© 2020 Journal of Pharmacy & Pharmacognosy Research, 8 (6), 569-579, 2020
ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

Effect of sex differences in antinociceptive, antipyretic, hypoglycemia, hepatoprotective and antidiarrheal activities in mice model

[Efecto de las diferencias sexuales en las actividades antinociceptiva, antipirética, hipoglucemia, hepatoprotectora y antidiarreica en modelo de ratones]

Irin Karim#, Roni Roy#, Md Rafiul Hoque, Sazzad Hosen, Tanaya Bhowmik, Israt Jahan Liya, Afroza Akter, Mohammad Anwarul Basher*

Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.
#These two authors contributed equally.
*E-mail: basher@nstu.edu.bd

Abstract

Context: The use of animal models is a longstanding practice in biological research. Among different models, the mouse is the most widely used and accepted model. In designing the mouse model, a male mouse is generally preferred over a female to avoid the effect of changing hormonal state in females. However, it is not known whether mouse sex affects all experiments.

Aims: To determine the effect of mouse sex on pharmacological responses in antinociceptive, antipyretic, hypoglycemia, hepatoprotective and antidiarrheal experiments.

Methods: Antinociceptive study was performed by three different experiments. An antipyretic experiment was performed by yeast induced hyperthermia test. The effect on hypoglycemic response was assessed by an oral glucose tolerance test. The effect on the hepatoprotective study was evaluated by carbon tetrachloride-induced liver damage. The antidiarrheal study was conducted by a castor oil-induced diarrhea test.

Results: Antinociceptive studies demonstrated mixed effects. Hot plate test showed significant differences; the licking test showed variation only in the late phase, while no significant variation was observed. In the antipyretic experiment, female mice showed higher body temperature in both control and standard that varied significantly with male mice. Hypoglycemia and hepatoprotective tests did not show significant variation between sexes; however, liver enzymes levels were found higher in males while the percentage liver weight was higher in females. In the antidiarrheal test, the male mouse was observed to have higher responses than the female.

Conclusions: Antinociceptive and antipyretic investigations should be performed separately on both male and female mice. On the other hand, hypoglycemic, hepatoprotective and antidiarrheal tests can be conducted on any mouse sex, and findings on particular sex can be extrapolated to the opposite sex.

Keywords: drug discovery; sex dimorphism; Swiss albino mice.

Resumen

Contexto: El uso de modelos animales es una práctica de larga data en la investigación biológica. Entre los diferentes modelos, el ratón es el modelo más utilizado y aceptado. Al diseñar el modelo de ratón, generalmente se prefiere un ratón macho a una hembra para evitar el efecto del cambio de estado hormonal en las hembras. Sin embargo, no se sabe si el sexo del ratón afecta a todos los experimentos.

Objetivos: Determinar el efecto del sexo del ratón sobre las respuestas farmacológicas en experimentos antinociceptivos, antipiréticos, hipoglucémicos, hepatoprotectores y antidiarreicos.

Métodos: Se realizaron experimentos para demostrar efectos anti-nociceptivo (tres experimentos diferentes), antipirético (hipertermia inducida por levaduras), hipoglucémico (prueba de tolerancia a la glucosa oral), hepatoprotector (daño hepático inducido por tetracloruro de carbono) y antidiarreico (diarrea inducida por aceite de ricino).

Resultados: Los estudios antinociceptivos demostraron efectos mixtos. La prueba de la placa caliente mostró diferencias significativas; la prueba de lamido mostró variación solo en la fase tardía, mientras que no se observó variación significativa. En el experimento antipirético, las hembras mostraron una temperatura corporal más alta tanto en el control como en el estándar que varió significativamente con los ratones machos. Las pruebas de hipoglucemia y hepatoprotección no mostraron variación significativa entre sexos; sin embargo, los niveles de enzimas hepáticas se encontraron más altos en los machos mientras que el porcentaje de peso del hígado fue más alto en las hembras. En la prueba antidiarreica, se observó que el ratón macho tenía respuestas más altas que la hembra.

Conclusiones: Las investigaciones antinociceptivas y antipiréticas debían realizarse por separado en ratones machos y hembras. Por otro lado, las pruebas hipoglucémicas, hepatoprotectoras y antidiarreicas podrían realizarse en cualquier sexo de ratón, y los hallazgos sobre un sexo particular se pueden extrapolar al sexo opuesto.

Palabras Clave: dimorfismo sexual; investigación en medicamento; ratones albinos suizos.

ARTICLE INFO
Received: July 21, 2020.
Received in revised form: August 24, 2020.
Accepted: August 27, 2020.
Available Online: August 29, 2020.
Declaration of interests: The authors declare no conflict of interest.
Funding: This research was funded by Research Cell, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

AUTHOR INFO
ORCID: 0000-0001-8811-0340 (MAB).

INTRODUCTION

The use of an animal model in biological research is a well-established practice in the scientific world. Mammals such as mice, rats, rabbits, guinea pigs, cats, dogs and monkeys play a key role in mammalian genetic and biomedical research. Anatomical and physiological similarities between humans and other mammals used in animal model equip researchers to evaluate new drug candidates, understand the mechanism of drug action, identify adverse effects, and determine toxicity profiles, before these drug molecules are ready to administer to the human body. For example, the use of insulin to treat diabetes was first performed on the dog (Vecchio et al., 2018). Animal models, thus, provide similar experimental conditions to humans and this is a particularly valuable tool in pharmaceutical trials of newly designed drugs.

