ORIGINAL ARTICLE
Discovery Phytomedicine 2019, Volume 6, Number 1: 1-11

# In-vivo evaluation of Anti-nociceptive, Anti-inflammatory, Antipyretic, Hypoxia and Gastro-intestinal potentials of SwasKas Chintamani Ras

Tarequl Islam,[1] Md. Saddam Hussain,[2] Ashraful Mahmud Tuhin,[2] K. M. Rahat Maruf Jitu,[2] Abida Sultana,[3] Otun Saha,[1] Dos Mohammad,[3] Md. Shahadat Hossain,[3] Mohammad Nurul Amin[2,4*]

## ABSTRACT

**Background:** SwasKas Chintamani Ras (SKC) an Ayurvedic preparation used for respiratory disease was tested for the pharmacological study using small laboratory animals. The purpose of the experiment is to study the analgesic and anti-inflammatory, antipyretic activity, drug's property to modify the survival time, gastro-intestinal effects/side-effects.

**Method:** Analgesic and anti-inflammatory effect of the drug was carried out by three complementary test methods namely formalin induced paw licking test, xylene induced ear edema test and acetic acid writhing test. Antipyretic activity of the drug was determined by infra-red thermometry test. Drug's property to modify the survival time of mice was studied by hypoxia test. For investigating the gastro-intestinal effects / side-effects gastric emptying test, gastro-intestinal motility test, colon transit time test and castor oil induced anti-diarrhoea test were conducted. Mild analgesic and anti-inflammatory effect was observed by formalin induced paw licking test and xylene induced ear edema test in SKC treated animal that was more prominent in female mice.

**Results:** SKC at a dose of (100 mg/kg) showed significant analgesic effect, surprisingly reverse effect was observed at the higher doses. SKC treated male/female mice exhibit overall decrease in body temperature at dose 100 mg/Kg in the experimental period. SKC results in negligible decrease in gastric emptying at 2nd hour but increases in gastric emptying at 4th hour in experimental male mice. SKC at the dose of 100 mg/kg, showed an increase in the gut motility from the early 1sthrs to 4th hr. SKC also showed increase in colon transit time indicating possibility of potential laxative effect.

**Conclusion:** It can be said from this experiment that SKC is devoid of any prominent side effects, further in-depth toxicological study is recommended to validate the safety of this Ayurvedic drug.

**Key Words:** SKC, Analgesic, Anti-inflammatory, Antipyretic activity, Gastro-intestinal effects.

*Correspondence to:
Mohammad Nurul Amin,
Senior Lecturer & Coordinator,
Department of Pharmacy, Atish Dipankar University of Science and Technology, Bangladesh
amin.pharma07@gmail.com

**Cite This Article:**
Islam,T., Hussain, Md.S., Tuhin, A.M., Jitu, K.M.R.M., Sultana, A., Saha, O., Mohammad, D., Hossain, Md.S., Amin, M.N. 2019. In-vivo evaluation of Anti-onciceptive, Anti-inflammatory, Antipyretic, Hypoxia and Gastro-intestinal potentials of SwasKas Chintamani Ras. *Discovery Phytomedicine* 6(1): 1-11. DOI:10.15562/phytomedicine.2019.76

## INTRODUCTION

The look for ideal analgesic, anti-inflammatory, anti-diarrheal and antipyretic drugs continues to be on regardless of the creation of numerous new drugs over the last century. None of the available agents, but, have appreciable safety profiles.[1] Often patients turn to the conventional drugs for the treatment of continual inflammatory problems like osteoarthritis or rheumatoid arthritis. Scientific proof for the claimed medicinal residences of remedies or pills from the traditional structures of drugs is sparse.

SwasKas Chintamani Ras is an Ayurvedic medicinal drug available in tablet or pill form, used in the treatment of respiratory diseases. This formulation contains heavy metal substances, subsequently must most effective be taken underneath strict clinical supervision. This medicine is maximum usually used in north Indian Ayurvedic exercise.[2] It is used in the Ayurvedic treatment of bloodless, cough, bronchitis, bronchial asthma, and such other breathing diseases. One 125 – 250 mg a few times an afternoon, before or after food or as directed by way of an Ayurvedic doctor. It is advised in conjunction with lengthy pepper and honey.[2] It is run for a duration of one month. Self-medicine with this remedy might also show to be risky because it contains the heavy metal component. Take this medication in precise dose and for the restrained time period, as advised by health practitioner. Over-dosage may additionally purpose server toxic impact. It is high-quality avoided in being pregnant, lactation and in children.[2]

Pain is an unsightly sensory and emotional experience associated with real or potential tissue

[1]Department of microbiology, Noakhali Science and Technology University, Bangladesh
[2]Department of pharmacy, Noakhali Science and Technology University, Bangladesh
[3]Department of Applied Chemistry and Chemical Engeering, Noakhali Science and Technology University, Bangladesh
[4]Department of Pharmacy, Atish Dipankar University of Science and Technology, Bangladesh

damage.[3] By appearing within the CNS or at the peripheral pain mechanism, analgesic compounds selectively relieves pain without large alteration of attention. Actually, analgesics are implemented whilst the noxious stimulus can't be eliminated or as adjuvant to extra etiological approach to ache.[4]

Inflammation is the reaction of dwelling tissues to damage. It entails a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and restores. Non-steroidal anti-inflammatory capsules (NSAID) are some of the maxima typically prescription drugs due to their steady effectiveness inside the remedy of ache, fever, inflammation and rheumatic issues. However, their use is related to negative consequences at the extent of the digestive tract, ranging from dyspeptic symptoms, gastrointestinal erosions and peptic ulcers to extra serious headaches, which include bleeding or perforation.[5] Therefore to triumph over the toxicity of NSAID, the improvement of new anti-inflammatory tablets remains necessary and the natural product inclusive of medicinal flora may want to lead in coming across new anti-inflammatory tablets with less unwanted outcomes.[6]

