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ARTICLE

Pain Evaluation in Ovariohysterectomised Albino Wistar Rats using Physiological, Behavioural and Biochemical Indices

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SUMMARY

Twenty adult female albino wistar rats assigned randomly to four groups (GP1 – 4) were used to study the identification and measurement of post ovariohysterectomy (OVH) pain using physiological, behavioural and biochemical parameters. GP1 rats were given pentobarbitone (60mg/Kg body weight intraperitoneally) alone. GP2 rats were administered pentazocine at 10mg/kg (intramuscularly) body weight and pentobarbitone at the same dose as GP1. OVH was not performed on rats in GP1 and GP2. GP3 and GP4 rats were anaesthetised as in GP1 and GP2 respectively and OVH was carried out. Blood glucose, daily feed intake, daily defeacation were measured once daily, while behavioural changes were measured twice daily and the mean indices for the day recorded. Data obtained from the different groups were subjected to ANOVA and post hoc LSD and values of $p < 0.05$ were considered significant. The rates of defeacation and the scores for feed intake and movement were significantly higher in GP1 and GP2 than in GP3 and GP4 while those for aggression were higher in GP3 and GP4 than GP1 and GP2. Pentazocine seemed to have improved these indices significantly in GP3. The scores for avoidance of ventral abdominal contact and refusal to stand on hind limbs were significantly higher in GP3 and GP4 and did not differ significantly between the operated groups. Blood glucose levels and the scores for squinting of the eyes were not significantly different among the groups. These results appear to indicate that physiological and behavioural parameters can be used to identify pain in rats. They were however not sensitive enough to differentiate between the degree of these pain in rats.

Keywords: Pain assessment. Rats. Ovariohysterectomy.

INTRODUCTION

Pain is an aversive sensory and emotional experience. It represents awareness by animals or man of damage or threat to the integrity of thetissues which may change their physiological and behavioral patterns from normal in order to reduce or avoid further damage, reduce the likelihood of recurrence and promote recovery (Molony and Kent, 1997). The detection and assessment of pain in animal depends on the observance and measurement of changes in these physiological and behavioral deviations from normal.

The need to identify and manage pain in animals has been clearly demonstrated and is acutely relevant in most disease and surgical conditions in animals (Vadivelu *et al,* 2010).The presence of pain and its management in animals kept in farms, colonies and homes are useful indicators of their welfare, and veterinarians depend on them in their assessment of the welfare status of animals (Omamegbe and Ukweni, 2010).

Pain accompanies most traumatic conditions, including trauma caused by surgery but is usually difficult to identify and/or measure even in verbal humans and more so in non-verbal humans and in animals. For this reason, several scales have been designed to aid clinicians in the identification and measurement of pain in animals. These include the simple Visual Analogue Scale (VAS) to the more complex scales which combine the assessment of alterations in physiological and behavioural parameters in animals in various ways and combinations. However, the parameters used for the identification and assessment of pain in animals in any particular setting depend on the condition affecting the animal. For instance, parameters ideal for the identification and assessment of pain caused by aural procedures in dogs may be inappropriate for the assessment of pain after the repair of femoral fracture in a cat. Other factors which influence the identification and measurement of pain in animals are inter- and intra-observer variability, non-linearity and sensitivity of parameters employed in pain studies and non-validation of such parameters. The use of arbitrary definitions of pain descriptors has also been identified as a limiting factor in the identification and measurement of animal pain. Because of these limiting factors, none of the scales used presently to identify and measure pain in animals has been found to meet all the requirements for an ideal pain scale and none is easily applicable in clinical settings.

There is therefore some urgent need for a simple, easy-to-use and user-friendly scale for the assessment of pain in animals undergoing identified systemic or specific surgical conditions. Such considerations lead to the design of the short Form of the Glasgow Composite Measure Pain scale (GCMP-SF) (Murrell et al, 2008). Attempts to apply the GCMP-SF show that in spite of its simplicity compared to other scales, it requires a lot of clinic time to assess a patient completely if it is to be applied comprehensively.

