*pipette and pump *Capillary tube *test tubes and rack	*EWU Lab (Chemicals and Instruments)

Chapter 5: Method

ANALGESIC ACTIVITY
(WRITHING TEST)

5.1 ANALGESIC ACTIVITY
(WRITHING TEST)
5.1.1 Evaluation of analgesic property

Pain is probably the most prevalent symptom in clinical practice, and characterization of pain is of major importance in the diagnosis and choice of treatment (Thumshirn *et al*., 1999). In the treatment of diseases associated with pain, the clinical effects typically guide the selection of the analgesics and titration of the dose. However, in practice, the different symptoms of the underlying diseases confound the characterization of pain. These confounders may include complaints relating to psychological, cognitive and social aspects of the illness, as well as systemic reactions such as fever and general malaise (Drewes *et al*., 2003).

Furthermore, treatment with analgesics often causes sedation and other side effects. This may bias the clinical evaluation, as the patients tend to interpret other effects of the medication– such as an effect on the anxiety and depression relating to the disease – as a relief of pain (Le Bars *et al*., 2001).

Because of these confounding factors, *experimental pain models* are often advantageous in preclinical investigations of analgesics. With these models, the investigator can control the experimentally induced pain (including the nature, localization, intensity, frequency and duration of the stimulus), and provide quantitative measures of the psycho-physical, behavioral or the neurophysiological responses (Drewes *et al*., 2003).

Experimental pain models have been used in *animal studies.* In these experiments, the neuronal nociceptive activity can be recorded or behaviour can be assessed (Sengupta & Gebhart 1994). However neuronal recordings or reactions do not reveal all aspects of pain, since pain is the net effect of complex

multidimensional mechanisms that involve most parts of the central nervous system (Le Bars *et al*., 2001).

Nociceptive reflexes or electrophysiological recordings from selected pathways in the animal nervous system are important in basic research and screening of analgesics. However, animal experiments typically suppress central pain mechanisms and associated complex reactions seen in man. Furthermore, the neurobiology of nociceptive systems differs between species, and this limits the extrapolation of findings from animal studies to man even further (Le Bars *et al*., 2001).

5.1.3 Mechanism of Pain Induction in Acetic Acid Induced Writhing Method of Analgesic Activity Screening

Intra-peritoneal administration of acetic acid (0.7%) causes localized inflammation in mice. Following inflammation, there is biogenesis of prostaglandins (from cyclooxygenase pathway) and leukotrienes (lipooxygenase pathway). The released prostaglandins, mainly prostacyclin (PGl_2) and prostaglandin-E have been reported responsible for pain sensation.

The exact mechanisms by which prostaglandins produce pain are not still clear but there are a number of proposed mechanisms of action:

1. All kinds of pain or noxious stimuli (nociception) are conveyed by specific nerves called un-myelinated C fibers and myelinated A-delta fibers, the former

being slow conducting and the latter being fast conducting. It has been investigation that un-myelinated C fibers are the most usual conveyer of two.

2. The prostaglandin and other liberated products of inflammation serve as noxious stimuli. They are supposed to sensitize C fibers and subsequently reduce pain threshold. The C fibers get stimulated and cause enhanced release of tachykinins, mainly substance P and neurokinins. It is the substance P released in excessive amount following the stimulation of C fibers that has been held responsible for sensation of pain in animal (Le Bars *et al*., 2001).

3. The precise mechanism by which substance P arouses pain sensation is not well documented. But upon release, substance P and other tachykinins bind to specific receptors (NK_1, NK_2, NK_3) that are G-protein coupled. Among these receptors, substance P is specifically bound to NK_1 (Crul *et al*., 2000). After binding of substance P to the respective receptor, there is stimulation of phospholipase C resulting in the formation of two second messengers-Inositol triphosphate (IP_3) and Diacylglycerol (DAG). IP_3 causes the exocytotic release of Ca^{++} stored intra-cellularly and DAG activates protein kinase C which then causes the influx of Ca^{++} through the voltage gated Ca^{++} channel. These happenings may have role in the neural processing of the pain sensation and its subsequent conveyance to higher centers of the brain.