In the pursuit of discovery of new drugs, natural sources, especially plants, offer profuse supply of bioactive compounds. Traditional medicine based on the use of crude preparations of plant parts occupies an important position in the healthcare system worldwide, particularly in developing countries. Such anecdotal uses of plants against various diseases guide the researchers to delve into the plants with appropriate chemical and biological tools. For example, the bark of *Cinchona officinalis* had long been used against fever and later potent antimalarial drug- quinine was isolated from the plant. Potent bronchodilator drug, cromolyn was developed from khellin of *Ammi visnaga*; the plant is traditionally used against asthma (Fabricant and Farnsworth, 2001). Various *Taxus* species are abundant in paclitaxel, an important anticancer drug (Cragg et al., 2011). In the investigation of either crude plant extract or isolated bioactive compounds on experimental animals, murine model (*Mus musculus*) is the widely used model; in 2017, around 61% of animal studies was performed on mouse model (European Union, 2020). Despite the visible differences in phenotypic characteristics, mouse and human share 99% identical genes making it an ideal organism to study the functions of human genes in a normal physiological state as well as in different disease states such as cancer, cardiovascular diseases, diabetes and arthritis. Molecular structures that control cellular function and differentiation are similar in mouse and human. Also, the mouse genome exhibits mutations in protooncogenes and tumour suppressor genes that are similar to those observed in human cancers (Balmain and Harris, 2000).

During preliminary pharmacological investigations using crude plant extract on a mouse model, the choice of animal sex is rarely justified. Most researchers avert sex dimorphism and prefer male over female assuming possible interference of fluctuating hormonal state in females; thus, the putative pharmacological responses may be affected. Apart from variations in responses due to the estrous cycle, subtle anatomical differences between male and female are also anticipated to influence the experimental findings (Wald and Wu, 2010; Beery and Zucker, 2011,). Potential therapeutic effects of plants are frequently evaluated against diverse pathological conditions such as pain, fever, inflammation, liver damage, diabetes, diarrhea, anxiety, depression, epilepsy, kidney damage, hypertension and arthritis. In these studies, most scientists rely on male mouse model. However, variations in therapeutic effects in wild-type male and nulliparous female mouse model have not been substantially studied. In the present study, we aimed to investigate the effect of mouse sex on antinociceptive, antipyretic, hyperglycemic, hepatoprotective and antidiarrheal experiments and strived to find whether experimentation on particular mouse sex can be extrapolated to its opposite sex. Any differences in pharmacological responses between males and females warrant the use of both sexes in relevant experiments; on the other hand, insignificant variation between sexes permits random use of either male or female or even combination of the male and female mouse in a single group. Moreover, the freedom to choose a mouse of any sex ease the use of littermates; thus, availability and experimental cost can be easily reduced. It is always recommended to use mini-

mal number of animals per experiment with refinement of the existing experimental protocols.

MATERIAL AND METHODS

Chemicals and drugs

Acetic acid (98%) (Loba Chemie, India) was used to prepared 0.6% (v/v) solution. Formaldehyde (37%) (Loba Chemie, India) was used for the preparation of 2.5% formalin. Saline solution (0.9% NaCl) used for preparing Baker's yeast was a product of Orion Infusions Limited, Bangladesh. Standard drugs- diclofenac, ketorolac, paracetamol, loperamide, and silymarin were collected from different pharmaceutical companies in Bangladesh.

Animal

Swiss albino mice (25~30 g) of both males and females were collected from the International Centre for Diarrhoeal Disease Research, Bangladesh. The animals were housed in cages (40 cm × 30 cm × 17 cm) made up of polypropylene base with stainless steel net. Temperature and humidity of the vivarium were maintained at 23-25°C and 50-55% respectively under the illumination of 12/12 h dark/light cycle. Formulated pelleted food and water were allowed *ad libitum* during the entire study period. In all experiments, forty-eight mice were used and equally divided into four groups: 1) control group for male (CM), 2) control group for female (CF), 3) standard group for male (SM) and 4) standard group for female (SF); thus, each group consisted of twelve mice. The use of animals in the project had been primarily approved by institutional research cell and later by the Institutional Ethics Committee (25/2020). Animals were handled humanely to minimize pain and discomfort, and sacrificed by cervical dislocation after antinociceptive, antipyretic, hypoglycemic and antidiarrheal experiments.

Experimental procedures

Antinociceptive study by formalin-induced hind paw licking test

In formalin-induced licking test, 2.5% formalin (0.02 mL/each mouse) was injected subcutaneously into the plain surface of left hind paw after 1 h of oral administration of test samples- water for control group and diclofenac (10 mg/kg.bw) for the standard group (Dubuisson and Dennis, 1977). Here, concentration of prepared diclofenac stock solution was 1 mg/mL and administered volume of this solution for each mouse was calculated according to the individual weight. Licking and biting of the injected paw were considered as signs of nociception. Time spent in licking and biting was recorded in two phases: at first 0-5 min and last 15-30 min after formalin injection. The results were expressed as percent inhibition of licking response (PIL) [1](Mascolo et al., 1993):

$$\%PIL = \frac{\text{Time spent licking}_{(control)} - \text{Time spent licking}_{(standard)}}{\text{Time spent licking}_{(control)}} \times 100 \quad [1]$$

