Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.13, No.6, 2021

Comparative Antioxidant Effects of Two Separate Ethanolic Extracts Fed Female Albino Rats on Some Tissue Markers

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Abstract
Garcinia kola and *Curcuma longa* possess antioxidant, anti-inflammatory and anti-toxic properties which makes them ideal choices in traditional alternative medicine. Kolaviron and curcumin ethanoic extracts are from *Garcinia kola* and *Curcuma longa* respectively and have shown ample beneficial health effect in animal models of diseases and also in the prevention of hepatoxicity induced by several toxins. The aim of the study was to compare the separate effects of kolaviron and curcumin ethanoic extracts on blood parameters in female Wister albino rats. Eighty-four female albino rats were used in this study and were grouped into fertile, infertile and pregnant rats in the investigation. Anti-oxidant markers such as catalase, superoxide dismutase (SOD), glutathione s-transferase (GST) were assayed for in this study.The result shows that both kolaviron and curcumin, possess antioxidant properties and the results from the three groups investigated, showed that the extracts had similar influence (anti-oxidants effect) on pregnancy, as kolaviron showed a bit more "potency". In the infertile rats, both extracts present competing anti-oxidants effects on the organs. In conclusion, kolaviron presented a more effective anti-oxidants property across the three subject groups studied compared to curcumin.
Keywords: *Garcinia Kola*, Turmeric, Kolaviron, Curcumin, Haematological, antioxidants Parameters
DOI: 10.7176/JNSR/13-6-04
Publication date: March 31st 2021

INTRODUCTION

Garcinia Kola is widely used in traditional medicine in Africa with most parts of the plant being used and a wide range of ailments being treated, from which several medically active compounds have been identified, (Hernandez-Reif *et al.*, 2019). Tannins, a reducing sugar and traces of an alkaloid have been detected in the bark, flavonins are also present, while the whole plant being, extremely bitter, resinous and astringent. A number of pharmacological actions have also been demonstrated, as the fruits, seeds, nuts, and bark of the plant have been used for centuries in folk medicine to treat ailments from cough to fever (Priyadarsini, 2014).

Extracts of stems, roots and seeds (kolaviron) have been shown to have strong anti-hepatotoxic and hepatotropic activity. Petroleum ether and acetone extracts were found to be markedly anti-microbial (Farombi *et al.,* 2002, 2013). The seeds are said to be an antidote, antitussive, aphrodisiac, astringent and vermifuge. Mastication of the seeds is said to relieve coughs, hoarseness, and bronchial and throat troubles. They are taken dry as a remedy for dysentery. They are said to provide an antidote against Strophanthus poisoning. The active principle, or principles, in the nut remains enigmatic. Caffeine which is present in the true kola is absent, a trace of alkaloid has been reported in Nigerian materials, but absent in other samples (Farombi *et al.,* 2002).

Turmeric (*Curcuma longa*) belongs to the family Zingiberaceae includes more than 80 species of rhizomatous perennial herbs and has widespread existence in the tropics of Asia, Africa, and Australia, (Priyadarsini, 2014). *C. longa*, commonly known as turmeric (Haldi), is a well-known plant which is used as a drug in Ayurvedic and Unani system of medicine (Stohs and Ray, 2019). The World Health Organization has suggested the use of turmeric as a spice, (Srivastava *et al.,* 2016). Comprehensively, *Curcuma* attainment is importance as a growing source of new drug(s) to fight a variety of ailments as the species contain molecules validated with anti-fungal properties (Hernandez-Reif *et al.*, 2019), anti-inflammatory, hepatoprotective, antitumor, antiviral, (Ammon *et al.,* 1991) and anticancer activities (Stohs, and Ray, 2019). Curcumin (diferuloylmethane) is a natural yellow polyphenolic pigment isolated from the rhizomes of the plant *Curcuma longa*, (Owaga *et al.,* 2014). It is commonly used as a food additive and it shows a wide spectrum of biological and pharmacological effects, such as anti-inflammatory, antioxidant, antimicrobial, anti-hepatoxic, hypolipidemic, and anticancer properties (Srivastava *et al.,* 2016). Curcumin also has immunomodulatory and anti-allergic activities (Owaga *et al.,* 2014).