Pyrexia or fever is generally induced as a secondary effect of infection, tissue harm, irritation, graft rejection and malignancy or other disease states. The frame by using its herbal protection mechanism creates an environment in which infectious agent or damaged tissue cannot survive. Generally, inflamed or damaged tissue initiates the improved formation of seasoned-inflammatory mediators (cytokines like interleukin 1β, α, β and TNF-α) which increase the synthesis of prostaglandin E2 (PGE2) close to preoptic hypothalamus place and thereby triggering the hypothalamus to raise the body temperature.[7] Most of the antipyretic capsules usually save you or inhibit COX-2 expression to lessen the extended frame temperature with the aid of inhibiting PGE2 biosynthesis. Moreover, those artificial marketers irreversibly inhibit COX-2 with excessive selectivity that is poisonous to the hepatic cells, glomeruli, cortex of mind and heart muscle groups, while natural COX-2 inhibitors usually have decreased selectivity with fewer side effects.[8] Our important intention became to evaluate the in vivo analgesic, anti-inflammatory, antipyretic, hypoxia and gastrointestinal potentials of SKC to validate its traditional uses. After reviewing the current literature, it becomes discovered that there had been no research executed to validate claims of SKC as a whole aggregate for anti-inflammatory, antipyretic, and analgesic activities.[8,9] Hence the present examine became planned to assess an analgesic, anti-inflammatory, antipyretic, hypoxia and gastrointestinal potentials of commercially to be had general coaching of SKC, which turned into prepared as per the recommended approach in Ayurveda. Keeping in view the opportunity of simultaneous consumption of SKC with modern anti-inflammatory-analgesic capsules through sufferers, their combined activities were evaluated in experimental models of inflammation.[8,9] The consequences of in advance studies in which SKC has continuously shown efficacy in fashions of acute inflammation hinted on the opportunity of prostaglandin synthesis inhibition because of the probable mechanism of motion.[9] By virtue of the identical. If found to be effective, SKC may be a beneficial therapeutic adjuvant or opportunity for prevention of respiration dysfunction. The possibility of analgesic, anti-inflammatory, antipyretic, hypoxia and gastrointestinal potentials of SKC is hitherto unreported and untested. Hence an in-vivo look at turned into carried out to test analgesic, anti-inflammatory, antipyretic, hypoxia and gastrointestinal potentials of SKC.

## MATERIALS AND METHODS

### Collection of the Ayurvedic Formulation

For the analgesic and anti-inflammatory, antipyretic, hypoxia and gastrointestinal effects assessment SwasKas Chintamani Ras (SKC) gathered from Sree Kundeswari Aushadhalaya Ltd, Chittagong, Bangladesh.

### Experimental Animal

Male mice (Swiss-Webster strain, 20-40 gm body weight) bred in the Animal House of the Department of Pharmacy, Jahangirnagar University, were used for the pharmacological experiments. They were kept in cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cage provided with standard laboratory food and tap water '*ad libitum*' and maintained at natural day night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka). Before starting an experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular mouse prior to and after the administration could be noted separately.

### Doses Used In Different Experiments

For Formalin test (Dose: 100mg/kg body wt.), For Xylene induced ear edema in mice (Dose: 100mg/kg body wt.), For Acetic Acid writhing test (Dose: 100, 200 and 400mg/kg body wt.), For Infrared thermometry test (Dose: 100mg/kg body wt.),

For Hypoxia test (Dose: 100mg/kg body wt.), For Gastric emptying test (Dose: 100mg/kg body wt.), For GI Motility test (Dose: 100mg/kg body wt.), For Colon Transit Time test (Dose: 100mg/kg body wt.), For Castor oil induced anti-diarrhea test (Dose: 100mg/kg body wt.).

## Pharmacological experiments

### *Formalin test*

Formalin 1% was administered to mice by intraplantar route, and immediately the licking time was registered for 5 min (first phase, neurogenic). Twenty minutes after the beginning of the experiment (second phase, inflammatory) the licking time was registered for other 5 min. Experimental drug was administered 60 min before the formalin injection.[9]

### *Xylene-induced ear oedema in mice*

Male Swiss mice were divided into groups of ten mice each. After 30 min of the i.p. injection of the extract, xylene (0.03 ml) was applied to the anterior and posterior surfaces of the right ear. Mice were sacrificed 2 h after xylene application and both ears were removed. Circular sections of both treated and untreated ears were taken using a 7 mm diameter cork borer and weighed. The difference in weight between left untreated ear sections and right treated ear section was calculated.[10]

### *Acetic acid induced abdominal writhing assay (Non-Narcotic Analgesic Activity)*

Muscular contraction was induced by the intraperitonial injection of 0.6% acetic acid (AA) (0.25ml/animal). The test preparations were administered orally 30 minutes before the intraperitonial injection of acetic acid. Mice were cased individually to count number of writhes (painful muscular contraction) after 15 minutes of AA injection for 5 minutes. The average number of writhes and the percent protection were calculated and then compared between the animals of the experimental groups and the animals of the Control group.[11]

Percent protection was calculated as follows: -

% Protection= 100-(treated mean/control mean) x 100

### *Infrared thermometry*

The back skin temperature was obtained using a standard infrared thermometer in mice. Back temperatures were shown to be close to and consistent with rectal temperatures and measured temperatures at these sites were almost constant. These results of back skin temperatures obtained using a convenient and non-invasive infrared thermometer was safer and less stressful to animal subjects, compared to standard rectal temperature measurements.[12]

### *Hypoxia test*

In this experiment the method of *Caillard* et al. was employed.[13] Three set of ten mice per groups were used. 2 hr. after the treatment, the hypoxia time was recorded individually for all the animals. The animals were placed in an empty glass jar of 300 mL capacity attached with an electronic watch, the jars were made air tight with greased glass stoppers and the time until the onset of convulsion was recorded.