Antinociceptive study by hot plate test

For hot plate test, mice were pre-screened by putting each one on the hot plate (55 ± 0.5°C); animals that showed nociceptive responses within 10 s were chosen for the final experiment (Woolfe and MacDonald, 1944). Water (0.1 mL/each mouse) and ketorolac (10 mg/kg.bw) were used as control and standard drug, respectively, and were administered orally. Preparation of ketorolac stock solution and administered volume of ketorolac were same as licking test. Each mouse was then placed on the hot plate, and the response of mice to this thermal stimulus was recorded at 0, 0.5, 1 and 2 h posttreatment. Recording of response was commenced when the animal first licked its paws or started to jump. The cut-off time was set to 20 s to avoid mouse tissue damage. Results were expressed as mean percent maximal effect (% MPE) [2]:

$$\% \text{ MPE} = \left(\frac{\text{Post drug latency}_{(t\ hr)} - \text{Pre drug latency}_{(0\ h)}}{\text{Cut-off time} - \text{Pre drug latency}_{(0\ h)}} \right) \text{x } 100 \quad [2]$$

Antinociceptive study of acetic acid-induced writhing test

In the acetic acid writhing experiment, 0.6% acetic acid/each mouse (i.p) was used to induced writhing (Siegmund et al., 1957; Koster et al., 1959). Water (0.1 mL) and diclofenac (10 mg/kg.bw) were used in control and standard group, respectively. Preparation of diclofenac stock solution and administered volume of diclofenac were same as licking test. The number of squirming or stretching of the abdomen was considered as the inception of pain, and these responses were recorded for 15 min. Results were expressed as mean percent inhibition of writhing (PIW) [3]:

$$\text{PIW} = \frac{\text{No. of Writhes (control)-No. of Writhes (sample)}}{\text{No. of Writhes (control)}} \text{ x } 100 \quad [3]$$

Antipyretic study

In an antipyretic test, basal rectal temperature was first measured, followed by a subcutaneous injection of 20% Baker's yeast suspended in 0.9% saline at a concentration of 10 mL/kg.bw of the mouse to induce hyperthermia (Tomazetti et al., 2005). The test animals were then kept in fasting condition for 18 h; animals that exhibited an elevated body temperature of 0.83°C were selected for the experiment. These appropriate animals were orally treated with 10 mg/kg.bw 1% Tween 80 (control group) and paracetamol (150 mg/kg.bw) (standard group). Here, concentration of prepared paracetamol stock solution was 15 mg/mL and required volume of this solution for each mouse was calculated according to the individual weight. Rectal temperatures of all experimental animals were measured at intervals of 0, 0.5, 1, 2, 3 and 4 h. The results were expressed as mean body temperature with standard error of mean of each group.

Hypoglycemia test

In oral hypoglycemia assay, all animals were fasted overnight before the experiment (Mia et al., 2019). After 18 h fasting, blood glucose concentration was measured, followed by immediate oral administration of water (10 mL/kg.bw, control) and glibenclamide (10 mg/kg.bw, standard). Concentration of glibenclamide stock solution was 1 mg/mL and required volume of glibenclamide was calculated according the individual mouse weight. All mice were then rested for next 1 h. After this period, all mice were treated with a dextrose solution (2 g/kg.bw; stock solution concentration, 200 mg/mL). Blood was collected from the tail vein at every 1 h interval of 1, 2 and 3 h. The glucose level was measured by Accu-Check electronic glucometer (Roche, Germany). The results were expressed as mean blood glucose concentration with standard error of mean of each group.

Hepatoprotective test

For the hepatoprotective assay, a normal control group was formed receiving distilled water (10 mL/kg.bw) (Yoshitake et al., 1991). The toxicant group was also treated with distilled water (10 mL/kg.bw) while the standard group received silymarin (100 mg/kg.bw). Here, silymarin stock solution was prepared as 10 mg/mL and administered volume of silymarin was measured according to individual animal weight. Animals of all three groups received their respective sample for 7 consecutive days. To assess the hepatoprotective effect, the animal liver was damaged by oral administration of a single dose of 0.2% carbon tetrachloride (8 mL/kg.bw) on day 5 to the toxicant and standard groups. On day 7, after 2 h of last treatment with the respective test sample, mice were anesthetized with chloroform and then dissected. Blood was collected through cardiac puncture followed by separation of serum. Measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin was performed using a standard commercial kit (Humann, Germany). The weight of the liver was also measured.

Antidiarrheal test

The antidiarrheal activity in mice model was evaluated as described (Awouters et al., 1978) with slight modifications. The control and standard groups were treated with saline (10 mL/kg.bw) and loperamide (5 mg/kg.bw) respectively. Loperamide stock solution was prepared as 0.5 mg/mL and required volume of loperamide was calculated according to individual mouse weight. Castor oil (0.2 mL/each mouse) was used for the induction of diarrhea and was administered 30 min prior saline or standard treatment. Each animal was placed in separate cage with a floor lined with blotting paper, immediately after castor oil administration and observed for 5 h. Time to initial evacuation was recorded. The evacuations were categorized as 1 (normal stool), 2 (semi-solid stool), and 3 (watery stool) and frequency of each category was counted. From these data, evacuation index (EI) value was calculated according to the following formula [4]:

$$E\ I = 1 \times (\text{No 1. stool}) + 2 \times (\text{No 2. stool}) + 3 \times (\text{No 3. stool}). \quad [4]$$

Percentage of inhibition (PI) of diarrhea was calculated as [5]:

$$PI = (\text{EI of vehicle} - \text{EI of sample}) \times 100/(\text{EI of vehicle}). \quad [5]$$

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 8.4.3). Data were represented as mean ± SEM (n = 24). Level of significance was determined using appropriate statistical test and test results are presented as *p<0.05, **p<0.01 and ***p<0.001. 'ns' stands for nonsignificant comparing between male and female in each group separately.