METHODS

Collection of Plant materials and Authentication

Garcinia kola and fresh roots of *Curcuma longa* were used in this study. The *Garcinia kola* was obtained from a local farm in Osun state, Nigeria. While the roots of the *Curcuma longa* were procured from a local farm in Kaduna, Nigeria. Authentication for both plant samples was carried out at the Herbarium unit of Botany department, University of Lagos.

Extraction of Curcumin and Kolaviron

The outer coats of the *Curcuma longa* and *Garcinia kola* were removed and the seeds and roots respectively were cut into small pieces, washed and air dried. The dried seeds (roots) were grounded to fine powder and extraction was done separately, using 70% ethanol in a Soxhlet extractor. The yield was concentrated by evaporation in a rotary evaporator and dried solid was obtained.

Collection and Acclimatization of Animals

Female albino rats weighing between 70-100g were obtained from the animal house of the Department of Biochemistry, University of Ibadan, Oyo state. The rats were kept in wire meshed cages for two weeks to acclimatize. The animals were fed with commercial rat feed. The rats that were selected for administration of extracts weighed between 130g – 150g having an average weight of 140g.

Chronic Toxicity Test

Chronic toxicity test was performed on the animals to determine the actual dose to be administered to avoid high rate of deaths in the groups. In summary, a total of eighteen (18) rats were selected at random and observed for the toxicity test, nine (9) for kolaviron in a three (3) rats per cage grouping, and nine (9) for curcumin in another three (3) rats per cage grouping, making up six (6) cages, each cage received different doses of plant extract.

The doses administered for the toxicity test for Kolaviron: 400mg/kg for cage 1, 600mg/kg for cage 2, and 800mg/kg for cage 3

The doses administered for the toxicity test for Curcumin: 400mg/kg for cage 1, 500mg/kg for cage 2, and 600mg/kg for cage 3.

Animal Groupings

In order to know the actual dose to be administered to each animal in the group, calculation was done according to the average body weight before administration. All animals in the groups were repeatedly administered by oral gavages to the respective dose of kolaviron for 14 days. Concentrated kolaviron was dissolved in corn oil while curcumin was dissolved in olive oil.

Group 1 served as the control and the other test group that received administrations with oral gavage. Prior to treatment of, rats in groups 2, 5, 6, 7 and 8, that were made infertile with postinor-2. Rats in group 2 continued using the postinor-2, three days interval throughout the experimental period. Also, rats in groups 9, 10 and 11 were mated and were made pregnant with male rats procured along with the female at the beginning.

GROUPS

1 Control (Distilled Water), 2 Infertile Group, 3 Kolaviron only (200 mg/kg)
4 Curcumin only (100 mg/kg), 5 Low-Dose Kolaviron (200 mg/kg)
6 High-Dose Kolaviron (400 mg/kg), 7 Low-dose Curcumin (100 mg/kg)
8 High-dose Curcumin (400 mg/kg), 9 Pregnant Group,
10 Pregnancy+ Kolaviron (200 mg/kg), 11 Pregnancy+ Curcumin (100 mg/kg)

Extraction/collection of blood samples and storage

After fourteen days of treatment, the rats were then anaesthetized using diethyl-ether and were sacrificed followed by the collection of blood samples and extraction of organs. Blood samples were collected by cardiac puncture, into well labeled EDTA and plain bottles, then stored frozen in a refrigerator until ready for use. The kidney, liver and ovary of each of the rats were extracted immediately after blood collection. The organs were preserved in phosphate buffer (pH 7.4) before being homogenized. After homogenization, the homogenate were then centrifuged at 3000 rpm. The supernatants were decanted in well labeled plain bottles and then stored in a refrigerator until ready for use.