4. Prostaglandins also potentiate the pain producing activity of bradykinins and other autacoids (Rang, 2003).

5.1.4 Principle

In this method (Koster *et al.*, 1959; Whittle, 1964; Vogel & Vogel, 1997; Ahmed *et al.*, 2001) acetic acid is administered intra-peritoneal to the experimental animals to create pain sensation. As a result, the animals squirms their body at regular interval out of pain. This squirm or contraction of the body is termed as “writhing”. As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing of animals within in a given time frame.

5.1.5 Experimental Design

Five experimental animals were randomly selected and received a particular treatment i.e. control and the dose of the drug respectively. Prior to any treatment, each mouse was weighed properly and the dose of the test sample and control materials was adjusted accordingly.

5.1.6 Preparation of Test Materials

In order to administer the drug at dose of 100 mg/kg body weight of mice and was triturated unidirectional way by the addition of small amount of suspending agents Tween-80. After proper mixing of drug and suspending agent, normal saline was slowly added. The final volume of the suspension was made 2.5 ml.

To stabilize the suspension, it was stirred well by vortex mixture. For the preparation of Sorobin at the dose of 100-mg/kg-body weight, was taken and a suspension of 2.5 ml was made.

5.1.7 Method of identification of animals

Each group consists of five animals. It was difficult to observe the biological response of five mice at a time receiving same treatment. It is quite necessary to identify individual animal of groups during treatment. The animals were individualized in the following way i.e. marked as M1=mice 1, M2=mice 2, M3=mice3, M4=mice 4 & M5=mice 5.

Indication of mice:

Mouse-1 Mouse-2 Mouse-3 Mouse-4 Mouse-5

Figure 7: Identification of test animals for analgesic property screening

5.1.8 Procedure

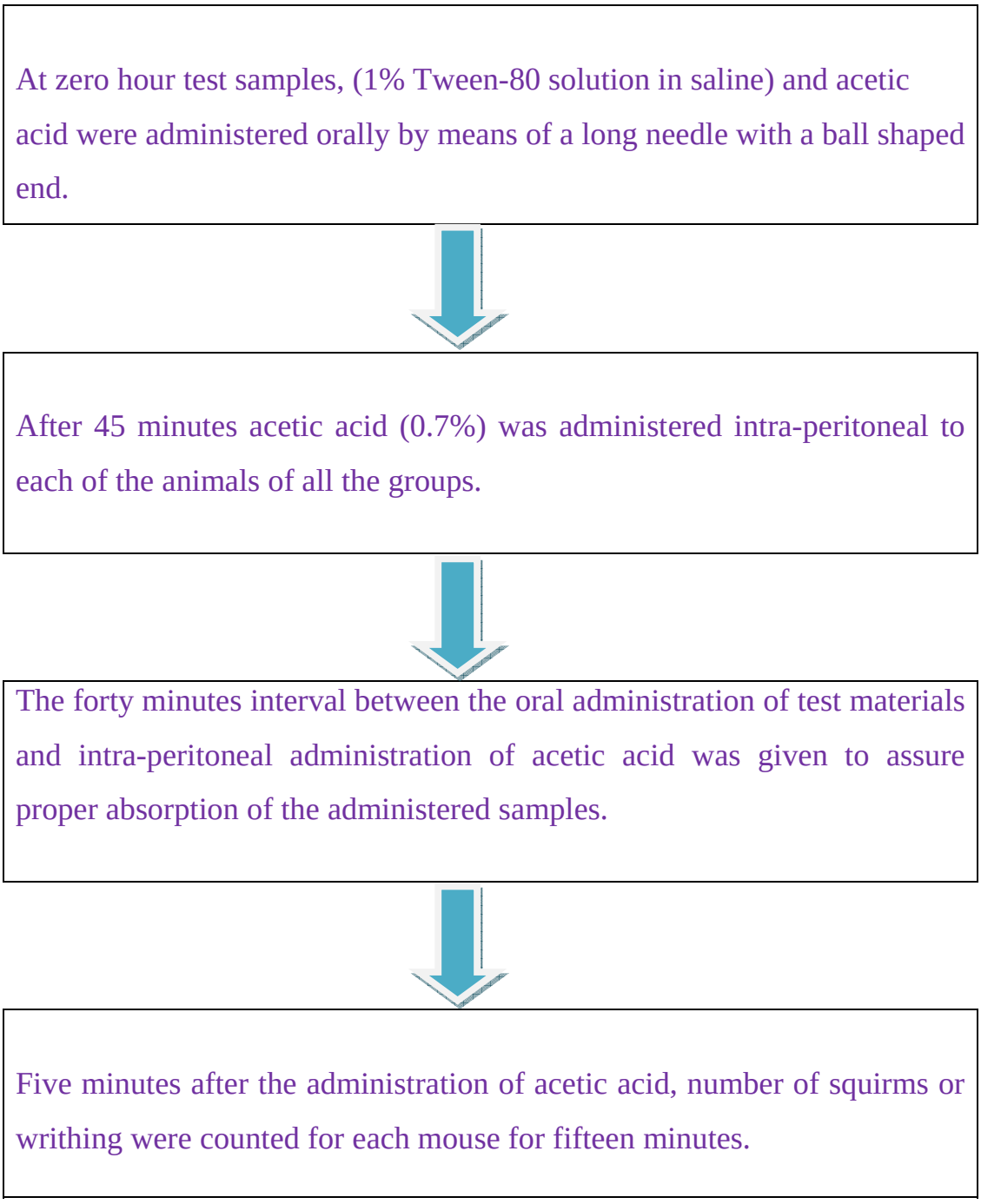


Figure 4: Schematic representation of procedure for screening of analgesic property on mice by Acetic Acid Induced method for Sorobin

Fig 8: Mice with writhing.

5.1.9 Counting of writhing

Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intra-peritoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half- writhing. Accordingly two half- writhing were taken as one full writhing.

5.1.10 Statistical Analysis

Here ANOVA or F test used because of three groups.

The various degrees of significance on activity were determined by analyzing aall the data obtained using SPSS (Statistical Package for Social Science) for WINDOWS (VERSION 12). Data were presented Mean ± SD (standard deviation)

For all the data analyzed, $p<0.05$ was assigned as the level of significance; $p<0.01$ was assigned to represent a high level of significance; $p<0.001$ was assigned to represent a high level of significance.

Equation For,

$$\% \text{ of Writhing} = (\text{mean of treated group} \div \text{mean of control group}) \times 100$$

$$\% \text{ of Writhing inhibition} = (100 - \% \text{ of Writhing})$$

ANTIOXIDANT ACTIVITY

(Determination of DPPH radical scavenging assay)

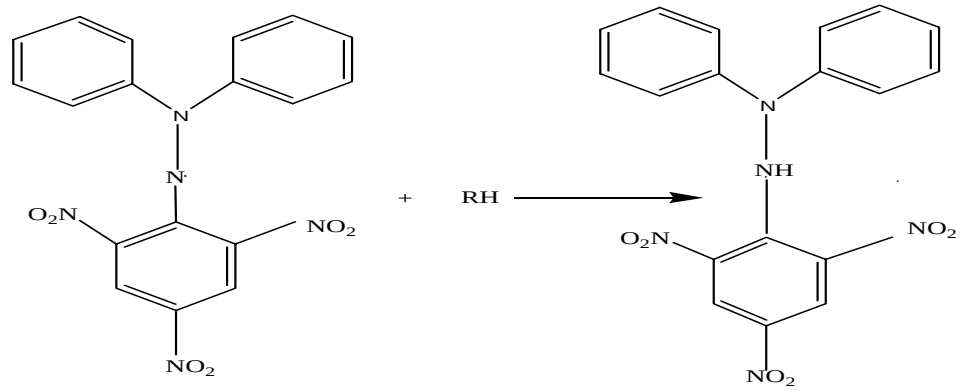
5.2 Antioxidant Activity
(Quantitative Analysis)

5.2.1. Determination of DPPH radical scavenging assay

5.2.1.1 Introduction

The free radicals species contain unpaired electrons. The oxygen radicals, icluding superoxide radical (O^{2-}), hydroxyl radical (OH-) and non-free radical species, such as H_2O_2 and singlet oxygen ($1O_2$), are various forms of activated oxygen (Gulcin *et al*., 2002; Yildirim *et al*., 2000), generated in many redox processes. These radicals are trapped and destroyed by specific enzymes, such as superoxide dismutase, catalase and glutathione peroxidase. Overproduction of free radicals, together with A, C and E avitaminosis and a reduced level of the above mentioned enzymes, is considered to be the main contributor to oxidative stress (Ellnain-Wojtaszek *et al*., 2003).