RESULTS

Antinociceptive study

The difference in nocifensive behaviors between male and female mice are shown in Fig. 1. In the hot plate test, a significant difference (p<0.001) in effects between males and females was observed in the standard group; the control group also showed significant variation (p<0.001) at 1 and 2 h. Differences in effects between males and females were more evident after calculating the mean percent of the maximal effect. In the control group, male mice showed a low response (12%) after 2 h, while female mice did not have any change of effect over time. In the standard group, the percent maximal effect was gradually increased and reached 74.3% after 2 h in males, while only 22% maximal effect was observed in females after the same period. On the other hand, the hind paw licking test exhibited significant differences (p<0.001) in licking time only in the late phase. Both males and females showed an equal magnitude of inhibition of licking (~15%) in the early phase; effect in the late phase was more increased in females (46.3%) than males (37.2%). In the case of the acetic acid-induced writhing test, no significant variation was observed in writhing responses between males and females. However, female mice showed more writhing in the control group that decreased in greater magnitude, as observed in the standard group. Consequently, the percent inhibition of writhing was substantially higher in females (48.6%) than male mice (16.1%).

Antipyretic study

Fig. 2A depicted the differences in body temperatures between male and female mice. Female animals showed higher body temperatures in both control and standard group. In the control group, body temperatures in female mice markedly elevated from 38.1 to 38.8°C while male mice showed a mild increase (37.5 to 37.9°C); differences in temperatures significantly (p<0.01, p<0.001) differed at all time points. On the other hand, reduction in temperature after administration of paracetamol was more pronounced in male mice than females. Here, male mice showed a reduction by 1.5°C after 4 h, while temperature reduced by only 0.6°C was seen in female mice.

Hypoglycemia test

Fig. 2B showed the variations in glucose concentrations in male and female mice. Statistical

analysis of blood glucose data in the control group and standard group (glibenclamide) exhibited nonsignificant differences (control: $p<0.09$ to $p<0.53$; standard: $p<0.08$ to $p<0.77$) between male and female animal.

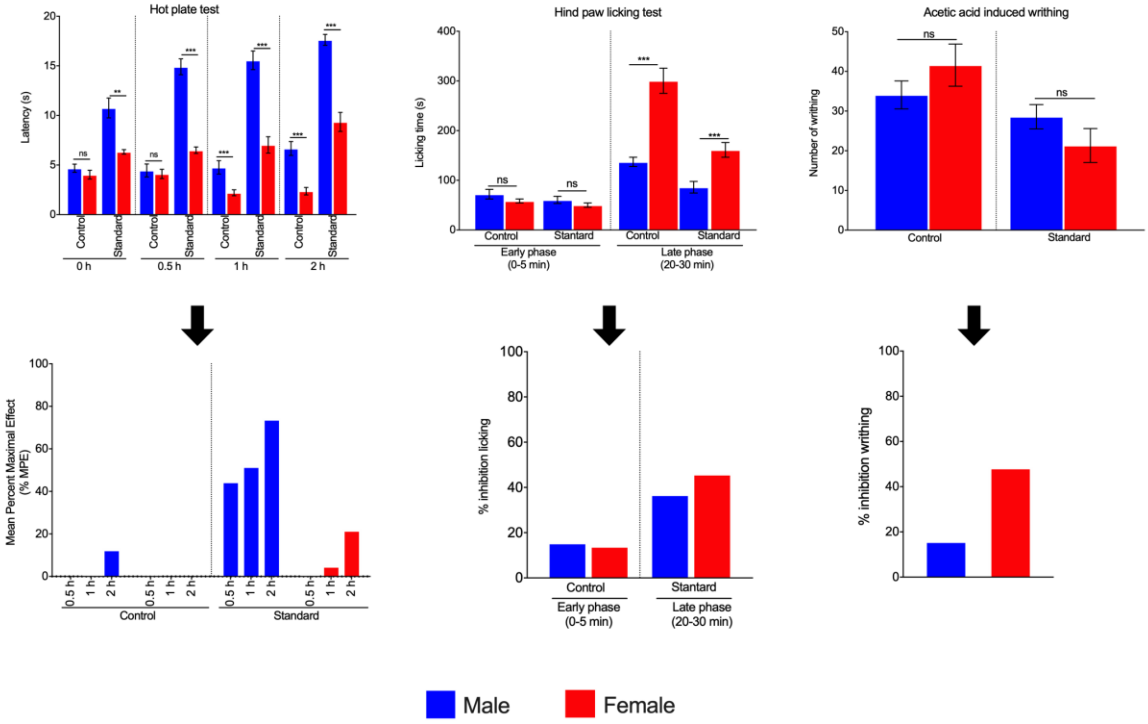

Figure 1. Variations in antinociceptive responses between male and female mice (n = 24).

Data were presented as mean ± SEM. Statistical significance was measured by the 't' test with a non-parametric test followed by the Mann-Whitney test comparing male versus female. Statistical significance of test results is presented as *p<0.05, **p<0.01 and ***p<0.001. 'ns' stands for nonsignificant, comparing between male and female in each group separately.