## Gastric emptying measurements

Gastric emptying of the solid nutrient meal measured in experimental mice by minor modifications of the two techniques previously described.[14]

Sixteen Swiss-Webster male mice were fasted for 18 hours prior to the experiment. Out of the 16, 8 were randomly chosen as the CR (drug) group and the remaining 8 as the Control group. Fasted animals had free access to water and pre-weighed solid food (solid: water ratio being 60:40) for a period of 1 hour. At the end of the 1 hour period, the remaining food was weighed, and adjustment for spillage was taken into consideration. The difference between the initial and final food weights gives the total food intake. Immediately after the 1 hr. feeding period, CR was orally administered to the mice of CR group at 200mg/kg (2 x doses) while their Control group counterparts were fed distilled water. The percentage of the gastric emptying of the ingested food was assessed 2 hours after the administration of the drug CR. The mice were sacrificed by cervical dislocation and the stomach removed by cutting off the cardiac and pyloric ends. The stomach was weighed in an electronic balance (Shimadzu) and opened; the gastric content was washed with tap water and the remaining gastric wall was blotted dry and weighed.

The gastric content was calculated as the difference between the total weight of the stomach with contents and the weight of the gastric wall after the contents were washed out. Percent gastric emptying (%GE) was calculated as-

$$\%GE = 1 - \frac{\text{Gastric contents}}{\text{Total food intake}} \times 100$$

### *Gastro-intestinal motility test*

The experiment was carried out by the method previously described by Chatterjee.[15] $BaSO_4$ milk was prepared by adding $BaSO_4$ at 15% w/v in 0.5% CMC suspension. The milk was given to a group of 12 mice 15 minutes after the administration of the test drug. The treated mice were divided into two sub-groups and were sacrificed after 15 and

30 minutes after the administration of the milk. The distance traversed by BaSO4 milk were measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileococcal junction). The test drug was compared with the control group administered with distilled water.

### *Colon transit time*

One hour after drug administration, a single glass bead, 2 mm in diameter was inserted into the distal colon of each mouse at 2 cm from the anus, after which the mice were returned to their respective cages and observed closely. Distal colonic transit time was determined by monitoring the time required for expulsion of the glass bead (bead latency).

### *Castor oil induced diarrhea*

The method of Niemeegeers et al, as adopted by Yegnanarayan et al. was followed.[16,17] Mice, body weight 25-30gm, were employed for this study. They were all screened initially by giving 0.5 ml of castor oil orally and only those showing diarrhoea were selected for further study. Drug suspension pretreatment was given orally 1 hour before the mice were administered with the standard dose of 0.5 ml of castor oil. The animals were caged individually and examined for the presence of diarrhoea every hour for 6 hours after the castor oil challenge. Diarrhoea was defined as the presence in the stool of fluidy material which stained the absorbent paper placed beneath the cage. The numbers of respondents, the number of stool pellets passed during the 6-hour period were noted for each mouse.

### Statistical analysis

Data were presented as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 11) was applied for the analysis of data. P<0.05 was taken to be the level of significance.

## RESULTS

### Formalin induced paw licking (analgesic + inflammation) test

SKC at dose 100 mg/kg mildly exerted both analgesic and anti- inflammatory activity in the formalin induced paw licking test in female mice compared to the corresponding control group (Table-1). However, all of the results were statistically insignificant.

### Xylene induced ear edema test

At dose 100 mg/kg, SKC treated female mice exerted decrease effect in inflammation in the xylene induced ear edema test when compared to the corresponding control group (Table-2). This increase was not statistically significant. From the findings of this experiment, it can be suggested that SKC has mild anti- inflammatory activity (probably).

### Acetic acid induced writhing test

SKC (100 mg/kg) treated female mice exerted a decrease in writhing response compare to the control group from the initial 1$^{st}$ min to 5$^{th}$ min. Only at 3$^{rd}$ min, the result was statistically significant.(p=0.015*) At the dose of 200 mg/kg, SKC treated female mice showed increase in writhing response compare to the control group, Results were found both of the 3$^{rd}$ min was statistically significant.(p=0.012*) and at 5$^{th}$ min the result was also exposed statistically significant.(p=0.031*). At the dose of 200mg/kg, SKC treated female mice showed increase in writhing response compare to the control group from the initial 1$^{st}$ min to 4$^{th}$min, but only the 5$^{th}$ min shown decrease response (Table-3).

### Infra- red thermometry test

At dose 100 mg/Kg, SKC treated female mice exerted some interesting observations in body temperature in the infra-red thermometry test. The SKC treated group showed overall decrease effect in body temperature in the whole study period except at min 30 & min 180 (Table-4). Thus the result was found to be statistically very highly significant (p=0.001**) at min 30. Noticeable result was also observed at min120 (p=0.065).

### Hypoxia test

At the dose 100 mg/kg, SKC treated male mice, showed an increased effect in the survival time of the hypoxia test when compared to the corresponding control group. Though the result was not found to be statistically significant, it was interesting. So it was suggested to further investigations of SKC (Table-5).

### Gastric emptying test

At the dose of 100mg/kg, SKC treated male mice exerted negligible increase in gastric emptying at 2$^{nd}$ hour (Table-6). But interestingly, SKC was found to decrease gastric emptying at 4$^{th}$ hour in experimental male mice when compared with that of the control group. But none of the results were found to be statistically significant. Noteworthy to mention with lapse of time the difference with the control increases i.e. with passage of time there was comparatively decreased gastric emptying.

**Table 1** The effect of SKC (100 mg/kg) in the Formalin Induced Paw licking (Analgesic +Inflammation) Test

| Group | Analgesic(1st Phase) | Inflammation(2nd Phase) |
|---|---|---|
| Ctrl (n=10) | 66.70±7.54 | 4.40±2.45 |
| SKC (n=10) | 48.60±7.28 | 1.30±0.76 |
| t/p | 1.73/0.101 | 1.21/0.243 |
| 95% Confidence Interval | -3.928 to 40.128 | -2.299 to 8.499 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

**Table 2** The effect of SKC (100 mg/kg) in Xylene induced ear edema Test

| Group | Inflammation |
|---|---|
| Ctrl (n=10) | 0.00700 ± 0.000596 |
| SKC (n=10) | 0.00650 ± 0.000428 |
| t/p | 0.681/0.504 |
| 95% Confidence Interval | -0.001042 to 0.002042 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