Besides, excessive generation of ROS, induced by various stimuli and which exceed the antioxidant capacity of the organism, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity, and cancer (Kourounakis *et al*., 1999, Gulcin *et al*., 2002, Gulcin *et al*., 2003). Numerous methods are available for determining the presence and quantification of the degree of antioxidant activity present in the plants extracts. Most of these methods make use of a color reaction and indicator to assess the degree of antioxidant activity. DPPH assay is a qualitative indicator of a free radical scavenging activity. DPPH is reduced from a stable free radical that is purple in color to diphenyl picryl hydrazine that is yellow, in the presence of an antioxidant (Roberta *et al*., 1999).



*DPPH (Oxidized form)

DPPH (Reduced form)

5.2.1.2. Principle

A rapid, simple and convenient method to measure free radical scavenging capacity of antioxidants involves the use of the free radical, 1,1-Diphenyl-2-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. DPPH is a stable nitrogen centered free radical with purple color and the odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm. When the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H, then the color turns from purple to yellow as the molar absorptivity of the DPPH radical reduces from 9660 to 1640 at 517 nm. Scavenging of DPPH free radicals by antioxidants decreases the absorbance. The lower the absorbance at 517 nm, the greater the free radical scavenging capacity of the crude extracts.

5.2.1.3. Methods

- 2.0 ml of a methanol solution of the extract at different concentration (2, 4, 6, 8, 10µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml).
- After 30 min reaction period at room temperature in dark place the absorbance was measured against at 517 nm against methanol as blank by using a UV- visible spetrophotometer.
- Inhibition free radical DPPH in percent (I%) was calculated as follows:

  $(I\%) = (1 - A_{\mathbf{sample}}/A_{\mathbf{blank}}) \times 100$

Where $A_{\mathbf{blank}}$ is the absorbance of the control reaction (containing all reagents except the test material).

- Extract concentration providing 50% inhibition *(IC_{50})* was calculated from the graph plotted inhibition percentage against extract concentration.
- L-Ascorbic acid was used as positive control.
- Tests carried out in triplicate and average value was taken. 2.0 ml methanolic solution of extract (conc. 2, 4, 6, 8, 10µg/ml) 3.0 ml DPPH methanolic solution (conc. 20µg/ml) Purple colour

Calcualtiion of *IC_{50}* value from the graph plotted inhibition percentage against extract concentration Absorbance measured at 517 nm using methanol as blank Decolorization of purple color of DPPH

Reaction allowed for 30 minutes in absence of light at room temperature

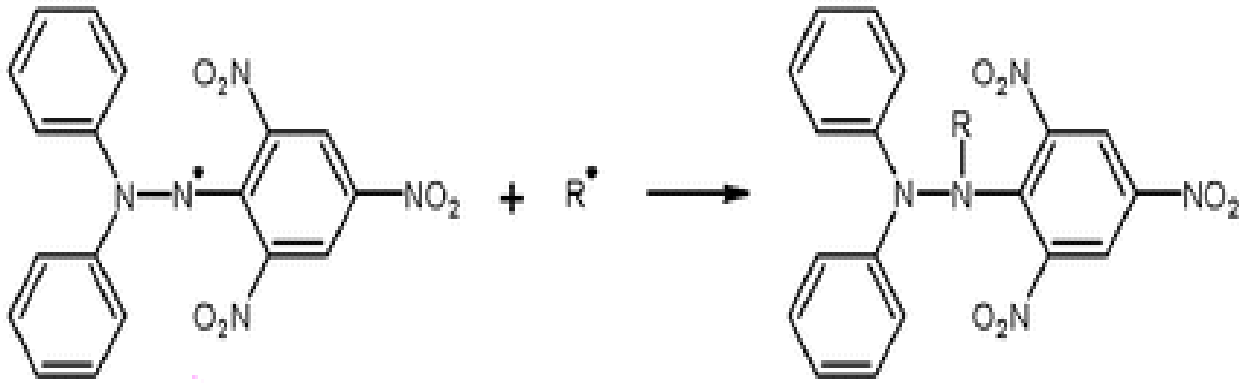


Figure: 9 Schematic representation of the method of assaying free radical scavenging activity. (Badarinath,A. V. et.,2010)