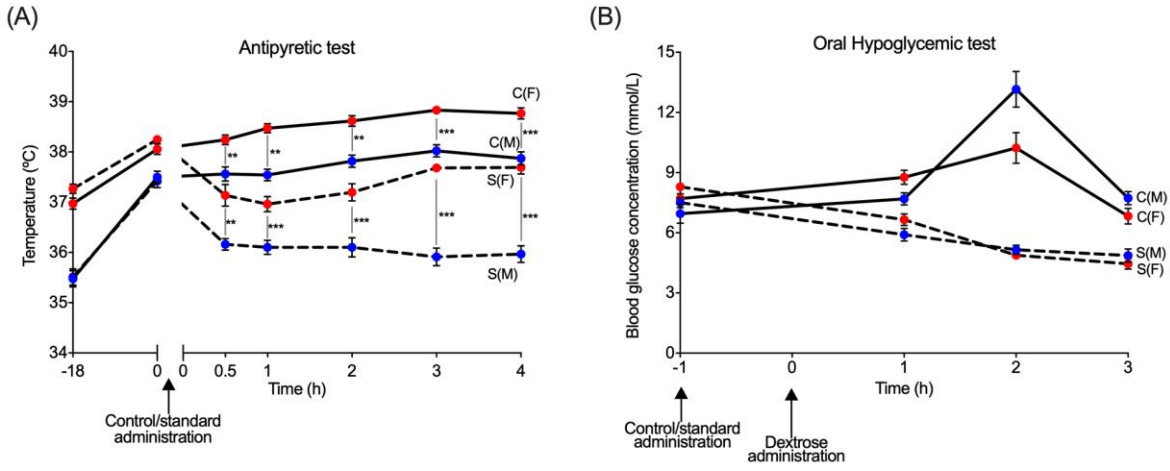

Figure 2. Variation in responses between male and female mice in **(A)** antipyretic test, and **(B)** oral hypoglycemia test.

Data were presented as mean ± SEM (n=24). 2-way ANOVA with Tukey multiple comparisons test was performed comparing male (M) *versus* female (F) for control (C) and standard (S) group separately to determine the statistical significance of test results; here, *p<0.05, **p<0.01 and ***p<0.001. In the oral hypoglycemic test, no statistical difference was observed; thus, (ns: nonsignificant) was not included in the figure due to space constraints between adjacent data points. Points in blue or red represent males or females, respectively.

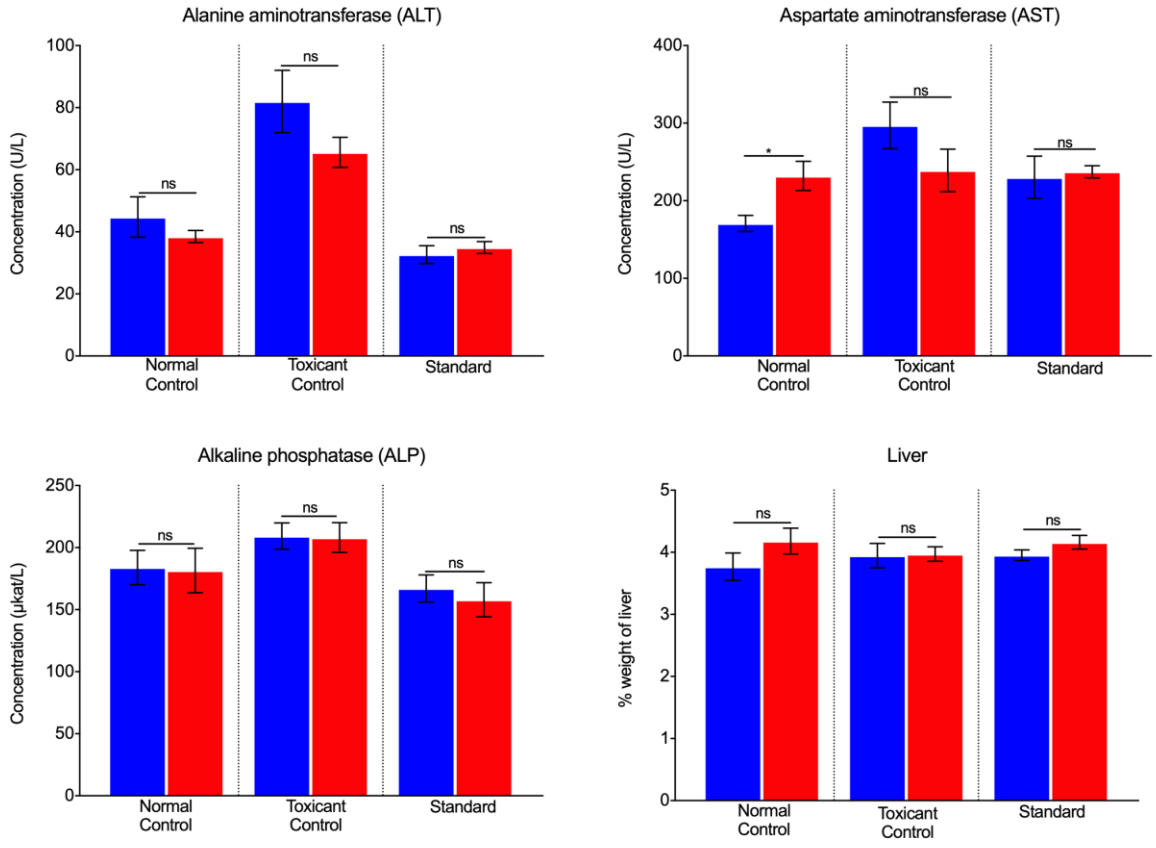

Figure 3. Variations in the hepatoprotective experiment between male and female mice.

Data were presented as mean ± SEM (n=24). Statistical significance was measured by the 't' test with a non-parametric test followed by the Mann-Whitney test comparing male versus female. Statistical significance of test results is presented as *p<0.05, **p<0.01 and ***p<0.001. 'ns' stands for nonsignificant, comparing between male and female in each group separately. Bars in blue or red represent males or females, respectively.