In this study, an attempt was made to investigate the correlation between changes of a simple easily determinable biochemical parameter like blood glucose level which can be determined using a commercial glucometer with changes in pre-determined physiological and behavioural parameters which are routinely measured in the clinic following OVH using albino Wistar rats as model.

MATERIALS and METHODS

Twenty non-pregnant, non-lactating female albino wistar rats weighing between 156.4±12.8 grams wereused in this study. They were assigned to four groups (GP1 – 4) of five rats per group, with GP1 serving as the control. The rats were obtained from the University of Nigeria, Nsukka and were acclimatized for two weeks. They were housed individually in large wooden confinements measuring 7ft x 3ft x 3ft to avail for easy access, observation and to prevent interference between the rats.

Each rat in each group was anaesthetised with pentobarbitone sodium (Sodium Pentobarbitone 6%, Kyron Lab (Pty) Ltd, South Africa) at a dose of 60 mg/kg b.w. intraperitoneally and subjected to a predetermined treatment pattern as detailed below (Table 1). No pre-anaesthetic medication was administered to the rats in any of the groups

TABLE I. SUMMARY OF THE TREATMENT GROUPS

GROUP	No of animals	Pentobarbitone (60mg/kg bwt)	Ovario hysterectomy	Pentazocine (10mg/kg bwt)
GP1	5	YES	NO	NO
GP2	5	YES	NO	YES
GP3	5	YES	YES	NO
GP4	5	YES	YES	YES

The five rats in GP1 were neither ovariohysterectomized nor received any pentazocine. Rats in this group served as the control for this experiment. GP2 rats were not ovariohysterectomized, but received pentazocine at a dose of 10mg/kg body weight intramuscularly twice daily. The pentazocine administration started immediately after recovery from anaesthesia. Ovariohysterectomy was carried out in animals in GP3 as described for canines (Venugopalan, 2000). They were not administered pentazocine post operatively. The five rats in GP4 were also ovariohysterectomised routinely as for GP3 and were subsequently treated postoperatively with pentazocine as in GP2.

Parameters like feed intake, number of feacal droppings and blood glucose were measured daily. Other pre-determined parameters were observed and measured twice daily and the mean value for each parameter recorded. These parameters were measured or observed approximately 30 minutes after the administration of pentazocine postoperatively in those groups where pentazocine was administered (GP2 and GP4) or at such times in rats in the other groups. These other parameters were rate of movement, squinting of the eyes, standing on hind limbs (vertical stretching), abdominal contact with underlying surface and aggression to handling. Each parameter was weighted subjectively according to its adjudged relevance to the detection of pain following abdominal surgery in animals. The parameters measured were subjectively assigned scores (Table 2). Scoring was on the all-or-none bases, as each parameter was scored either zero or the maximum score for that parameter. The score for feed consumption was based on the reported average daily feed consumption of more than 5-10g/100g body weight per day (Sanchez-Mateos *et al,* 2007)

The blood glucose levels were determined in each rat before the commencement of the experiment and twice daily on alternate days using a glucometer used in the determination of blood glucose in humans (Acu-Check Active glucometer (Roche, Germany). The directives given by the manufacturers were adhered to at all times in the use of the glucometer.

The data obtained for these parameters were analyzed statistically within and between the groups using ANOVA and post hoc LSD (SPSS version 16). Values of $p < 0.05$ were considered significant.

TABLE II: WEIGHTED SCORE FOR PARAMETERS

S/NO	PARAMETER	ADJUDGED SCORE
1	Aggression to handling	3
2	Refusal to stand on hind limbs	3
3	Avoidance of contact with underlying surface	6
4	Squinted eyes	6
5	Movement	6
6	Feed intake	9

RESULTS

FEED INTAKE

The operated rats (GP3 and GP4) ate significantly less ($p < 0.05$) feed on days 1 and 2 postoperatively than the control group (GP1). There was no significant difference in feed intake between GP3 and GP4 on day 1. On the second day, rats in GP3 ate significantly ($p < 0.05$) less feed than those in other groups. The feed consumption of rats in GP4 was significantly less than the other groups, but more than those of GP3. The feed intake was not significantly different ($p<0.05$) among the groups after day 3 postoperatively.