**Table 3** The effect of SKC (100mg/kg, 200 mg/kg, 400 mg/kg100 mg/kg) on Percentage of Protection on acetic acid induced writhing test

| Group | 100 mg/kg | | 200 mg/kg | | 400 mg/kg | |
|---|---|---|---|---|---|---|
| | Parameter | | Parameter | | Parameter | |
| Female mice | Min (0-5) | % Protection | Min (0-5) | % Protection | Min (0-5) | % Protection |
| Control(n=10) | 6.90±0.84 | | 7.30±1.55 | | 7.60±3.03 | |
| SKC (n=10) | 5.20±0.90 | 132.69% | 12.20±2.23 | 59.84% | 11.80±3.04 | 64.41% |
| t/p value | 1.38/0.18 | | -1.81/0.09 | | -0.98/0.34 | |
| 95% confidence Interval | -0.887 to 4.287 | | -10.596 to 0.796 | | -13.217 to 4.817 | |

N.B: * (£ 0.05) = Significant, ** (£ 0.01) = highly significant, *** (£ 0.001) =Very highly significant

**Table 4** The effect of SKC (100 mg/kg) in the Infra-red Thermometry Test

| Group | Min_30 | Min 30 | Min 60 | Min120 | Min180 | Min 240 |
|---|---|---|---|---|---|---|
| Ctrl (n=10) | 79.83±1.08 | 79.45±0.821 | 151.12±71.93 | 83.14±0.760 | 81.93±0.318 | 81.87±0.686 |
| SKC(n=10) | 80.6±0.944 | 83.82±0.738 | 81.68±0.774 | 81.13±0.696 | 82.53±0.867 | 81.65±0.745 |
| t/p | 0.538/0.597 | 3.957/0.001** | 0.965/0.360 | 1.950/0.067 | 0.650/0.529 | 0.217/0.831 |
| 95% Confidence Interval | 3.785 to 2.241 | 6.690 to 2.049 | 93.289 to 232.169 | 0.155 to 4.175 | 2.624 to 1.424 | 1.908 to 2.348 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

**Table 5** The effect of SKC (100mg/kg) in the Hypoxia Test

| Group | Survival Time( in sec) |
|---|---|
| Ctrl(n=10) | 2123.20±114.59 |
| SKC(n=10) | 2353.60±108.01 |
| t/p | -1.46/0.16 |
| 95% Confidence Interval | 561.24 to 100.44 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

### Gastro-intestinal activity

Treatment with SKC result in negligible decrease in gastric emptying at 2nd hour. But interestingly, increase in gastric emptying at 4th hour in experimental male mice were observed. In the Gastro intestinal motility test, SKC at the dose of 100 mg/kg, showed an increase in the gut motility from the early 1sthrs to 4thhr (Table: 7.1-7.4). However a decrease in gut motility was observed from first 15 min for few hours. SKC also showed increase in colon transit time indicating possibility of potential laxative effect.

### Colon transit time test

At the dose of 100mg/kg, the drug SKC treated male mice showed decrease in bead latency time in the colon transit time test when compared to the respective control group. The result was found to be statistically significant (p=0.023*) (Table-8).

### Castor oil induced diarrhoea test

At the dose 100 mg/Kg, female mice were treated by SKC, in the castor oil induced diarrhea test and the result have been represented at Tabe-9. The solid stool count was observed as increased in the SKC treated group from the initial 1sthr to till 4thhr as comparison to the respective control group but were statistically significant. On the contrary, the semi-solid stool count was decreased from the initial 1st hour to till 6th hour. All of these results were also found to be statistically insignificant. Interestingly, the liquid stool count was found to be increased from the initial 1sthr to 4thhr except 5th and 6th hour. The result of increasing effect of 2nd hour was found to be statistically highly significant (p=0.001**) and also 3rd hour (p=0.02*) and 4th hour (p=0.03*) were statistically significant. But other results were statistically insignificant. From

**Table 6 The effect of SKC (100mg/kg) on Gastric Emptying Test after 2nd and 4th hour study**

| | 2nd hour study | 4th hour study |
|---|---|---|
| Group | % GE (Mean ± S.E.M) | % GE (Mean ± S.E.M) |
| Control 2hr GE | 7.63±1.95 | 10.78±1.48 |
| SKC 2hr GE | 9.72±3.33 | 8.64±1.48 |
| t/p | -0.54/0.59 | 1.02/0.32 |
| 95% Confidence interval | -10.202 to 6.026 | -2.262 to 6.530 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

**Table 7.1 The effect of SKC (100 mg/kg) on the Gastrointestinal Motility Test utilizing Female mice**

| | 1st hour 15 minutes | | | 1st hour 30 minutes | | |
|---|---|---|---|---|---|---|
| Group | Total Length | $BaSO_4$ Length | % Traversed | Total Length | $BaSO_4$ Length | % Traversed |
| Ctrl (n=8) | 59.86±1.21 | 29.00±2.28 | 48.54±3.84 | 57.25±1.62 | 52.00±2.11 | 90.97±3.15 |
| SKC (n=8) | 52.87±1.04 | 20.87±2.20 | 39.77±4.51 | 59.12±1.88 | 54.56±1.43 | 92.59±2.31 |
| t/p Value | 4.366/0.001*** | 2.562/0.023* | 1.480/0.161 | -0.754/0.463 | -1.003/0.333 | -0.417/0.683 |
| 95% Confidence Interval | 3.554 to 10.420 | 1.322 to 14.927 | -3.942 to 21.487 | -7.205 to 3.455 | -8.039 to 2.914 | -10.014 to 6.756 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

**Table 7.2 The effect of SKC (100 mg/kg) on the Gastrointestinal Motility Test utilizing Female mice**

| | 2nd hour 15 minutes | | | 2nd hour 30 minutes | | |
|---|---|---|---|---|---|---|
| Group | Total Length | $BaSO_4$ Length | % Traversed | Total Length | $BaSO_4$ Length | % Traversed |
| Ctrl (n=8) | 55.37±1.71 | 29.56±5.00 | 54.73±9.43 | 58.06±2.00 | 43.19±2.89 | 74.29±4.11 |
| SKC (n=8) | 57.12±2.00 | 39.51±2.75 | 69.60±5.40 | 53.57±2.99 | 37.69±3.75 | 69.61±4.69 |
| t/p Value | -0.664/0.517 | -1.743/0.103 | -1.368/0.193 | 1.195/0.252 | 1.161/0.265 | 0.749/0.466 |
| 95% Confidence Interval | -7.401 to 3.901 | -22.197 to 2.297 | -38.183 to 8.448 | -3.424 to 12.049 | -4.659 to 15.659 | -8.714 to 18.059 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