Determination of anti-inflammatory markers

Determination of superoxide dismutase (SOD) activity

The percentage inhibition of SOD was determined using the method of José, *et al.,* (2013). The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

Determination of catalase activity

Catalase activity was determined according to the method of Odewabi *et al.,* (2014). The changed in the colour of the solution to stable green due to formation of chromic acetate. After cooling at room temperature, the volume of the reaction mixture was made to 3ml and the optical density measured with a spectrophotometer at 570nm.

Estimation of glutathione S-transferase activity

Glutathione S-transferase activity was determined according to the method of Pérez - Chaca *et al.,* (2014). The

medium for the estimation was prepared and the reaction was allowed to run for 60 seconds each time before the absorbance was read against the blank at 340nm. The temperature was maintained at approximately 31^0C.

Statistical Analysis

All statistical analysis was performed using Graph pad prism 5 (statistical software). The results were first expressed as mean ± standard errors of mean (SEM) with a 95% confidence interval for mean. The data were then analyzed using one-way analysis of variance (ANOVA) followed by comparison using Tukey's Honest Significant Difference (HSD) post-hoc test. Values of $P<0.05$ were considered statistically significant.

The data were then analyzed using two-way analysis of variance (ANOVA) followed by comparison between the two extracts using Bonferroni post-hoc test.

RESULTS

Table 1: Results showing the amount of phytochemicals (quantitative) present in *Garcinia kola* and *Curcuma longa*

phytochemical	*Garcinia kola* (mg/g)	*Curcuma longa* (mg/g)
Phenols	0.23	21.27
Alkaloids	0.72	8.54
Tannins	0.26	42.49
Saponins	9.41	11.06
Flavonoids	1.04	57.80

The result of the chronic toxicity test performed. on the animals to determine the actual dose to be administered to avoid high rate of deaths in the groups, showed that no death was recorded during the toxicity test. The result in figure 1 shows data obtained for the SOD activities in the kidney of all the groups treatment compared for the two extracts administered.

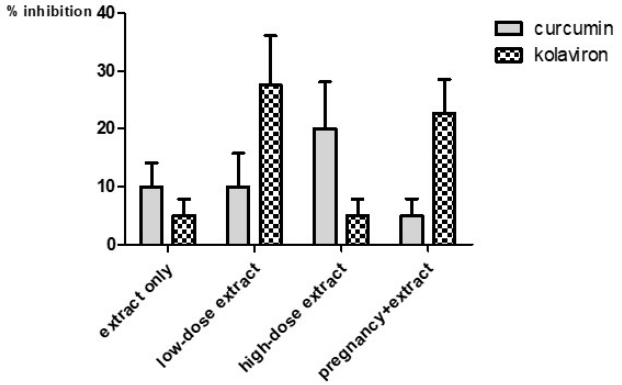

Fig. 1: Effect of extracts on SOD activity in the kidneys of female albino rats. Each bar represents mean ± SEM of 6 rats.

This result shows no significant difference in the SOD activities of all the comparisons made. Rats treated with "curcumin only" however had a higher inhibition value (10.00 %) when compared with those treated with "kolaviron only" (5.00 %). Also, infertile rats treated with high-dose curcumin had a higher inhibition value (20.00 %) when compared with those treated with high-dose kolaviron (5.00 %). However, infertile rats treated with low-dose kolaviron had a higher inhibition value (27.50 %) when compared with those treated with low-dose curcumin (10. 00 %). Also, pregnant rats treated with kolaviron had a higher inhibition value (22.73 %) when compared with the pregnant rats treated with curcumin (4.32 %).

The result in figure 2 shows data obtained for the SOD activities in the liver of all the groups treatment compared for the two extracts administered.