TABLE III. FEED INTAKE SCORE. A HIGHER SCORE INDICATES A REDUCTION IN FEED INTAKE

Groups	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	7.20 ± 0.22	9.00 ± 0.00	5.40 ± 0.42	9.00 ± 0.00	7.20 ± 0.22
_	6.75 ± 0.37	7.20 ± 0.22	9.00 ± 0.00	7.20 ± 0.22	9.00 ± 0.00
3	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	6.75 ± 0.37	6.00 ± 0.30	9.00 ± 0.00
4	0.00 ± 0.00^{a}	4.50 ± 0.64^{b}	7.50 ± 0.26	9.00 ± 0.00	7.50 ± 0.26

Different superscripts indicate significant difference between groups. $P < 0.05$

NUMBER OF FEACAL DROPPINGS PER DAY

The rate of defeacation was significantly lower in the operated rats (GP3 and GP4) than those in GP1 and GP2 on the first ($p < 0.001$) and second ($p < 0.05$) postoperative days. On the third postoperative day, the frequency of defeacation was significantly higher ($p < 0.05$) in GP3 than in GP4 only. No other statistically significant difference was observed between the groups on that day.

TABLE IV: AVERAGE NUMBER OF FEACAL DROPPINGS PASSED PER DAY

Groups	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	39.20±4.63	30.80±4.74	25.60±6.63^{ab}	42.40±8.76^{b}	28.80±4.73
2	35.80±3.74	33.80±5.83	26.60±7.73^{ab}	50.00±10.73^{b}	34.70±8.83
3	2.25±1.73^{a}	15.36±4.35^{a}	37.25±5.23^{a}	23.25±6.05^{a}	47.25±6.92
4	3.60±0.53^{a}	12.60±2.60^{a}	20.80±3.62^{b}	30.80±9.37^{ab}	33.60±7.53

Different superscripts indicate significant difference between groups. $P < 0.05$

AVOIDANCE OF CONTACT OF VENTRAL ABDOMEN WITH UNDERLYING SURFACE

The score for avoidance of contact of ventral abdomen with the underlying surface was significantly higher ($p < 0.05$) on days 1, 2 and 3 in the operated rats (GP3 and GP4) than in the unoperated rats. There was no significant difference in the scores between GP3 and GP4. On day 4, the score for contact was significantly higher in GP4 than in other groups.

TABLE V: SCORE FOR AVOIDANCE OF CONTACT WITH UNDERLYING SURFACE. A HIGHER SCORE INDICATES A MORE FREQUENT AVOIDANCE.

Groups	DAY 1	DAY 2	DAY 3	DAY 4	DAY5
1	0.00±0.00	0.00±0.00	1.20±0.17	1.20±0.17	2.40±0.73
2	0.00±0.00	1.20±0.26	0.00±0.00	3.60±1.03	2.40±0.73
3	4.50±0.72^{a}	3.00±0.23^{a}	3.00±0.23^{a}	3.00±0.23	3.00±0.23
4	2.57±0.81^{a}	4.29±0.53^{a}	5.00±1.21^{a}	5.00±1.21^{a}	1.00±0.14

Different superscripts indicate significant difference between groups. $P < 0.05$

AGGRESSION TO HANDLER

Rats in the group that was operated but did not receive analgesics (GP3) had a significantly higher score for aggression to handling than rats in the other groups on days 1, 2 and 3 postoperatively. There was no difference between the groups on days 4 and 5.

TABLE VI: SCORE FOR AGGRESSION TO HANDLER. A HIGHER SCORE INDICATES A HIGHER FREQUENCY OF AGGRESSION.