Hepatoprotective test

In the hepatoprotective study, concentrations of three different enzyme- alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in blood, and percentage liver weights were measured (Fig. 3). No significant differences were observed in these enzyme levels except in the normal group of AST ($p<0.05$). The concentration of ALT was observed higher in males in both normal control and toxicant control group; for males, the concentrations were 44.8 and 82.0 U/L, whereas the female group showed 38.5 and 65.6 U/L in normal and toxicant group, respectively. Treatment with silymarin in the standard group showed a greater reduction of enzyme level in males (32.7 U/L) than females (35 U/L). In the case of AST, enzyme concentration in the normal control group was significantly ($p<0.05$) higher in female mice (232 U/L) than male (171 U/L). In male mice, induction of hepatotoxicity by paracetamol showed a noteworthy increment of enzyme level (297 U/L) that sharply dropped upon standard drug treatment (230 U/L). On the contrary, in females, liver damage by paracetamol showed little increase in the enzyme (239 U/L); also, the standard drug didn't show notable change having AST concentration of 237 U/L. Blood levels of ALP were observed with nearly equal concentrations in all three groups in both male and female mice. The control group showed 184 and 182 µkal/L for male and female mice,

Table 1. Differences in diarrheal response between male and female mice in castor oil-induced diarrheal test.

Group	Animal sex	Initial evacuation (min)	Evacuation number			Evacuation index	Inhibition of diarrhea (%)
			Liquid	Semi-solid	Solid		
Control	Male	85.0 ± 14.7	1.58 ± 0.26	1.42 ± 0.19	4.33 ± 0.33	11.92	16.78
	Female	80.5 ± 11.3^{ns}	0.50 ± 0.15**	2.00 ± 0.49^{ns}	3.08 ± 0.47^{ns}	8.58	
Standard	Male	94.8 ± 13.3	0.25 ± 0.13	2.83 ± 0.35	3.50 ± 0.31	9.92	2.91
	Female	88.2 ± 11.5^{ns}	0.25 ± 0.13^{ns}	2.17 ± 0.24^{ns}	3.25 ± 0.22^{ns}	8.33	

Data are expressed as mean ± SEM (n=24). Statistical significance was measured by the 't' test with a non-parametric test followed by the Mann-Whitney test comparing between male and female for control and standard group separately. Statistical significance of test results is presented as **p<0.01. 'ns' stands for nonsignificant, comparing between male and female in each group separately.

respectively. Concentrations were increased to 209 and 208 µkal/L upon hepatic damage. The standard group in both sex groups showed a reduction in concentrations to 167 and 158 µkal/L. Comparing percentage liver weights revealed that damaged liver in the toxicant control group and animals treated with silymarin in the standard group did not vary with the normal control group. Differences in percentage liver weights between male and female mice was observed statistically insignificant in all groups; however, female mice showed higher percentage weights than male.

Antidiarrheal test

Variations in symptoms and responses in castor oil-induced diarrhea are presented in Table 1. Male mice showed statistically insignificant but longer evacuation time than females in both control and standard. Also, male mice were always exhibited equal or higher defecation frequency in all stool categories except in the semi-solid of the control group. Consequently, males had a greater evacuation index of 11.92 and 9.92 for control and standard, respectively, while female mice showed 8.58 and 8.33. Greater changes in evacuation indices in male mice thus showed higher percentage inhibition of diarrhea (16.78%) than females (2.91%).

DISCUSSION

In antinociceptive studies, mouse sex imparted mixed effects in different experiments. In the hot plate test, a significant difference in effects between males and females was observed in both control and standard group. Thermal stimulus from hot plate initiates L-arginine-nitric oxide signaling pathway to a greater extent in males than females; inhibition of this signal pathway resulted in diminished pain perception only in male mice (Fatehi-Hassanabad et al., 2005). Thermal stimuli also elicit spinal analgesia via different types of opioid receptors. It was reported that the activity of opioid receptors significantly varies in male and female mice; opioids acting on µ and κ receptors were reported more effective in male mice than female (Pathan and Williams, 2012). In the formalin-induced hind paw licking test, pain is manifested in two phases having a specific underlying pathway. The early phase indicates neurogenic pain generally mediated through C-fibre activation, whereas various cytokines such as prostaglandins, leukotrienes and tissue necrosis factor are released in peripheral tissues eliciting pain (Linhart et al., 2003). It was reported that initiation of pain differs in male and female mice; in the early phase, injuries to peripheral nerves stimulate microglia for pain sensation in male mice, whereas

T cell is responsible for controlling pain in female mice (Sorge et al., 2015). Differential responses in the late phase of the experiment are attributed to a varied rate of cytokine formation. In male mice, prostaglandin is synthesized to a greater extent than leukotriene; the opposite phenomenon is observed in female mice with higher leukotriene formation (Pace et al., 2017). Thus, selective inhibition of either prostaglandin or leukotriene synthesis display preferential attenuation of pain in a particular sex of mouse. In the writhing experiment, acetic acid causes the release of prostaglandins resulting in acute inflammation in the abdominal cavity; this, in turn, stimulates sensory nerve endings in the peritoneal area (Berkenkopf and Weichman, 1988). Findings in the present work showed no significant difference in nociceptive manifestations between male and female mice. This finding partially discords with the report that described variable writhing responses in male and female mice due to the differences in prostaglandin synthesis pathways (Ballou et al., 2000).