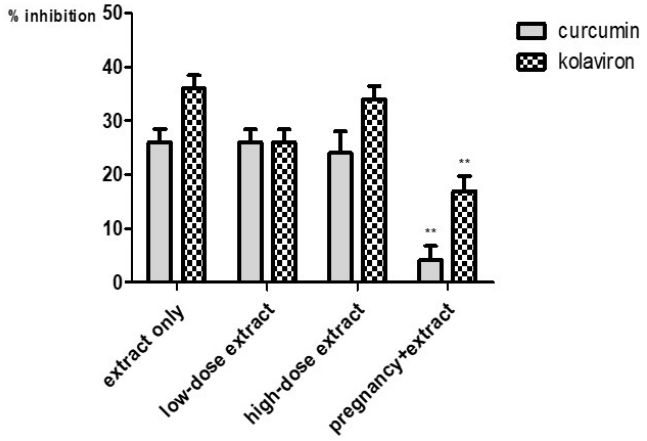

Fig. 2: Effect of extracts on SOD activity in the livers of female albino rats. Each bar represents mean ± SEM of 6 rats. Mean bars having "**" in the same dosage differ significantly at $p<0.01$.

This result shows that pregnant rats treated with kolaviron had a significantly higher inhibition value (16.97 %) when compared with pregnant rats treated with curcumin (4.18 %). There was however no significant difference in the remaining comparisons made in this result; although, rats treated with "kolaviron only" had a higher inhibition value (36.00 %) when compared with those treated with "curcumin only" (26.00 %). Also, infertile rats treated with high-dose kolaviron had a higher inhibition value (34.00 %) when compared with those treated with high-dose curcumin (24.00 %). Infertile rats treated with low-dose kolaviron had the same inhibition value (26.00 %) as those treated with low-dose curcumin.

The result in figure 3 shows data obtained for the SOD activities in the ovaries of all the groups treatment compared for the two extracts administered.

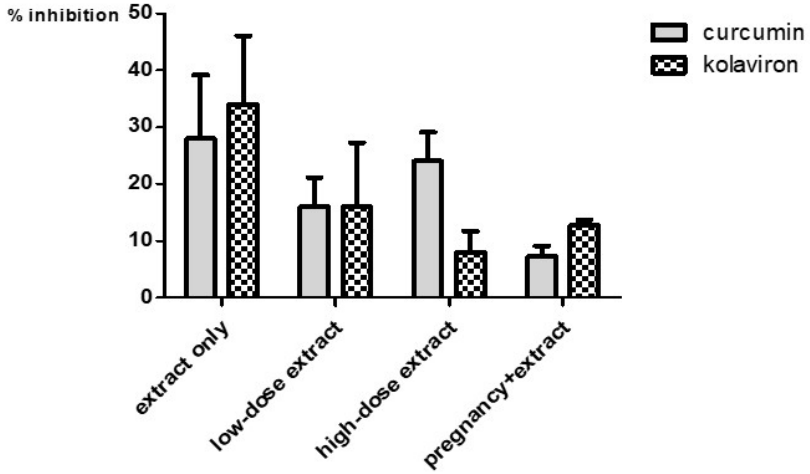

Fig. 3: Effect of extracts on SOD activity in the ovaries of female albino rats. Each bar represents mean ± SEM of 6 rats.

This result shows no significant difference in the SOD activities of all the comparisons made. Rats treated with "kolaviron only" however had a higher inhibition value (34.00%) when compared with those treated with "curcumin only" (28.00%). Also, pregnant rats treated with kolaviron had a higher inhibition value (12.73%) when compared with the pregnant rats treated with curcumin (7.27%). However, infertile rats treated with high-dose curcumin had a higher inhibition value (24.00%) when compared with those treated with high-dose kolaviron (8.00 %). Infertile rats treated with low-dose curcumin had the same inhibition value (16.00%) as those treated with low-dose kolaviron.

The result in figure 4 shows data obtained for the GST activities in the kidney of all the groups treatment compared for the two extracts administered.

