SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name: **British Journal of Medicine and Medical Research**

Manuscript Number: **2013 BJMMR_4217**

Title of the Manuscript: **Antinociceptive effects of ethanolic extract of Hybanthus enneaspermus leaf in male albino rat**

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
This manuscript was intended to investigate the Antinociceptive effect of ethanolic extract of Hybanthus enneaspermus leaf (EEHE) in rats by using tail flick and formalin tests. The results showed that EEHE (500 mg/kg and 1000 mg/kg) significantly reduced the paw licking time and significantly increased the tail flick latency. This manuscript is a preliminary. Specific comments are addressed as follows : **The method of formalin test is different from the method described in many articles. The results of formalin test (Figure 1 and Figure 2) are different from that described in the method of formalin test section. Please cite references in the formalin test section.**	The Method of Hunskar and Hole (1987) cited is widely used in studies incorporating the formalin test. Virtually all studies that involve formalin test cite this same method a few studies using this same method and citing Hunskar and Hole are: (i) Awe et al 2005 (ii) Bukahri et al 2004 (iii) Oyadeyi et al 2007 (iv) Zala et al 2012 **Figure 1** depicts the change in tail flick latency occurring after treatment with the reference drug and the extracts. It is not for the formalin test which is shown in figures 2 and 3. Figure 1 shows that in the acetaminophen (AMP), HEE 500mg/Kg and HEE 1000mg/Kg treated rats the tail flick latency increased significantly after treatment. **Figure 2** shows the duration of paw licking reaction to plantar injection of formalin in the early phase of formalin test. It is **Table2** that shows the calculated percentage inhibition of paw licking caused by the administration of the reference drug and

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	the extracts.
1. **Table 1:** 1. **The duration (sec) of AMP before treatment is lower than the control group. Why?** 2. **Is there any significant difference between control and AMP groups after treatment? The value of 4.04 in control group is near to the value of 4.33 in AMP group. Both of the 4.04 and 4.33 should not be significant.**	**(1)** The observed difference in the "before treatment" values of the four groups not only the AMP group can be due to the inherent variation in animals. The particularly low value of tail flick latency before treatment in the AMP group may be due to random selection bias. However, the method of statistical analysis used in this case the paired t-test nullifies any difference between the groups as the before and after format used makes each group its own control! **(2)** As stated above we did not compare the groups directly but compared the increase in tail flick latency after treatment. Thus to demonstrate antinociceptive effect of AMP the tail flick before (2.76 ± 0.42) and after (4.33± 0.3) were compared by paired t-test. This clearly shows that administration of the reference drug had significant effect. Similar effect was recorded for HEE 500mg/Kg and HEE 1000mg/Kg.
3. **The statistical analysis method (ANOVA followed by Tukey**	The One way ANOVA was used for the comparison of the

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	multiple range test) used by authors is suitable to more than three groups. The authors seem to compare the statistical significant between before treatment and after treatment. Oneway ANOVA is unsuitable to compare the statistical significant between before treatment and after treatment.	differences in the tail flick latencies before and after the reference drug and the extracts were given. In this case the differences in the latencies of the control, the AMP, the HEE 500mg/Kg and the HEE 1000mg/Kg treated rats were analysed using theOne way ANOVA test.