Elevation of body temperature in mammals plays a crucial role to combat against pathogens. Increased thermogenesis opposes pathogen viability facilitating the host defense system to combat infections (Lwoff, 1971). Despite this general mechanism of febrile response, variation in body temperatures between male and female mice can be attributed due to their inherent neuroanatomical differences in the preoptic area of the hypothalamus; this region of the hypothalamus contains EP receptors such as EP_1, EP_3, and EP_4 that upon binding with prostaglandin (PGE_2) elevates body temperature (Stitt, 1973). Synthesis of PGE_2 in brain vasculature is also varied and differentially influenced in male and female mice (Gregory et al., 2000).

Glibenclamide is a potent antidiabetic drug of sulfonylurea group that stimulates pancreatic beta cells resulting release of insulin. The drug also enhances insulin sensitivity mediated either through the improvement of metabolic control or via a direct peripheral effect (Kaur et al., 2016). In the present experiment, insignificant differences in glycemic conditions in male and female mice were observed whether treated with glibenclamide or not. However, Kaikaew et al. (2019) reported sex-dependent variation in insulin resistance in mice priorly exposed to high doses of corticosteroids. Also, hyperinsulinemia was reported only in obese male C57BL/6J-Daruma mice, whereas wild-type mice of both sexes did not exhibit any differences (Suzuki et al., 2017).

Diarrhea is manifested as excess loss of fluid in feces results from an imbalance in the secretory and absorptive mechanisms in the gastrointestinal tract (Field et al., 1989). Ricinoleic acid, the metabolic product of castor oil, induces diarrhea by decreasing fluid absorption with increased secretion in the small intestine and colon. It also affects GI muscle contractility leading to enhanced propulsion of food contents (Mascolo et al., 1993). In this experiment, male and female mice showed a mild and statistically insignificant difference in diarrheal symptoms and responses. This finding is in agreement with the previous report where male and female wild-type mice did not show any difference in *Salmonella* colitis with features of diarrhea (Woo et al., 2008). However, the antidiarrheal drug loperamide showed a stronger effect in males than in females. Loperamide acts on the μ opioid receptors in myenteric plexus of the large intestine resulting in decreased peristaltic movement of intestinal content and hence longer evacuation time (DeHaven-Hudkins et al., 1999). The number of μ opioid receptors varies in mouse sexes that corresponds to altered effect in male and female mice (Craft, 2003). Variations in effect can also be explained by factors affecting drug action. The pH of the fundus is significantly lower in females than males, while fluid buffer capacity and surface tension throughout the intestinal tract are similar in both sexes (Afonso-Pereira et al., 2018). On the other hand, male rats have higher gastric blood flow than females (Shore et al., 2017).

Limitations of the study

In this study, effects of sex differences on different pharmacological studies were evaluated in male and female mouse model. It was tried to use mice of the same litters to minimize their innate differences. Male and female mice were housed in separate cages to restrict mating. All the female

mice were nulliparous to reduce the effect of fluctuating hormonal states. However, the experiments were performed on swiss albino male and female mice. Laboratory mice of different strains differ in genetic factors that influence their phenotypes and they have different research applications. Mice of other strains need to be investigated for sex differences. Also, the findings of the current study can be extended to rat model to determine the effects of rat sexes in pharmacological evaluations.

CONCLUSIONS

The experimental findings demonstrated that antinociceptive and antipyretic tests should be performed separately on both male and female mice. On the other hand, tests on hypoglycemia and liver damage do not require to perform on a particular sex, and random combination of both males and females in a group can be accepted. In the case of the diarrheal experiment, males usually demonstrate a greater effect than female mice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This research work was funded by Research Cell, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

REFERENCES

Afonso-Pereira F, Dou L, Trenfield SJ, Madla CM, Murdan S, Sousa J, Veiga F, Basit AW (2018) Sex differences in the gastrointestinal tract of rats and the implications for oral drug delivery. Eur J Pharm Sci 115: 339–344.

Awouters F, Niemegeers CJ, Lenaerts FM, Janssen PA (1978) Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. J Pharm Pharmacol 30: 41–45.

Ballou LR, Botting RM, Goorha S, Zhang J, Vane JR (2000) Nociception in cyclooxygenase isozyme-deficient mice. Proc Natl Acad Sci USA 97: 10272–10276.

Balmain A, Harris C (2000) Carcinogenesis in mouse and human cells: parallels and paradoxes. Carcinogenesis 21: 347–371.

Beery AK, Zucker I (2011) Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev 35: 565–572.

Berkenkopf JW, Weichman BM (1988) Production of prostacyclin in mice following intraperitoneal injection of acetic acid, phenylbenzoquinone and zymosan: its role in the writhing response. Prostaglandins 36: 693–709.

Craft RM (2003) Sex differences in opioid analgesia: "From mouse to man". Clin J Pain 19: 175–186.

Cragg GM, Kingston DG, Newman DJ (2011) Anticancer agents from natural products. CRC press.

DeHaven-Hudkins D, Burgos LC, Cassel J, Daubert J, DeHaven R, Mansson E, Nagasaka H, Yu G, Yaksh T (1999) Loperamide (ADL 2-1294), an opioid antihyperalgesic agent with peripheral selectivity. J Pharmacol Exp Ther 289: 494–502.

Dubuisson D, Dennis SG (1977) The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4: 161–174.

European Union (2020) Report of European Union, The Statistics on the Number of Animals Used for Experimental and Other Scientific Purposes.

Fabricant DS, Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 109: 69–75.

Fatehi-Hassanabad Z, Jafarzadeh M, Fafehi M, Razavi-Tossi MT (2005) Sex affects the feeling of pain in the mice, possible involvement of nitric oxide. DARU J Pharm Sci 13: 116–119.