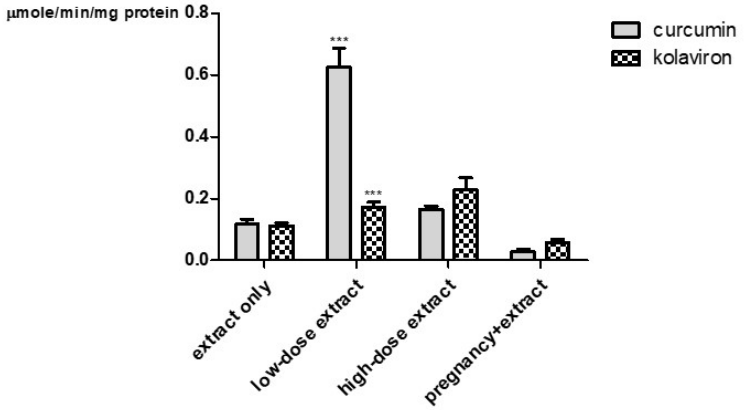

Fig. 4: Effect of extracts on GST activities in the kidneys of female albino rats. Mean bars having "***" in the same dosage differ significantly at $p<0.001$.

This result shows that infertile rats treated with low-dose curcumin had a significantly higher value (0.626 µmole/min/mg protein) when compared with those treated with low-dose kolaviron (0.1728 µmole/min/mg protein). There was however no significant difference in the remaining comparisons made in this result; although, infertile rats treated with high-dose kolaviron had a higher value (0.2295 µmole/min/mg protein) when compared with those treated with high-dose curcumin (0.1653 µmole/min/mg protein). Also, pregnant rats treated with kolaviron had significantly higher value (0.058 µmole/min/mg protein) when compared with those treated with curcumin (0.028 µmole/min/mg protein). Rats treated with "curcumin only" had a higher value (0.118 µmole/min/mg protein) when compared with those treated with "kolaviron only" (0.1123 µmole/min/mg protein).

The result in figure 5 shows data obtained for the GST activities in the liver of all the groups treatment compared for the two extracts administered.

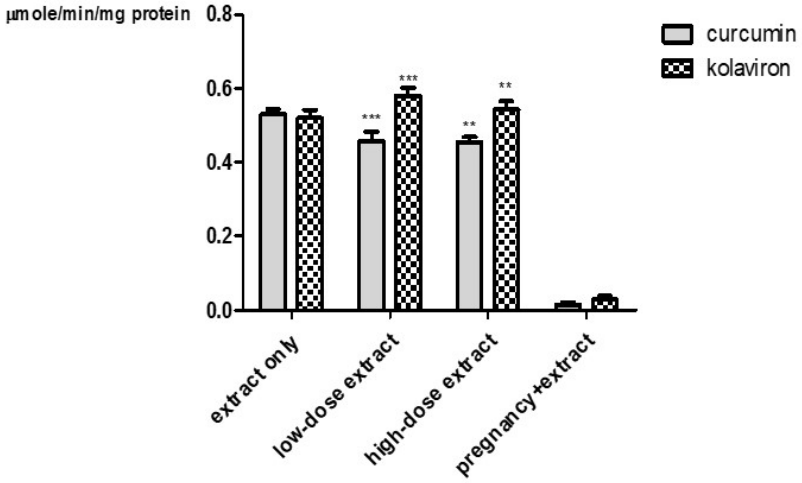

Fig. 5: Effect of extracts on GST activities in the livers of female albino rats. Each bar represents mean ± SEM of 6 rats. Mean bars having "***" in the same dosage differ significantly at $p<0.001$ while bars having "**" in the same dosage differ at $p<0.01$.

This result shows that infertile rats treated with low-dose kolaviron had a significantly higher value (0.5798 µmole/min/mg protein) when compared with those treated with low-dose curcumin (0.4568 µmole/min/mg protein). Also, infertile rats treated with high-dose kolaviron had a significantly higher value (0.5425 µmole/min/mg protein) when compared with those treated with high-dose curcumin (0.4544 µmole/min/mg protein). There was no significant difference between pregnant rats treated with kolaviron (0.029 µmole/min/mg protein) when compared with those treated with curcumin (0.0148 µmole/min/mg protein). Also, there was no significant difference between rats treated with curcumin (0.5298 µmole/min/mg protein) when compared with those treated with kolaviron (0.5194µmole/min/mg protein).