Chapter 6: Results and Discussion

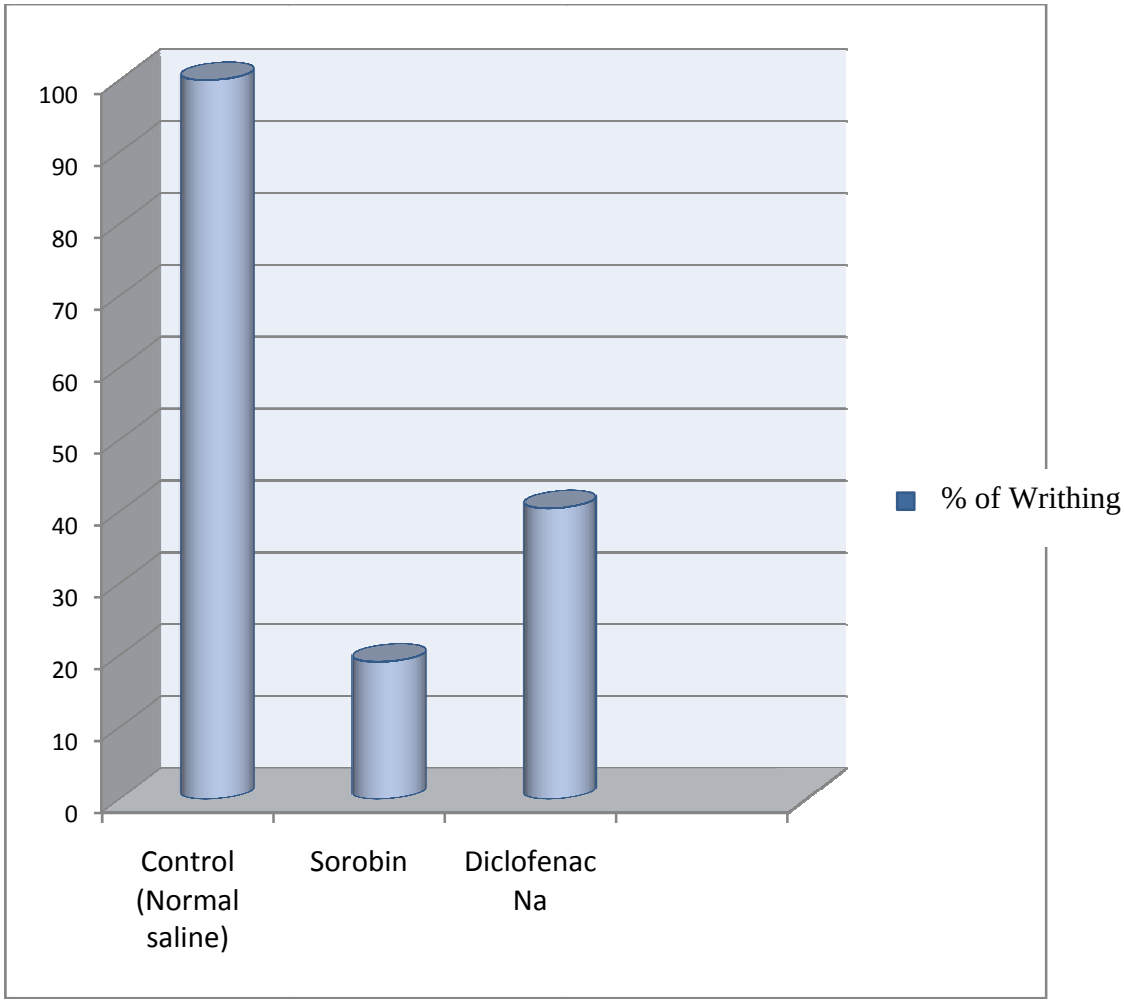
6.1 Analgesic activity of Sorobin

Table: 3 Analgesic activity of Sorobin

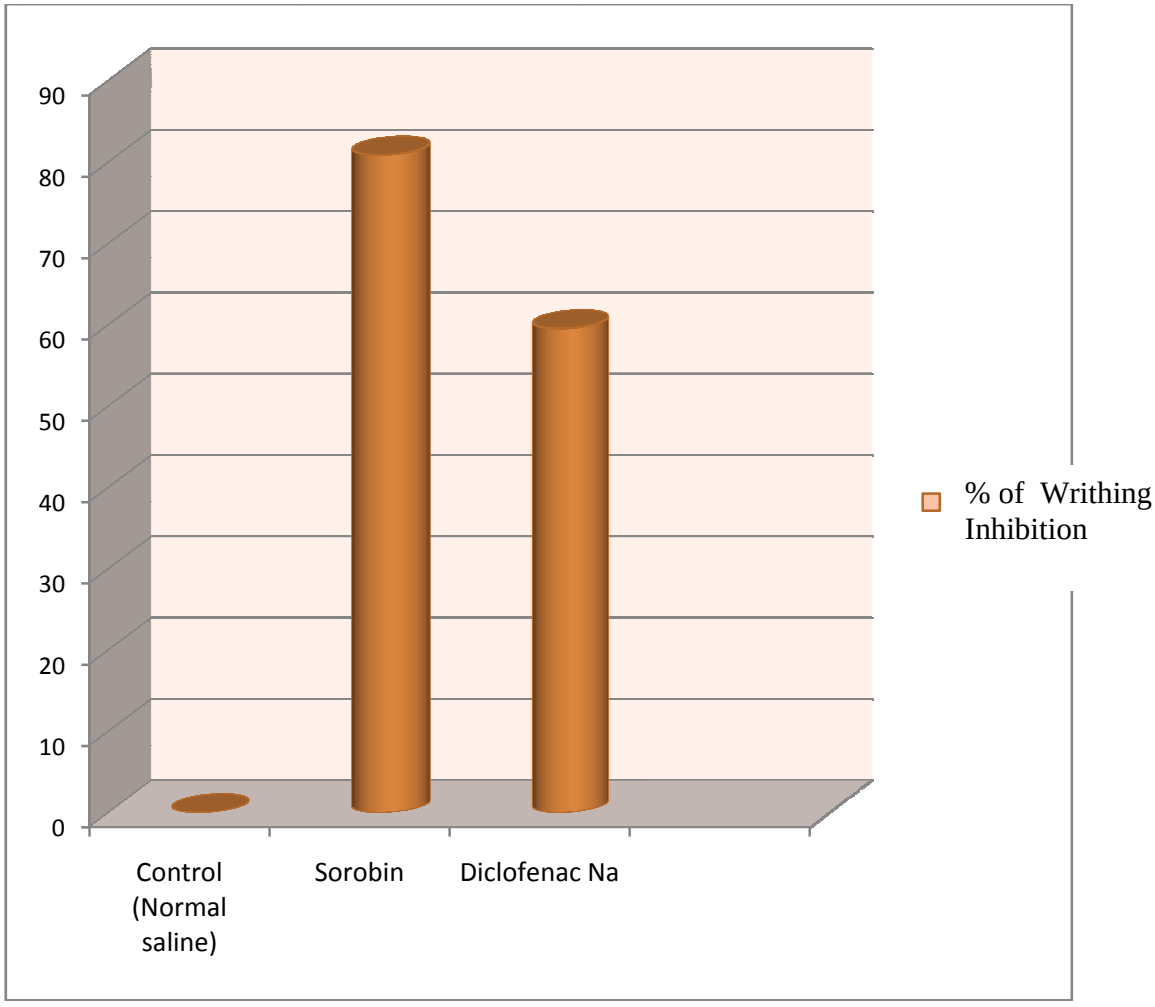
Animal Group	Mean	Standard deviation	% of writhing	% of inhibition
Control (Normal saline)	17.8	7.2	100	0
Sorobin	3.4	2.8	19.101	80.898
Diclofenac Na	7.2	3.7	40.449	59.550

Grouped Data:

Mice	M1	M2	M3	M4	M5
Control	14	16	20	19	20
Standard	10	7	6	5	8
Sorobin	4	2	6	2	3



Graph 1: Group Vs % of Writhing



Graph 2: Group Vs % of Writhing Inhibition

The diagram (1) showed relation between groups and % of writhing given by Swiss albino mice. Control (normal saline) , Standard (Diclofenac Na) , Experimental (Sorobin) represent the groups.