**Table 7.3** **The effect of SKC (100 mg/kg) on the Gastrointestinal Motility Test utilizing Female mice**

| | 3rd hour 15 minutes | | | 3rd hour 30 minutes | | |
|---|---|---|---|---|---|---|
| Group | Total Length | $BaSO_4$ Length | % Traversed | Total Length | $BaSO_4$ Length | % Traversed |
| Ctrl (n=8) | 56.75±1.13 | 51.44±1.12 | 90.95±2.94 | 48.31±3.39 | 31.19±5.51 | 64.89±9.97 |
| SKC (n=8) | 53.94±1.63 | 46.37±3.02 | 86.41±5.59 | 58.00±3.35 | 54.69±3.79 | 94.10±2.56 |
| t/p Value | 1.420/0.178 | 1.152/0.151 | 0.708/0.494 | -2.03/0.061 | -3.513/0.003** | -2.239/0.022* |
| 95% Confidence Interval | -1.435 to 7.060 | -2.233 to 12.358 | -9.659 to 18.744 | -19.905 to 0.530 | -37.846 to -9.153 | -52.985 to -5.436 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

**Table 7.4** **The effect of SKC (100 mg/kg) on the Gastrointestinal Motility Test utilizing Female mice**

| | 4th hour 15 minutes | | | 4th hour 30 minutes | | |
|---|---|---|---|---|---|---|
| Group | Total Length | $BaSO_4$ Length | % Traversed | Total Length | $BaSO_4$ Length | % Traversed |
| Ctrl (n=8) | 57.19±1.78 | 49.44±2.41 | 86.38±2.95 | 60.37±2.09 | 36.37±3.30 | 59.91±4.43 |
| SKC (n=8) | 57.87±1.35 | 35.75±4.03 | 61.46±6.62 | 54.62±2.23 | 40.00±3.64 | 72.63±5.34 |
| t/p Value | -0.308/0.763 | 2.912/0.011* | 3.436/0.007** | 1.877/0.082 | -0.737/0.473 | -1.83/0.088 |
| 95% Confidence Interval | -5.482 to 4.107 | 3.606 to 23.768 | 8.686 to 41.153 | -0.821 to 12.321 | -14.178 to 6.928 | -27.61 to 2.16 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

**Table 8** **The effect of SKC (100mg/kg) in the Colon Transit Time Test**

| Group | Bead latency time (Sec) |
|---|---|
| Ctrl(n=20) | 671.25±77.05 |
| SKC(n=20) | 403.45±82.75 |
| t/p | 2.37/0.023* |
| 95% confidence interval | 38.904 to 495.695 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

the findings it can be suggested that SKC is devoid of anti- diarrhea effect.

Thus it can be said from this experiment that SKC is devoid of any prominent side effects, further in-depth toxicological study is recommended to validate the safety of this Ayurvedic drug.

## DISCUSSION

The quantity of animal fashion which stimulates organic and biochemical traits of pain and inflammation is to be had for the screening of analgesic and anti-inflammatory markers. We selected time examined fashions of an acute exudative segment of infection like paw oedema, which brought on by way of the injection of the phlogistic agent like xylene[18] and acetic-acid or formalin.[19] These models are touchy to steroidal and non-steroidal anti-inflammatory drugs. Granulation tissue formation is the important thing function in chronic infection and has been chosen for screening anti-arthritic pills. Injection of an irritant like acetic acid in the peritoneal cavity develops writing, that's a totally sensitive version for screening analgesic agents that mainly act peripherally.[11,20,21] In the acetic acid, xylene or formalin precipitated paw oedema test, these agents are claimed to induce peritonitis and in writhing fashions. Pretreatment utilized in those models evaluated the prophylactic potential of SKCs, in the prevention of prompted infection and ache. Our results showed that SKC tested similar anti-inflammatory and analgesic outcomes as shown via aspirin. In formalinor acetic acid triggered paw edema, prostaglandins are concerned in mediating inflammation.[22,23,24] The granulomatous chronic irritation is likewise driven by means of prostaglandins, which are liberated from the inflammatory neutrophils and macrophages migrating to the site of overseas bodies like cotton pellets.[25] It is in all likelihood that SKC probably has the identical

**Table 9** **The effect of SKC (100 mg/kg) on Hourly solid stool count, Hourly Semi Solid stool count, Hourly liquid stool count, Hourly Total stool count in Castor Oil Induced Diarrhea Test**