The result in figure 6 shows data obtained for the GST activities in the ovaries of all the groups treatment compared for the two extracts administered.

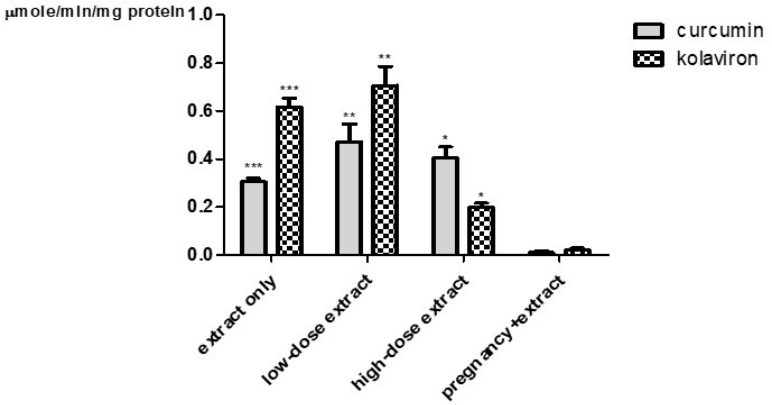

Fig. 6: Effect of extracts on GST activities in the ovaries of female albino rats. Each bar represents mean ± SEM of 6 rats. Mean bars having "***" in the same dosage differ significantly at $p<0.001$ while bars having "**" in the same dosage differ at $p<0.01$ and those having "*" differ significantly at $p<0.05$.

This result shows that rats treated with "kolaviron only" had a significantly higher value (0.6183 µmole/min/mg protein) when compared with those treated with "curcumin only" (0.3050 µmole/min/mg protein). Also, infertile rats treated with low-dose kolaviron had a significantly higher value (0.7068 µmole/min/mg protein) when compared with those treated with low-dose curcumin (0.4723 µmole/min/mg protein). However, infertile rats treated with high-dose curcumin had a significantly higher value (0.4048 µmole/min/mg protein) when compared with those treated with high-dose kolaviron (0.199 µmole/min/mg protein). There was no significant difference between pregnant rats treated with kolaviron (0.02025 µmole/min/mg protein) and those treated with curcumin (0.0105 µmole/min/mg protein).

The result in figure 7 shows data obtained for the catalase activities in the kidneys of all the groups treatment compared for the two extracts administered.

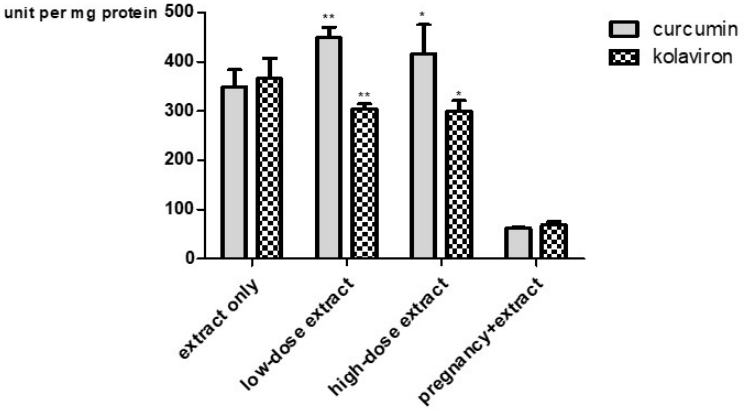

Fig. 7: Effect of extracts on catalase activities in the kidneys of female albino rats. Each bar represents mean ± SEM of 6 rats. Mean bars having "**" in the same dosage differ at $p<0.01$ while those having "*" differ significantly at $p<0.05$.