Groups	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	1.20±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	1.20±0.28	0.00±0.00	0.00±0.00
3	3.00±0.00^{a}	3.00±0.00^{a}	3.00±0.00^{a}	1.50±0.50	1.20±0.28
4	0.00±0.00	0.00±0.00	1.00±0.31	0.00±0.00	0.00±0.00

Different superscripts indicate significant difference between groups. $P < 0.05$

REFUSAL TO STAND ON HINDLIMBS (Reluctance to stretch vertically)

Operated rats (GP3 and GP4) had a significantly higher score ($p < 0.05$) for refusal to stand on their hindlimbs than the unoperated rats (GP1 and GP2) on the first and second postoperative days. However, the score on that day for the control group was significantly higher than that of GP2. Scores for the control group was significantly lower than scores in the other groups on the third postoperative day. No significant difference in scores was recorded on the fourth day between the groups. On day 5, the score for GP3 was significantly higher ($p<0.05$) than that for other groups. No other significant difference was recorded.

TABLE VII. SCORE FOR REFUSAL TO STAND ON THE HIND LIMBS. A HIGHER SCORE INDICATES A HIGHER RELUCTANCE TO STAND.

Groups	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	1.20±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	1.20±0.28	0.00±0.00	0.00±0.00
3	3.00±0.00a	3.00±0.00a	3.00±0.00a	1.50±0.50	1.20±0.28
4	0.00±0.00	0.00±0.00	1.00±0.31	0.00±0.00	0.00±0.00

Different superscripts indicate significant difference between groups. $p<0.05$

SQUINTED EYES

Results for scores for squinting revealed no significant difference between groups on the five postoperative days under consideration.

TABLE VIII: SCORE FOR SQUINTING OF THE EYES. A HIGHER SCORE INDICATES A HIGHER DEGREE OF SQUINTING

Groups	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	1.80±0.16	1.80±0.16	0.00±0.00	3.60±0.52	3.60±0.52
2	1.80±0.16	0.00±0.00	3.60±0.52	1.80±0.`6	0.00±0.00
3	2.25±0.21	2.25±0.21	2.25±0.21	2.25±0.21	2.25±0.21
4	3.00±0.00	2.57±0.18	0.00±0.00	6.00±0.00	1.50±0.20

Different superscripts indicate significant difference between groups. p<0.05

MOVEMENT

The results for movement revealed that there was a higher impairment of movement in animals that were operated on the first postoperative day, with the score for GP3 being significantly higher than those for the control and GP2 animals, and that for GP4 being significantly higher than GP1. The score for movement impairment was higher in GP3 than in other groups on day 2. GP2 and GP3 rats showed a significantly higher score than the other groups on day 3. No significant difference was recorded among the groups of rats on days 4 and 5,

TABLE IX: SCORE FOR MOVEMENT. A HIGHER SCORE INDICATES A MORE SLUGGISH MOVEMENT.

Groups	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	$0.00±0.00^{c}$	1.20±0.02	$0.00±0.00^{bc}$	0.00±0.00	0.00±0.00
2	$1.20±0.02^{bc}$	1.20±0.02	$3.60±0.17^{a}$	3.60±0.17	1.20±0.02
3	$6.00±0.00^{a}$	$6.00±0.00^{a}$	$4.50±0.25^{a}$	1.80±0.17	4.50±0.25
4	$3.43±0.11^{ab}$	3.43±0.11	$3.00±0.00^{ab}$	3.00±0.00	3.00±0.00

Different superscripts indicate significant difference between groups. p<0.05

BLOOD GLUCOSE

Results of blood glucose from rats in each of the groups showed no significant difference in the blood sugar levels among the groups in the four postoperative days under consideration.

TABLE *X: Blood glucose levels*

Groups	DAY 0	DAY 1	DAY 3	DAY 5
1	114.00±4.27	115.75±16.73	108.75±9.73	84.50±7.52
2	111.50±4.60	92.75±7.52	106.25±14.62	92.75±10.62
3	101.25±9.56	103.50±3.83	103.75±13.28	116.50±21.17
4	100.25±10.62	90.00±8.87	109.25±6.66	76.20±6.29

DISCUSSION

Previous results have shown that there is a close correlation between the perception of pain and the degree of pain felt following surgical trauma in the one hand and certain, physiological, behavioural and biochemical changes in animals on the other (Roughan and Flecknell, 2000). However, the administration of analgesia to such animals during the peri-operative period has been shown to modulate or eliminate such physiological and behavioural changes associated with post-operative pain in castrated calves (Molony *et al.,* 1997).