| Group | | Ctrl (n=9) | SKC (n=9) | t/p | 95% confidence interval |
|---|---|---|---|---|---|
| Hourly solid stool count | Hr1 | 0.00±0.00 | 0.67±0.44 | -1.51/0.17 | -1.683 to 0.350 |
| | Hr2 | 0.11±0.11 | 0.22±0.15 | -0.60/0.55 | -0.501 to 0.279 |
| | Hr3 | 0.00±0.00 | 0.33±0.33 | -1.00/0.35 | -1.102 to 0.435 |
| | Hr4 | 0.00±0.00 | 0.11±0.11 | -1.00/0.35 | -0.367 to 0.145 |
| | Hr5 | 0.00±0.00 | 0.00±0.00 | 0.00/0.00 | 0.000 to 0.000 |
| | Hr6 | 0.00±0.00 | 0.00±0.00 | 0.00/0.00 | 0.000 to 0.000 |
| Hourly Semi Solid stool count | Hr1 | 3.11±0.54 | 2.11±0.59 | 1.25/0.23 | -0.690 to 2.690 |
| | Hr2 | 1.67±0.53 | 1.00±0.37 | 1.03/0.32 | -0.701 to 2.035 |
| | Hr3 | 0.67±0.37 | 0.22±0.15 | 1.11/0.28 | -0.443 to 1.332 |
| | Hr4 | 0.33±0.23 | 0.00±0.00 | 1.41/0.18 | -0.210 to 0.876 |
| | Hr5 | 0.33±0.23 | 0.00±0.00 | 1.41/0.19 | -0.210 to 0.876 |
| | Hr6 | 0.22±0.22 | 0.00±0.00 | 1.00/0.35 | -0.290 to 0.734 |
| Hourly liquid stool count | Hr1 | 2.00±0.67 | 3.67±0.71 | -1.71/0.11 | -3.726 to 0.393 |
| | Hr2 | 1.00±0.29 | 5.22±1.01 | -4.01/0.001** | -6.450 to -1.993 |
| | Hr3 | 1.00±0.47 | 2.55±0.44 | -2.40/0.02* | -2.929 to -0.182 |
| | Hr4 | 1.00±0.29 | 2.67±0.64 | -2.36/0.03* | -3.221 to -0.111 |
| | Hr5 | 1.44±0.47 | 0.78±0.52 | 0.95/0.36 | -0.827 to 2.161 |
| | Hr6 | 1.22±0.43 | 1.00±0.44 | 0.36/0.72 | -1.089 to 1.533 |
| Hourly Total stool count | Hr1 | 5.44±1.17 | 6.67±1.04 | -0.78/0.45 | -4.538 to 2.094 |
| | Hr2 | 2.78±0.46 | 5.22±0.79 | -2.65/0.017* | -4.397 to -0.491 |
| | Hr3 | 1.67±0.44 | 3.11±0.59 | -1.96/0.060 | -3.002 to 0.113 |
| | Hr4 | 1.33±0.33 | 2.78±0.70 | -1.86/0.08 | -3.148 to 0.259 |
| | Hr5 | 1.78±0.52 | 0.89±0.61 | 1.11/0.28 | 0.813 to 2.591 |
| | Hr6 | 1.44±0.58 | 1.00±0.44 | 0.61/0.55 | -1.100 to 1.98 |

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

mechanism of motion as aspirin i.e prostaglandin inhibition. In comparison of SwasKas Chintamani Ras found effective acetic-acid-induced peritonitis and writhing fashions in mice in contrast to within the other two models i.e acetic acid induces rat paw oedema and Xylene induced ear oedema test.

Our study findings corroborated the proof generated via a number of the latest research executed in ultimate 2 years by means of other researchers. In one look at, SKC confirmed the widespread reduction in acetic acid or formalin induced rat paw oedema and the tremendous reduction in Xylene induced ear oedema.[26]

In all the above-referred to research, distinct formulations of SKC had been used. The dose used in this test was pre-examined. We preferred to adhere to components and doses described in the Ayurvedic pharmacology. Lower degrees of doses are used for arishta formula.[27,28,29] Arishta method is favoured clinically over kwath as it is more palatable and effective in long-time period use.[30] An extra spectrum of SKC was evaluated in the present study at which has not been studied in advance. Ayurvedic texts mention useful results of SKC specially the kwath preparation in various respiratory disease.[18] The look at findings implies that SKC may be beneficial in the scientific control of situations like respiratory disease. SKC may be a more secure alternative remedy. It may be possible to apply it as an upload-on remedy to aspirin that allows you to assist clinicians to obtain higher efficacy and decrease doses of aspirin. Future experimental and medical studies are had to discover this opportunity. Further exploratory research with SKC to evaluate its outcomes on cyclooxygenase, inflammatory mediators, and other anti-aggregatory mechanisms.

The use of tympanic thermometry is appealing in primary care, but a latest systematic evaluation highlighted the paucity of data comparing tympanic thermometry with conventional methods. We record a observe of ninety-four preschool children offering to primary care in the United Kingdom (UK) with an acute cough in whom tympanic infrared and axillary

mercury thermometry are compared. Infrared thermometry showed poor settlement, negative sensitivity and high specificity. Infrared thermometry is simply too insensitive for use as a screening take a look at for fever, but when fever is already suspected, for example via touch, it may be useful as a 'rule in' assay.[31] During our present study SKC treated female mice exerted some interesting observations in body temperature in the infra-red thermometry test. The SKC treated group showed overall decrease effect in body temperature in the whole study period except at min 30 & min 180. Another aim of the present study was to investigate the effects of SKC on hypoxia models mice and to find the possible mechanism of its anti-hypoxic actions so as to elucidate the anti-hypoxia activity and provide scientific basis for the clinical use of SKC. Given orally, the SKC at doses of 100 mg/kg could dose-dependently enhance the survival time of mice in both of the normobaric and chemical hypoxia models. That was may be due to the activity of the glycolysis enzymes and the level of ATP were higher than those of the control. Our present study indicating that the observed anti-hypoxic activity was unlikely due to sedation or motor abnormality. This present study data is being supported by a previous study.[32] The ayurvadic preparation become properly tolerated with the aid of the animals when administered orally, no signal of acute toxicity like restlessness or seizures had been found over the length of statement. There have been no deaths recorded after the oral management of SKC. Deaths were no longer recorded after the intraperitoneal management of SKC. Castor oil become used in this observe to brought on diarrhea. It is nicely documented that castor oil produces diarrhea due to its most active metabolite, ricinoleic acid with the aid of hyper secretory reaction, which stimulates peristaltic activity within the small gut, main to modifications in the electrolyte permeability of the intestinal mucosa.[33,34] Its action additionally stimulates the release of endogenous prostaglandins E and F which motive belly cramp and diarrhoea due to the effect at the smooth muscle and secretion.[35,36] Among the several mechanisms proposed to provide an explanation for the diarrheal impact of castor oil are activation of adenylate cyclase or mucosal CAMP mediated energetic secretion,[37] stimulation of prostaglandin formation[38] and nitric oxide.[39] In this observe, there has been a statistically significant reduction inside the incidence and severity of diarrheal stool produced within the experimental animals. The extract examined at 100 mg/kg l. This end result is in support of preceding claims in respect of antidiarrheal herbs. The SKC inhibited gastrointestinal propulsion inside the castor oil triggered transit than in regular transit. This makes it useful as a preventive agent. Antidiarrheal remedy in patient is performed through the goal of the therapy which includes increasing resistance to flow (segmental contraction and reduce propulsion) and elevated mucosal absorption or lowering secretion.[40] This is indicative of the capacity of the plant to regulate regular peristaltic motion and hence lower the movement of substances within the intestinal tract permitting extra time for absorption. In the fluid accumulation check, the extract appreciably decreased each the weight and quantity of intestinal content. This may sell reabsorption of materials within the intestine due to lower propulsion of fabric in the intestinal tract, and the extract would possibly have exerted its antidiarrheal movement by way of antisecretory mechanism. The SKC additionally inhibited normal gastric emptying; this impact may be related to lessen gastrointestinal propulsion. Decrease in intestinal transit time by means of morphine and atropine is linked to postpone in gastric emptying.[41,42] This suggests that the SKC can also have morphine-like motion in exerting its antidiarrheal activity.