This result shows that infertile rats treated with low-dose curcumin had significantly higher value (448.5 per mg protein) when compared with those treated with low-dose kolaviron (304.7 per mg protein). The result also shows that infertile rats treated with high-dose curcumin had significantly higher value (416.0 per mg protein) when compared with infertile rats treated with high-dose kolaviron (299.7 per mg protein). There was however no significant difference between the rats treated with "kolaviron only" (366.2 per mg protein) when compared to those treated with "curcumin only" (348.9 per mg protein). There was also no significant difference between the pregnant rats treated with kolaviron (69.13 per mg protein) and those treated with curcumin (62.29 per mg protein).

The result in figure 8 shows data obtained for the catalase activities in the liver of all the groups treatment compared for the two extracts administered.

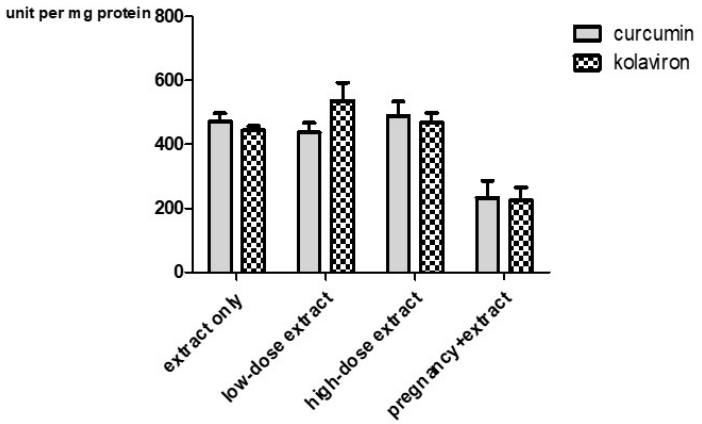

Fig. 8: Effect of extracts on catalase activities in the livers of female albino rats . Each bar represents mean ± SEM of 6 rats. This result shows no significant difference in the catalase activities of all the comparisons made.

This result shows no significant difference in the catalase activities of all the comparisons made. However, rats treated with "curcumin only" had a higher value (471.5 per mg protein) when compared with rats treated with "kolaviron only" (443.7 per mg protein). Also, pregnant rats treated with curcumin had a higher value (233.1 per mg protein) when compared with those treated with kolaviron (225.6 per mg protein). Infertile rats treated with high-dose curcumin also had a higher value (488.7 per mg protein) when compared with those treated with high-dose kolaviron (467.8 per mg protein). Infertile rats treated with low-dose kolaviron however had a higher value (536.2 per mg protein) when compared with those treated with low-dose curcumin (437.9 per mg protein).

The result in figure 9 shows data obtained for the catalase activities in the ovaries of all the groups treatment compared for the two extracts administered.

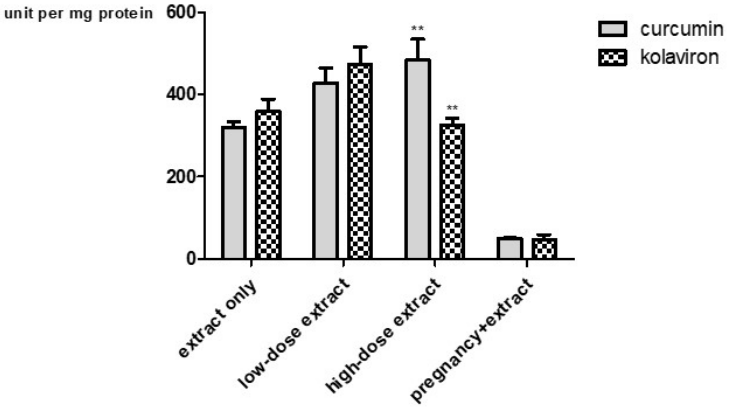

Fig. 9: Effect of extracts on catalase activities in the ovaries of female albino rats. Each bar represents mean ± SEM of 6 rats. Mean bars having "**" in the same dosage differ at $p<0.01$.