Some physiological, behavioural and biochemical changes associated with castration in calves were reduced to baseline values following the administration of analgesics The physiological and behavioural changes observed in ovariohysterectomized rats in this study disappeared before the fifth postoperative day whether pentazocine was administered postoperatively or not. This observation implies that these changes occurred in the acute phase of the pain and are in agreement with previous works (Molony and Kent, 1997; Liles and Flecknell, 1994Bateson, 2004).

The insignificant difference in the blood glucose levels of the different groups of rats recorded in this study contradicts the findings of similar works (Udegbunam *et al.,* 2012) in which significantly higher levels of blood glucose were recorded in animals that were operated but did not receive analgesics than in those that were operated and administered analgesics (piroxicam) postoperatively. The later however seems to be consistent with the notion that pain as a stressor induces an endocrine stress response which cause the release of cortisol, catecholamines, and other stress hormones like glucagon. Glucagon seems to be responsible for the hyperglycaemia in such cases. The reason for the confliction of the result of blood glucose in this study with results of other works (Liles and Flecknell, 1994; Udegbunam *et al.,* 2012) cannot be explained.

The reduction in feed intake during the immediate post operative period seems to correlate closely with the perception of pain in operated rats. Similar findings have been previously observed in rats postoperatively (Roughan and Flecknel, 2000). However, following the administration of analgesics, feed intake in such animals returned to normal values (Liles and Flecknell, 1994). The close association between the reduction in feed intake and the reduction in daily feacal output is an expected outcome and has been reported previously (Guyton and Hall, 2000; Randell *et al*., 1997). Pain, as a stressor is known to cause a transient reduction in the rate of food and water intake and consequently a reduction in the daily feacal mass (Omamegbe and Ukweni, 2010). The reduction in feed intake in the groups that received pentazocine compared to the control (GP1) may be due to pentazocine sedative and distractive effects. This may reflect the increase of gastrointestinal transit time generally by opioids. Although constipation has been associated with pentazocine in man, (Kohn *et al.,* 1997), it has not been reported in rats.

Subjective parameters such as increased aggression, squinting of the eyes, increased back arching, horizontal stretching, abdominal writhing, falling/staggering, reduced activity (like the rate of movement, grooming, and climbing on vertical objects or standing on hind limbs), poor gait, twitching and biting of cages (simulating aggression) are thought to be due to pain caused by most surgical procedures (Roughan and Flecknell, 2000). Avoidance of ventral abdominal contact with the underlying surface, increased aggression, reduced frequency of standing on hindlimbs and squinting of the eyes were also observed in all the groups of ratsovariohysterectomized in this study, but not in the control, and is thought to be due to the discomfort experienced by the rats due to the surgical pain. The degree at which these changes were manifested was mild in groups that were administered analgesics and moderate to severe in groups that did not receive analgesics postoperatively. This finding suggests that analgesics tend to modulate these changes downwards.

CONCLUSION

There appears to be an inconsistent relationship between pain and blood glucose levels. More work is therefore needed in this area to validate this relationship.Feed intake, fecal output, aggression, rate of movement, avoidance of contact of ventral abdomen (site of operation) with underlying surface, and standing on hind limbs are good tools for assessing acute postoperative pain in rats, and probably in other animals.

Most post-operative pain is acute, transient and self limiting. The use of analgesics pre-operatively, reduces the discomfort experienced by animals following surgical manipulations. The increase in such animals' activities including feeding would eventually lead to a better general outcome of surgical procedures, shortening of the catabolic phase of recovery from trauma, faster wound healing and a quicker recovery. Postoperative analgesia is therefore important in ensuring a better and faster recovery from surgery.

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