## CONCLUSION

The current study verified that SKC had anti-inflammatory, analgesic, anti-hypoxic results. Again, the result of the prevailing study suggests that the SKC possess great anti diarrheal potentiality due to its effect on reduction of number of diarrhea stool, delayed in gastrointestinal propulsion and inhibition of fluid accumulation inside the intestinal tract of rats. Further studies are ongoing to decide the precise compound(s) answerable for its antidiarrheal motion and create opportunity to search useful alternative to presently available non-steroidal anti-inflammatory drug.

## ABBREVIATIONS

AA: Acetic acid, Ctrl: Control, F: Female, GE: Gastric Emptying, GI: Gastrointestinal, Hr: Hour, i.p.: Intra-peritoneal, Kg: Kilogram, M: Male, Min: Minute, PI: Purging Index, p.o.: Per-oral, SKC: Svasa Kasa Cintamani, Wt: Weight, CNS: Central nervous system.

## FINANCIAL SUPPORT

We do not have any private or public organizational funding. All members of research group having personal contributions for fund.

## DISCLOSURES REGARDING REAL OR PERCEIVED CONFLICTS OF INTEREST

No competing interests in this scientific work.

## ACKNOWLEDGMENTS

The authors are thankful to all the stuffs of Department of pharmacy, Noakhali Science and Technology University for their outmost support during conducting this study.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

## REFERENCES

1. Furst DE, Ulrich RW, Prakash S. Non-steroidal Anti-inflammatory Drugs, Disease Modifying Anti-rheumatoid Drugs, Non-opiod Analgesics, and Drugs Used in Gout. In: Katzung BG, Masters SB, Trevor AJ, editors. Basic and Clinical Pharmacology. 12th ed. New Delhi: McGraw-Hill Companies. 2012; 635–57.
2. Islam T, Hussain MS, Amin MN, Tuhin, AM. Evaluation of psychopharmacological and neurosafety profile of SwasKas Chintamani Ras (SKC) in Swiss-Webster mice. *Avicenna. J. Phytomed.* 2018; 8(1): 85-95. [PubMed].
3. Loeser JD, Treede RD. The Kyoto protocol of IASP basic pain terminology. *Pain.* 2008; 137(3):473–477. doi: 10.1016/j.pain.2008.04.025. [PubMed] [Cross Ref]
4. Dewan SMR, Amin MN, Adnan T, Uddin SMN., Shahid-Ud-Daula AFM., Sarwar G Hossain MS. Investigation of analgesic potential and in vitro antioxidant activity of two plants of Asteraceae family growing in Bangladesh. *J. Pharm. Res.* 2013; 6(6):599–603. doi: 10.1016/j.jopr.2013.05.016. [Cross Ref]
5. Hossain H, Shahid-Ud-Daula AFM, Hasan K, Mansur AA, Haq MM. Anti-inflammatory activity, total flavonoids and tannins content from the ethanolic extract of *Spilanthes paniculata* leaf growing in Bangladesh. *Int. J. Pharm.* 2002; 2(2):271–277.
6. Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human disease: where are we now? J. Lab. *Clin. Med.* 1992; 119(6):598–620. [PubMed]
7. Spacer CB, Breder CD. The neurologic basics of fever. *N. Engl. J. Med.* 1994; 330(26):1880–1886. doi: 10.1056/NEJM199406303302609. [PubMed] [Cross Ref]
8. Luo C, Bohlin l. Is COX-2 a perpetrator or a protector? Selective COX-2 inhibitors remain controversial1. *Acta. Pharmacologica. Sinica.* 2005; 26:926–933. doi: 10.1111/j.1745-7254.2005.00150.x. [PubMed] [Cross Ref]
9. Tjolsen A, Berge OD, Hunskaar S, Rosland JH, Hole K. The formalin test — an evaluation of the methods. *Pain.*1992; 51(1):5–17.
10. Tang XC, Lin ZG, Cai W, Chen N, Shen L. Anti-inflammatory effect of 3-acetylaconitine. *Zhongguo. Yao. Li.Xue. Bao.* 1984; 5(2):85-9. [PubMed] PMID: 6235717.
11. Koster R, Anderson M, Beer DEJ. Acetic acid for analgesic screening. *Proc. Soc. Exp. Biol. Med.* 1959; 18:412-5.
12. Saegusa Y, Tabata H. Usefulness of infrared thermometry in determining body temperature in mice. *J. Vet. Med. Sci.*2003; 65(12): 1365-1367.
13. Caillard C, Menu A, Plotkin M, Rossignol P. Do anticonvulsant drugs exert protective effect against hypoxia? *Life. Sci.* 1975; 16(10): 1607-1612.
14. Martinez V, Wang L, Rivier JE, Vale W, Tache Y. Differential actions of peripheral corticotropin-releasing factor (CRF), Urocortin II, and Urocortin III on Gastric Emptying and Colonic transit in mice: Role of CRF receptor subtypes I and II. *J. Pharmacol. Exp. Ther.* 2002; 301(2): 611-617.
15. Chatterjee TK. Handbook on Laboratory Mice and Rats. Department of Pharmaceutical Technology, Jadavpur. University. 1st edn.1993; 157.
16. Niemeegeers CJE, Lenaerts FM, Aouters F. Synthetic Anti diarrhoeal Drugs. Van Baver W. and Lal H. (eds.), New York, Marcel Dekker.1976; 65-114.
17. Radha Y, Shrotri DS. Comparision of Antidiarrhoel Activity of Some Drugs in Experimental Diarrhoea. *Indian. J. Pharmacol.* 1982; 14(4): 293-299.
18. Bagad AS, Joseph JA, Bhaskaran N, Agarwal A. Comparative Evaluation of Anti-Inflammatory Activity of Curcuminoids, Turmerones, and Aqueous Extract of Curcuma longa. *Adv. Pharmacol. Sci.* 2013; 805756. doi: 10.1155/2013/805756.
19. Tomisawa S, Sato NL. Preventive and curative effects of non-steroidal anti-inflammatory drugs on experimental peritoneal inflammation in mice. *Jpn. J. Pharmacol.* 1973; 23(4): 453–8.
20. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *Br. J. Pharmacol.* 1964; 22(2):246–53. [PMC free article] [PubMed]
21. Baul S, Amin MN, Hussain MS, Mukul MEH, Millat MS, Rashed MSU et al. Phytochemical Nature and Pharmacological Evaluation of Chloroform Extract of *Pandanus fascicularis* L. (Fruits): An *in vivo* Study. *J. Bioanal. Biomed.* 2017; 9(4): 223-228. doi:10.4172/1948-593X.1000183
22. Buodonpri W, Wichitnithad W, Rojsitthisak P, Towiwat P. Synthetic curcumin inhibits carrageenan-induced paw edema in rats. *J. Health. Res.*2009; 23(1): 11–6.
23. Aiyelero OM, Ibrahim ZG, Yaro AH. Analgesic and anti-inflammatory properties of the methanol leaf extract of *Ficusingens* (Moraceae) in rodents. *Niger. J. Pharm. Sci.*2009; 8(2): 79–86.
24. Paschapur M, Patil S, Patil S, Kumar R, Patil MB. Evaluation of the analgesic and antipyretic activities of ethanolic extract of male flowers (inflorescence) of *Borassus flabellifer L.* (Arecaceae). *Int. J. Pharm. Pharm. Sci.*2009; 1(2):98–106.
25. Srinivasan K, Muruganandan S, Lal J, Chandra S, Tandan SK, Prakash VR. Evaluation of anti-inflammatory activity of *Pongamia pinnata* leaves in rats. *J. Ethnopharmacol.* 2001; 78(2-3):151–7.
26. Singh RS, Ahmad M, Wafai ZA, Seth V, Moghe VV, Upadhyaya P. Anti-inflammatory effects of Dashmula, an Ayurvedic preparation, versus Diclofenac in animal models. *J. Chem. Pharm. Res.* 2011; 3(6):882–8. 34.
27. Tripathi B. editor. Sharangdhara-Samhita. Varanasi: Chaukhambha Surbharti Prakashan. Sharangdhacharya. Sandhan-Kalpana. 2001; 247–62
28. Sharma PV. In: Dravyaguna-Vidnyan. Varanasi: Chaukhambha Surbharti Prakashan. Impurities of drugs and their purification. 1998; 353–9.
29. Khandal S. In: Rasa-Bhaishajya kalpana Vigyana. 3rd ed. Jaipur: Publication Scheme. SandhankalpanaVidnyan. 2000; 469–83.
30. Nandre BN, Bakliwal SR, Rane BR, Pawar SP. Traditional fermented formulations-Asava and Aristha. *Int. J. Pharm. Biol. Arch.* 2012; 3(6):1313–9.
31. Hay AD, Peters TJ, Wilson A, Fahey T. The use of infrared thermometry for the detection of fever. *Br. J. Gen. Pract.* 2004; 54(503): 448-450.
32. Chen CJ, Wang WY, Wang XL, Dong, LW, Yue YT, Xin HL et al. Anti-hypoxic activity of the ethanol extract from *Portulaca oleracea* in mice. *J. Ethnopharmacol.* 2009; 124(2):246-50.

