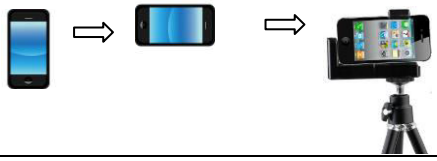
Script to prepare video clip(s)

Manuscript Title:	Neuroprotective potential of curcumin analogue: Therapeutic approach for neurodegeneration and neuroinflammation related diseases		
ID: 32384	Histopathology of rat brain		
Material:	Time:	Date:	Location: Your Lab

Position of your smart phone	
	Take the video using your smart phone in horizontal position instead of vertical Smart phone on the tripod reduces the shaking of picture

Develop neurodegerative change in rat			
Materials and Reagents			
	Methylmercury (II), rat		
Action			Subtitle
1	Show the vial containing methylmercury (II)	Close view	
2	Weigh methylmercury (II) and dissolve it into water		
3	Show rat within the cage		
4	Administer 20 mM methylmercury (II) solution for 4 weeks		
6	Observe the neurodegenerative symptoms through behavioral signs such as clasping score	Close view	

Collection of rat brain tissue			
Materials and Reagents			
	Rat brain, chloroform, petri dish, phosphate buffer		
Action			Subtitle
1	Sacrifice the rat on day 30 using chloroform anesthesis	Wide shot	
2	Decapitate the head and collect the brain		
3	Place the brain into a petri dish containing phosphate buffer		
4	Place the brain into fixative like formalin		

Embedding brain tissue		
Materials and Reagents		
	Gelatin, Sucrose, Deionized water, Microtome, rat brain, paraffin	
Action		Subtitle
1	Dissolve the 10 g gelatin in 100 mL warm water	
2	Add the sucrose	
3	Cool and keep in refrigerator. Gel is formed	
4	Heat gelatin gently over a steam bath until it melts	
5	While waiting for the gel to melt, remove the brain from the fixative	
6	Gently dab the brain dry	
7	Pour the melted gelatin into a mold and cool in ice water in a shallow dish of ice or in the refrigerator for several min	
8	Place brain into the gelatin, and with forceps, orient the brain (telencephalon down) until the gel hardens. Do not let the brain touch the edges of the mold	
9	Let the gel harden for about 20-30 min in the ice bath or until solidified in the refrigerator	
10	Remove the gel from the mold, and trim the block to a reasonably small cube around the brain and refrigerate until ready for sectioning	

Sectioning the brain tissue		
Materials and Reagents		
	Gelatin, Sucrose, Deionized water, Microtome, rat brain, paraffin	
Action		Subtitle
1	Fill the wells of approximately 4 histology trays with mounting medium	
2	Mounting medium consists of a 10:1 ratio of distilled water to phosphate buffer solution	
3	Letting brain sections or mounted tissue set in the undiluted phosphate buffer solution results in the tissue being exposed to too high a concentration of salt	
4	Collect dry ice	
5	Place the stage on the microtome and tighten the screw so that it sits in place without moving	
6	Fill the well of the stage approximately 2/3 full with 95% ethanol	
7	Remove the blade from its box and wipe with wiper to remove excess oil from storage	
8	Slide the blade into the microtome and tighten in place	
9	Put 2-3 pieces of dry ice into the stage well, and place the cover over the top	
10	When no more bubbling of the alcohol is heard, place another 2-3 pieces of dry ice into the well	

11	Continue until a thin layer of frosty white ice crystals form on top of the stage grate. Once this forms, draw 30% sucrose into a microtip pipet	
12	Drop sucrose onto the grate, so that the individual drops congeal together in the shape of a square, slightly larger than the cube of gelatin. Once the sucrose has frozen, it will turn white	
13	Lower the blade down until it is slightly below the surface of the sucrose square and slice off the top, making it level. Use wiper to unidirectionally wipe the now melting sucrose off of the blade	
14	Place the gelatin brain cube onto the now frozen sucrose square and wait for it to freeze; like the sucrose, the gelatin will turn an opaque white color when completely frozen	
15	Move the blade up using the right crank until even with the top of the gelatin cube, ensure that the base is set at 5 microns, and slowly click the left lever to move the blade down 5 microns. It may be necessary to remove excess gelatin before any brain tissue is visible in the cube	
16	To cut, move the blade forward with one fluid motion. Avoid jerky movements, and find the right balance between moving quickly enough so that the friction of the track does not cause it to stop mid-slice, and going so quickly that the piece of sliced tissue gets sucked underneath the blade, becoming unsalvageable. Once the brain tissue has reached the surface, you will see the tissue appearing on the blade	
17	Take a paintbrush and dip it into one of the filled histology tray wells so that it is damp. In one gentle motion, swipe the paintbrush down across the blade and over the brain tissue – scooping it up in the process, and keeping it from being pushed back against the blade and becoming accidentally sliced	
18	Once the tissue slice has been removed, slide the blade back over top of the brain cube, and lower it once it has reached its original position	