This result shows that infertile rats treated with high-dose curcumin had significantly higher value (484.0 per mg protein) when compared with those treated with high-dose kolaviron (325.3 per mg protein). There was however no significant difference in the remaining comparisons made in this result; although, infertile rats treated with low-dose kolaviron had a higher value (473.7 per mg protein) when compared with those of low-dose curcumin. Also, rats treated with "kolaviron only" had a higher value (359.1 per mg protein) when compared with those treated with "curcumin only" (320.0 per mg protein). Pregnant rats treated with curcumin also had a higher value (50.56 per mg protein) when compared with those treated with kolaviron (46.65 per mg protein).

DISCUSSION AND CONCLUSION

The findings from this study might as well be the first, in literature to compare the anti-oxidants properties of kolaviron and that of curcumin in any animal. This study showed that the groups treated with kolaviron generally had a higher increase in % inhibition of superoxide in hepatocytes (with no significant difference) when compared with, especially the pregnant groups treated with kolaviron which was significantly higher than the pregnancy group treated with curcumin. This suggest that during pregnancy, kolaviron protects the liver cells better than curcumin, by preventing it from cell damage induced by superoxide as it causes many types of cell damage when not acted upon by dismutase (Hayyan *et al.*, 2016; Desai and Bhilave, 2018). The result also suggests that kolaviron slows down lipid peroxidation more than curcumin in the ovaries by this comparisons made; most especially the

ovaries of infertile rats treated with kolaviron. This could indicate that kolaviron generally slows down the rate of oxidation in the system than curcumin. This could also mean that kolaviron will ameliorate oxidative stress (particularly induced by lipid peroxidation) faster than curcumin. In the kidneys of the treated groups, infertile group treated with low-dose curcumin had a significantly higher concentration of GST than the infertile group treated with low-dose kolaviron; indicating that curcumin detoxifies kidney cells (in infertile women) better than kolaviron because glutathione S-transferases (GST) are best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification and monitoring of cell injuries (Smith *et al.*, 2013; Stoddard *et al.*, 2019). In the livers of the infertile rats treated with the extracts (low-dose and high-dose), kolaviron increased the concentration of GSTs significantly when compared to curcumin at both doses, possibly indicating that kolaviron detoxifies the liver better than curcumin and could suggests that hepatocyte injury is better maintained by kolaviron. In the ovaries, this result indicate that kolaviron detoxifies the ovary of fertile rats better than with curcumin, it also indicates that ovarian cell injuries are better ameliorated with kolaviron. This study also suggests that infertile rats treated with low-dose kolaviron had their ovaries better detoxified than infertile rats treated with low-dose curcumin, although the reverse was the case when treated with high-dose of the extracts.

Furthermore, the result also indicates that curcumin might protects the kidney cells of infertile rats from oxidative damage better than kolaviron; as concentrations of catalase in infertile rats treated with curcumin were significantly higher than those treated with kolaviron, Since Catalase detoxifies hydrogen peroxide (H_2O_2) into oxygen and water, (Thimraj *et al.*, 2018), when H_2O_2 is present in high concentrations (Cao *et al.*, 2003; Day, 2009, Cho *et al*., 2019); this makes it a very important enzyme in protecting the cell from oxidative damage induced by reactive oxygen species (ROS). In the ovaries of infertile rats treated with high-dose of the extracts, the obtained result indicates that curcumin protects the ovarian cells from oxidative damage better than kolaviron.

In conclusion, this result indicates that kolaviron protects the liver and ovary more than curcumin which exhibits more protection of the kidney, hence kolaviron prevents oxidative stress better in the ovaries and livers of females while curcumin prevents oxidative stress better in the kidneys of females. It should also be noted that long term moderate oxidative stress has been linked to infertility, (Shi *et al.*, 2016; Bisht *et al.*, 2017), hence this study shows that kolaviron could assist to prevent this and could be useful therapeutically in the prevention of infertility caused by oxidative stress.

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