33. Zavala I, Barrera E, Nava A. Ceftriaxone in the treatment of bacterial meningitis in adults. *Chemotherapy*. 1988; 34 (Suppl 1): 47-52.
34. Hardman JG, Limbird LE. Drugs affecting gastrointestinal function. In The pharmacological basis of therapeutics. 10th edn. Goodman and Gilman's edn. New York. Macgraw Hill. 2001; 1023-1024.
35. Galvez J, Zavzuelo A, Crespo ME, Lorente MD, Ocete MA, Jimenez J. Anti-diarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoids constituent. *Planta. Med.* 1993; 59(4):333-336.
36. Saito T, Mizutani F, Iwanaga Y, Morikawa K, Kato H. Laxative and anti-diarrheal activity of polycarbophil in mice and rats. *Jpn. J. Pharmacol.*2002; 89(2):133 -141.
37. Capasso F, Tavares IA, Bennet A. PAF formation by human gastrointestinal mucosa/submucosa in vitro: release by ricinoleic acid, and inhibition by 5-aminosalicylic acid. *J. Pharm. Pharmacol.* 1992; 44(9):771- 772.
38. Capasso F, Mascolo N, 1zzo AA, Gaginella TS. Dissociation of castor oil-induced diarrhea and intestinal mucosal injury in rat: Effect of NG nitro-L-arginine methyl ester. *Br. J. Pharmacol.* 1994; 113(4): 1127-1130.
39. Uchida M, Kato Y, Matsuede K, Shode R, Muraoka A, Yemato S. Involvement of NO from nerves in diarrhea induced by castor oil in rats. *Jpn. J. Pharmacol*. 2000; 82(2): 168-170.
40. Burks TF. Gastrointestinal drugs In. Kist, K. (edn). Human pharmacology: molecular to clinical. Wolfe Publ. Itc London. 1991; 789-800
41. Izzo AA, Mascolo N, Capasso R, Germano MP, De pasuele R, Capasso F. Inhibitory effect of cannabinoid agonists on gastric emptying in rat. *Arch. Pharmacol*. 1999; 360(2):221-223.
42. Uchida M, Yoshida K, Shimizu K. Effect of atropine sulfate on gastric emptying and gastrocecal transit time evaluated by using the [1_13C] acetic acid and lactose [13C] ureide-breath test in conscious rats. *J. Breath Res*. 2009; 3(4):7003.