Control gave 100% writhing where Diclofenac Na and Sorobin gave 40.449 % and 19.101% Writhing respectively.

Diagram (2) showed % % of writhing inhibition occurred after passing several time. Control showed no inhibition of writhing. The capacity of writhing inhibition was better in Diclofenac Na (7.2±3.7) than Sorobin (3.4±2.8)

So, Sorobin (experimental) has analgesic activity as it showed % of writhing less than control. But its effect in writhing inhibition was less than Standard (Diclofenac Na).

Further experiment must be done to know the ingredient which is responsible for this effect.

ANOVA Test:

Single Factor

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	5	89	17.8	7.2
Diclofenac	5	36	7.2	3.7
Sorobin	5	17	3.4	2.8

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	556.9	2	278.466	60.9781	5.17E-07	3.8852
Within Groups	54.8	12	4.56666			
Total	611.7	14				

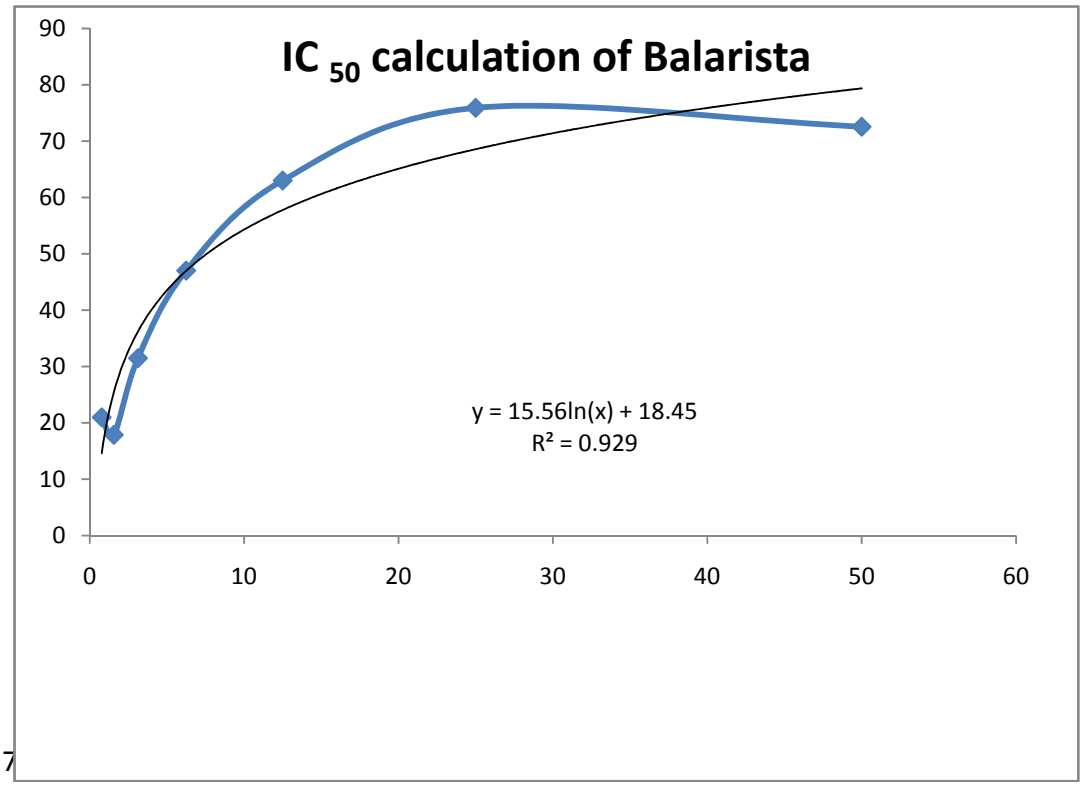
From above result it's clear that p value is very much less than 0.001which is assign to represent a high level of significance among the groups. So they are significantly different. The result occurred here are not by chance and if this experiment was repeated 100 times more than 99 times the experiment will yield these same results