Mounting		
Materials and Reagents		
	Microscope, phosphate buffer solution, petri dish, freezer, subbed slide, hand gloves, pencil, paint brush	
Action		Subtitle
1	Turn on the microscope	
2	Heat the 10:1 distilled water and phosphate buffer solution mixture until thermometer reads approximately 48°C	
3	Place bottom half of a petri dish in front of the histology microscope and fill to approximately ¾ full with heated solution	
4	Take subbed slides out of the freezer and allow them to warm to room temperature	
5	When ready to mount the tissue, remove one slide from the container with gloved hands, so as to not	

	fingerprint the glass, and label it with a pencil. The slide should be labeled with the Animal ID, your initials, the date, the histology tray number, and the rows within that tray from which the tissue sections were taken	
6	Remove trays of tissue from refrigerator and begin mounting tissue. Use a damp paintbrush to remove each tissue section from the wells	
7	Place the tissue section into the petri dish, allowing it to float and reorient easily in the liquid. You will see the gelatin melt away in the heated solution leaving only the tissue. Once this has happened, orient the tissue the way you wish it to appear on the slide, then flip it bottom over top, so that it is now upside down.	
8	Place the tissue section into the petri dish, allowing it to float and reorient easily in the liquid. You will see the gelatin melt away in the heated solution leaving only the tissue. Once this has happened, orient the tissue the way you wish it to appear on the slide, then flip it bottom over top, so that it is now upside down.	
9	Bring the paintbrush in from underneath and lay the brain horizontally along the paintbrush	
10	Remove the tissue section from the heated solution and take the paintbrush between your thumb and forefinger to enable rolling over the brush	
11	Align the brush-head so that the edge of the tissue slice is visible and place this edge firmly onto the slide	
12	Slowly roll the brush forwards away from yourself until you see the section sticking to the slide. Placement on the slide is important because the subbing recipe creates sticky slides, which do not afford many adjustments	
13	Mount sections in even rows so that slide photography for publication will be possible later	
14	Once the tissue is in the proper orientation, it is necessary to dry the individual tissue sections with wiper. Cut the wiper into four pieces and twist them at the corners to form widgets. It is important to twist them very tightly because if they are loose, they will unravel when they come in contact with the liquid surrounding the tissue section and tear the brain in the process	
15	Gently touch the edges of the tissue section with the wiper and continue until the shiny liquid ring surrounding the tissue has been absorbed	

Staining		
Materials and Reagents		
	Glass slide, staining holder, Hematoxylin-Eosin	
Action		Subtitle
1	Place slides into vertical slide-staining holder	
2	Stained with Hematoxylin-Eosin	

Cover slipping		
Materials and Reagents		
	Hand gloves, microtip pipette, toluene solution, coverglass, blotting paper	
Action		Subtitle
1	Wear gloves to avoid fingerprints on the slide or glass coverslip	
2	Take a microtip pipet and draw up toulene solution	
3	Place the slide on a piece of paper towel on a level surface and gently squeeze the toulene solution out of the pipet forming a T on the slide. It is necessary to make one long horizontal line on the upper half of the glass slide, and one small perpendicular line in the center of the slide	
4	Then take one piece of Microscopic coverglass and set it on top of the slide	
5	Use a curved tweezer to spread the toulene solution in between the layers; this allows you to press with a great amount of pressure on the coverglass, without scratching it	
6	Hold the slide up to the light and make sure there are no air bubbles. If air bubbles are present, press and slide the air bubbles unidirectionally towards one edge with the tweezer until they escape	
7	Blot the edges using blotting paper to remove excess toulene solution which has seeped out of the edges. Let slides dry before inspection under the microscope	

Images were captured with image J software under bright field microscope at magnification of 20X