Field M, Rao MC, Chang EB (1989) Intestinal electrolyte transport and diarrheal disease. N Engl J Med 321: 800–806.

Gregory MS, Duffner AL, Hahn LE, Hsin-Hsiung T, Faunce ED, Kovacs JE (2000) Differential production of prostaglandin E_2 in male and female mice subjected to thermal injury contributes to the gender difference in immune function: possible role for 15-hydroxyprostaglandin dehydrogenase. Cell Immunol 205: 94–102.

Kaikaew K, Steenbergen J, van Dijk TH, Grefhorst A, Visser JA (2019) Sex difference in corticosterone-induced insulin resistance in mice. Endocrinology 160: 2367-2387.

Kaur N, Kishore L, Singh R (2016) Attenuating diabetes: What really works? Curr Diabetes Rev 12: 259–278.

Koster R, Anderson M, de Beer EJ (1959) Acetic acid for analgesic screening. Fed Proceed 18: 412–417.

Linhart O, Obreja O, Kress M (2003) The inflammatory mediators serotonin, prostaglandin E2 and bradykinin evoke calcium influx in rat sensory neurons. Neuroscience 118: 69–74.

Lwoff A (1971) Protoza to bacteria and viruses-fifty years with microbes. Annu Rev Microbiol 25: 1–27.

Mascolo N, Izzo AA, Barbato F, Capasso F (1993) Inhibitors of nitric oxide synthetase prevent castor-oil-induced diarrhoea in the rat. Br J Pharmacol 108: 861–864.

Mia S, Roy R, Akter A, Barek MA, Basher MA (2019) Hypoglycemic effect of leaf extract of *Fimbristylis miliacea* in mice model. Clin Phytoscience 5: 26.

Pace S, Rossi A, Krauth V, Dehm F, Troisi F, Bilancia R, Weinigel C, Rummler S, Werz O, Sautebin L (2017) Sex differences in prostaglandin biosynthesis in neutrophils during acute inflammation. Sci Rep 7: 3759.

Pathan H, Williams J (2012) Basic opioid pharmacology: An update. Br J Pain 6: 11–16.

Shore R, Björne H, Omoto Y, Siemiatkowska A, Gustafsson J-Å, Lindblad M, Holm L (2017) Sex differences and effects of oestrogen in rat gastric mucosal defence. World J Gastroenterol 23: 426–436.

Siegmund E, Cadmus R, Lu G (1957) A method for evaluating both non-narcotic and narcotic analgesics. Proc Soc Exp Biol Med 95: 729–731.

Sorge RE, Mapplebeck JCS, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin J-S, Sotocinal SG, Chen D, Yang M, Shi XQ, Huang H, Pillon NJ, Bilan PJ, Tu Y, Klip A, Ji R-R, Zhang J, Salter MW, Mogil JS (2015) Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci 18: 1081–1083.

Stitt JT (1973) Prosaglandin E_1 fever induced in rabbits. J Physiol 232: 163–179.

Suzuki Y, Nakahara K, Ensho T, Murakami N (2017) Sex difference of hyperinsulinemia in the C57BL/6J-Daruma (obese) mouse. J Vet Med Sci 79: 1284–1293.

Tomazetti J, Avila DS, Ferreira AP, Martins JS, Souza FR, Royer C, Rubin MA, Oliveira MR, Bonacorso HG, Martins MA, Zanatta N, Mello CF (2005) Baker yeast-induced fever in young rats: characterization and validation of an animal model for antipyretics screening. J Neurosci Methods 147: 29–35.

Vecchio I, Tornali C, Bragazzi NL, Martini M (2018) The Discovery of Insulin: An Important Milestone in the History of Medicine. Front Endocrinol (Lausanne) 9: 613.

Wald C, Wu C (2010) Of mice and women: The bias in animal models. Science 327(5973): 1571–1572.

Woo H, Okamoto S, Guiney D, Gunn JS, Fierer J (2008) A model of *Salmonella colitis* with features of diarrhea in SLC11A1 wild-type mice. PLoS One 3: e1603.

Woolfe G, MacDonald AD (1944) The evaluation of the analgesic action of pethidine hydrochloride (demerol). J Pharmacol Exp Ther 80: 300–307.

Yoshitake I, Ohishi E, Kubo K (1991) Hepatoprotective effects of 1-[(2-thiazolin-2-yl)-amino]acetyl-4-(1,3-dithiol-2-ylidene)-2,3,4,5-tetrahydro-1H-1-benzazepin-3,5-dione hydrochloride (KF-14363) in various experimental liver injuries. Jpn J Pharmacol 57: 127–136.

AUTHOR CONTRIBUTION:

Contribution	Karim I	Roy R	Hoque MR	Hosen S	Bhowmik T	Liya IJ	Akter A	Basher MA
Concepts or ideas	x	x						x
Design	x	x						x
Definition of intellectual content	x	x						x
Literature search	x	x	x	x	x	x	x	x
Experimental studies	x	x	x	x	x	x	x	x
Data acquisition	x	x	x	x	x	x	x	x
Data analysis	x	x						x
Statistical analysis	x	x						x
Manuscript preparation	x	x	x	x	x	x	x	x
Manuscript editing								x
Manuscript review	x	x	x	x	x	x	x	x

Citation Format: Karim I, Roy R, Hoque MR, Hosen S, Bhowmik T, Liya IJ, Akter A, Basher MA (2020) Effect of sex differences in antinociceptive, antipyretic, hypoglycemia, hepatoprotective and antidiarrheal activities in mice model. J Pharm Pharmacogn Res 8(6): 569–579.