6.2 ANTIOXIDANT ACTIVITY OF BALARISTA:

Determination of DPPH radical scavenging assay

Table: 5 Antioxidant activity of Balarista

Blank	Concentration	Absorbance	Inhibition %
	50	0.115	72.55
	25	0.101	75.89
0.419	12.5	0.155	63.01
	6.25	0.222	47.02
	3.125	0.287	31.5
	1.5625	0.344	17.9
	0.78125	0.331	21

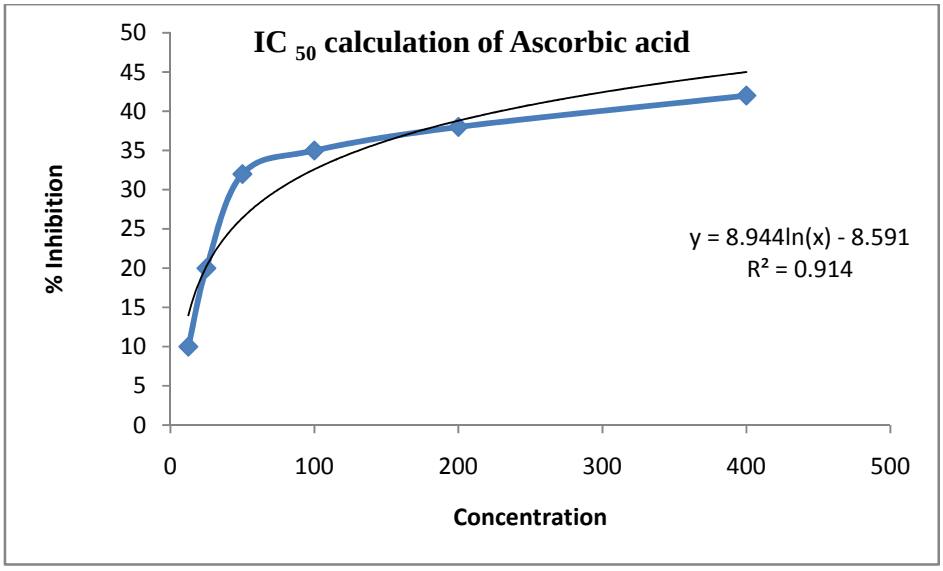


X= 7

Balarista has antioxidant activity which found by Quantitative analysis of DPPH method. We know that 1-50 microgram/ml range represent good activity and IC_{50} value of Balarista is 7.596. So it indicates that strong activity of the drug.

Antioxidant activity Test of Ascorbic Acid as a Standard

Blank	CONC	% Inhibition
	12.5	10
	25	20
	50	32
0.419	100	35
	200	38
	400	42



IC_{50} value of Ascorbic acid = 14.72

So Balarista has better activity than standard

Further experiment is required to know the ingredient which is responsible for this effect.

Chapter 7: Conclusion

“Unani” have their own basis and principles. Unani herbs or herbal extracts in different forms along with some ingredients of animal or mineral origin. In modern medicine or allopathy, treatment is based on symptoms, and is based on “cause and effect”.It focuses on what physical being causes the ailment and what can be done to “cause and effect”. It focuses on what physical being causes the ailment and what can be done to cure it. In a way, both systems have one basic role, treatment of ailments and keeping the body and mind well. Modern medicine allopathy has the advantage of extensive research. Unani lack this. All drugs included as unani should be proven to perform their specific actions and researched for side effects. Once there is proper specific actions and researched for side effects. Once there is proper scientific evidence and backing, unani drugs can be used in synergy with allopathic drugs or in compliment to each other. This will increase the credibility of the drugs. The writhing inhibition Sorobin (3.4±2.8) was less than Diclofenac Na (7.2±3.7). Balarista has antioxidant activity which was found by free radical scavenging activity using DPPH with IC_{50} value of 7.596.

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